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Inhibition of Voltage-Sensitive Sodium Channels in Neuroblastoma Cells by Antiarrhythmic Drugs

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SUMMARY

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The antiarrhythmic drugs lidocaine, quinidine, procainamide, and diphenylhydantoin block neurotoxin-activated sodium channels in neuroblastoma cells. At concentrations which effectively block sodium channels, these drugs have no effect on binding of [^{3}H]saxitoxin to its receptor site on sodium channels. Similarly, effective concentrations of lidocaine, quinidine, and diphenylhydantoin have no effect on specific binding of ^{125}I-labeled scorpion toxin to sodium channels. These results show that antiarrhythmic drugs do not occupy neurotoxin receptor site 1, the tetrodotoxin/saxitoxin receptor site, or neurotoxin receptor site 3, the scorpion toxin/sea anemone toxin receptor site, in inhibiting sodium channels. The antiarrhythmic drugs are competitive inhibitors of activation of sodium channels by the full agonist batrachotoxin, but are mixed inhibitors affecting both $K_{0.5}$ and V_{max} for activation by the partial agonist veratridine. These results are consistent with the conclusion that the antiarrhythmic drugs are allosteric competitive inhibitors of sodium channel activation by batrachotoxin and veratridine. It is likely that antiarrhythmic drugs act at site(s) distinct from neurotoxin receptor Site 2 and alter veratridine and bactrachotoxin action indirectly.

INTRODUCTION

The Class I (1) or local anesthetic-like antiarrhythmic drugs slow the rate of rise ($\dot{V}_{max}$) of the cardiac action potential by modifying the properties of voltage-sensitive sodium channels (see ref. 2 for a recent review). Their effects on $\dot{V}_{max}$ are complex and are modulated by resting potential and both frequency and duration of channel activation (2). In nerve, the action of local anesthetics, including some anesthetics used as antiarrhythmic drugs, is also dependent on the frequency and duration of sodium channel activation (3–5). These results have led Strichartz (3), Courtney (4), and Hille (5) to develop a modulated receptor hypothesis of local anesthetic action. In this model, a specific local anesthetic receptor site is assumed to bind local anesthetics more rapidly and with higher affinity when the sodium channel is in its active (conducting) state (3–5). This model can potentially explain many aspects of antiarrhythmic drug action in heart (2). The objective of the work described in this report was to gain further insight into the nature of the receptor site(s) for local anesthetic-like antiarrhythmic drugs by examining their interaction with neurotoxins that bind at specific receptor sites associated with sodium channels.

Neurotoxins act at three distinct receptor sites associated with sodium channels (reviewed in refs. 6 and 7). Receptor site 1 binds the inhibitors tetrodotoxin and saxitoxin (6, 7). Receptor site 2 binds grayanotoxin and the alkaloids veratridine, batrachotoxin, and aconitine. These toxins cause persistent activation of sodium channels (7). Receptor site 3 binds the polypeptides scorpion toxin and sea anemone toxin. These toxins slow or block sodium channel inactivation and enhance persistent activation of sodium channels by alkaloid toxins acting at receptor site 2 by an allosteric mechanism (7). The interaction of neurotoxins with each of these receptor sites can be studied by ligand binding or ion flux experiments on electrically excitable neuroblastoma cells (8–10). In these experiments, we have studied the effects of four clinically useful antiarrhythmic drugs, lidocaine, quinidine, procainamide, and diphenylhydantoin, on neurotoxin action at each of these three receptor sites associated with sodium channels in neuroblastoma cells.

EXPERIMENTAL PROCEDURES

Materials. The growth of clone N18 neuroblastoma cells and the sources of commercially available materials have been cited previously (8, 9). Batrachotoxin was provided by Dr. John Daly (Laboratory of Bio-Organic Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health,

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Bethesda, Md. Scorpion toxin from *Leiurus quinquestriatus* was purified and radioactively labeled by lactoperoxidase-catalyzed iodination as described previously (8). Monoiodo scorpion toxin was used in all experiments. Saxitoxin was obtained from the National Institutes of Health and was radiolabeled by the specific exchange procedure of Ritchie *et al.* (11) and repurified as described previously (120. The preparation of [^{3}H]saxitoxin used in these studies was 85% radiochemically pure and had a specific radioactivity of 18.6 Ci/mmole.

[^{3}H]saxitoxin binding measurements. Saxitoxin binding measurements were carried out as described previously (10). Cells were incubated for 20 min at 36° with antiarrhythmic drugs and 3 nM [^{3}H]saxitoxin in 0.25 ml of standard binding medium consisting of 5.4 mM KCl, 130 mM choline chloride, 50 mM Hepes[1] (adjusted to pH 7.4 with Tris base), 5.5 mM glucose, 0.8 mM $MgSO_4$, and 1 mg/ml of bovine serum albumin. The cells were then washed three times in 15 sec at 0° with 3 ml of wash medium consisting of 163 mM choline chloride, 5 mM Hepes (adjusted to pH 7.4 with Tris base), 5.5 mM glucose, 0.8 mM $MgSO_4$, and 1.8 mM $CaCl_2$. The cells were then suspended in 0.4 N NaOH and bound radioactivity was determined. In each experiment, nonspecific binding in the presence of 1 μM tetrodotoxin was subtracted from the results (10).

^{125}I-labeled scorpion toxin binding measurements. Scorpion toxin binding measurements were carried out as described previously (8). Cells were incubated for 60 min at 36° with antiarrhythmic drugs and 0.1 nM mono[^{125}I]iodo scorpion toxin in 0.25 ml of standard binding medium. The cultures were then washed three times for 1 min each with 3 ml of wash medium at 36°. The cells were suspended in 0.4 N NaOH and bound radioactivity was determined. In each experiment, nonspecific binding in the presence of 200 nM unlabeled scorpion toxin was subtracted from the results (8).

$^{22}Na^+$ influx measurements. In experiments in which sodium channels were activated with batrachotoxin, cell cultures were incubated with the concentrations of batrachotoxin and antiarrhythmic drugs indicated in Figs. 1–4 for 30 min at 36° in 0.25 ml of medium consisting of 135.4 mM KCl, 50 mM Hepes (adjusted to pH 7.4 with Tris base), 5.5 mM glucose, 0.8 mM $MgSO_4$, and bovine serum albumin, 1 mg/ml. After 30 min, this medium was removed and the cells were rinsed twice in 15 sec with 1 ml of standard binding medium. The cells were then incubated for 30 sec in assay medium containing the same concentrations of batrachotoxin and antiarrhythmic drugs and 5.4 mM KCl, 120 mM choline chloride, 10 mM NaCl, 5 mM ouabain, 50 mM Hepes (adjusted to pH 7.4 with Tris base), 5.5 mM glucose, 0.8 mM $MgSO_4$, and ^{22}NaCl, 1.0 μCi/ml. Finally, the cells were washed three times with 3 ml of medium consisting of 163 mM choline chloride, 5 mM Hepes (adjusted to pH 7.4 with Tris base), 5.5 mM glucose, 0.8 mM $MgSO_4$, and 1.8 mM $CaCl_2$. Previous experiments have shown that these procedures give an accurate estimate of sodium permeability at constant membrane potential (9).

[1] The abbreviation used is: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

In experiments in which sodium channels were activated with veratridine, cell cultures were incubated for 10 min at 36° with the concentrations of veratridine and antiarrhythmic drugs indicated Figs. 1–4 in standard binding medium. The cells were then incubated for 2 min in assay medium containing the same concentrations of veratridine and antiarrhythmic drugs and washed as above. Control experiments confirmed that these procedures give an accurate estimate of the permeability increase caused by veratridine at constant membrane potential.

RESULTS

Lidocaine, quinidine, procainamide, and diphenylhydantoin block the increase in sodium permeability caused by batrachotoxin or veratridine in cultured neuroblastoma cells. The inhibition of batrachotoxin activation of sodium channels by quinidine is illustrated in Fig. 1. Neuroblastoma cells were incubated with batrachotoxin in the presence of increasing concentrations of quinidine in medium containing 135 mM K^+ and the initial rate of $^{22}Na^+$ influx (●) was measured as described under Experimental Procedure. Half-maximal inhibition was obtained at 3×10^{-5} M quinidine. In heart muscle preparations, the action of antiarrhythmic drugs is dependent on stimulus frequency and voltage (2). In order to assess the effect of membrane potential on antiarrhythmic drug action under the experimental conditions employed in this work, we compared concentration-effect relationships for inhibition in 135 mM K^+ [V_M = 0 mV (8)] to those in 5 mM K^+ [V_M = −41 mV (8),]. The results for quinidine are illustrated in Fig. 1. Depolarization in this membrane potential range has no effect on quinidine action in neuroblastoma cells. The results of similar experiments with lidocaine, procainamide, and diphenylhydantoin are summarized in Table 1. The action of procainamide and diphenylhydantoin was unaffected by depolarization. The affinity for lidocaine was reduced 2- to 3-fold by depolarization. Thus, apparent binding constants for antiarrhythmic drugs are not markedly voltage-

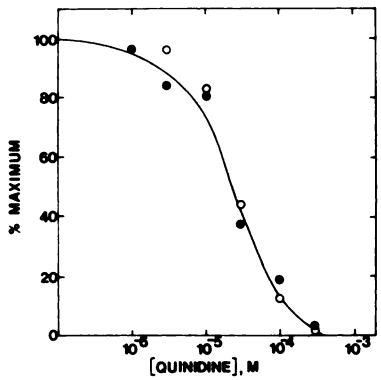

FIG. 1. *Inhibition of batrachotoxin activation of sodium channels by quinidine in 5 mM K^+ and 135 mM K^+*

Cell cultures were incubated for 30 min at 36° with 1 μM batrachotoxin and increasing concentrations of quinidine in standard binding medium (○) or medium with 135 mM KCl substituted for choline chloride (●). The initial rate of $^{22}Na^+$ influx was then measured in the presence of the same concentration of antiarrhythmic drug as described under Experimental Procedure.

TABLE 1

Effect of membrane potential on antiarrhythmic drug action

Drug	K_I	
	$V_{Max} = 0$ mV	$V_M = -41$ mV
Quinidine	3×10^{-5}	3×10^{-5}
Lidocaine	8×10^{-4}	3×10^{-4}
Procainamide	5×10^{-3}	5×10^{-3}
Diphenylhydantoin	1×10^{-4}	1×10^{-4}

dependent over this membrane potential range. These results are not in conflict with previous findings on heart muscle preparations and myelinated nerves (2–5), since the effects of membrane potential observed in those studies occurred at more negative membrane potentials not accessible in the present experimental system.

Lidocaine, quinidine, procainamide, and diphenylhydantoin are lipid-soluble and may alter membrane properties in a nonspecific manner in addition to any action at specific receptor sites. Therefore, in studying the interaction of these drugs at neurotoxin receptor sites, our approach was to correlate inhibition of sodium channel ion flux by antiarrhythmic drugs with block of specific neurotoxin binding. Neuroblastoma cells were incubated with batrachotoxin and antiarrhythmic drugs, and the initial rate of $^{22}Na^+$ was measured as described under Experimental Procedure. Under the conditions used, the initial rate of $^{22}Na^+$ influx is linearly proportional to sodium permeability, and is therefore an accurate measure of the fraction of active sodium channels (9). Concentration-effect curves for inhibition of batrachotoxin-activated sodium channels (●) are illustrated in Fig. 2A–D. Each of the four drugs tested blocked sodium channel ion flux completely. $K_{0.5}$ values ranged from 2.0×10^{-5} M for quinidine to 2.5×10^{-3} M for procainamide. In each case, the data were adequately fit by a simple Langmuir isotherm consistent inhibition of one sodium channel by one drug molecule.

In order to determine whether the antiarrhythmic drugs bind at receptor site 1, the tetrodotoxin/saxitoxin receptor site, during inhibition of the sodium channel, parallel experiments were carried out in which the effect of the drugs on specific [^{3}H]saxitoxin binding was measured. Figure 2A–D (△) shows that none of the antiarrhythmic drugs tested blocks [^{3}H]saxitoxin binding at concentrations sufficient to inhibit sodium channel ion flux. Some inhibition is observed with quinidine at a concentration 30-fold higher than is required for inhibition of ion flux (Fig. 2a; △). We conclude from these data that the antiarrhythmic drugs tested do not interact with receptor site 1 during inhibition of sodium channel ion flux.

The effect of antiarrhythmic drugs on specific ^{125}I-labeled scorpion toxin binding at receptor site 3 was assessed in the same way (Fig. 2A–D; ○). Diphenylhydantoin had no effect on ^{125}I-labeled scorpion toxin binding (Fig. 2D). Both lidocaine and quinidine inhibited scorpion toxin binding, but 10- to 50-fold higher concentrations were required to inhibit ^{125}I-labeled scorpion toxin binding as effectively as ion flux. We conclude that none of these three drugs interacts with neurotoxin receptor site 3 during inhibition of sodium channel ion flux.

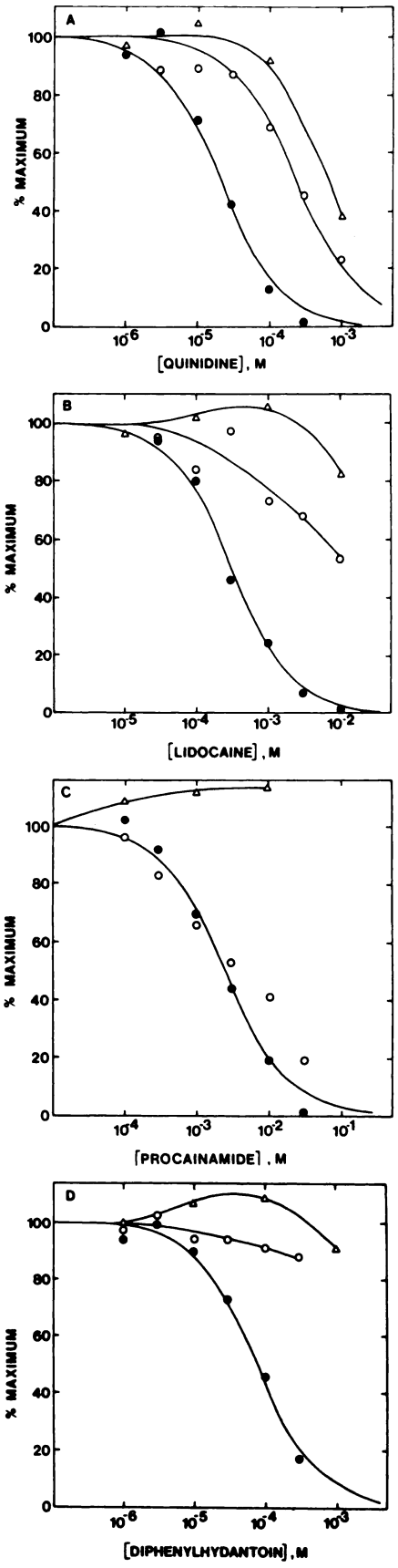

FIG. 2.

In contrast, procainamide inhibited ^{125}I-labeled scorpion toxin binding in the same concentration range as ion flux (Figure 2C). On the basis of this result alone, procainamide might bind at neurotoxin receptor site 3 in inhibiting sodium channels. However, data presented below argue against this possibility and suggest that the inhibition of scorpion toxin binding is unrelated to inhibition of ion flux.

At present, there is no toxin binding assay for receptor site 2 that can be applied to neuroblastoma cells. Therefore, the interaction of local anesthetics with this receptor site has been studied indirectly by using double reciprocal analysis of concentration-effect curves for alkaloid toxin activation of sodium channels. Titration curves for batrachotoxin were obtained under control conditions and in the presence of two different concentrations of antiarrhythmic drugs. Double reciprocal plots of these data are presented in Fig. 3A–D. In each case, the antiarrhythmic drug tested causes an increase in $K_{0.5}$ without significant change in V_{max}. These four antiarrhythmic drugs are therefore competitive inhibitors of activation of sodium channels by batrachotoxin.

Competitive inhibition patterns can result either from direct steric interaction at a common receptor site or from allosteric interactions at separate sites. Batrachotoxin and other alkaloid toxins are allosteric activators of sodium channels (9), and indirect allosteric inhibition of their action would not be surprising. On the basis of an allosteric model of alkaloid toxin action presented previously, some classes of allosteric inhibitors would be expected to give competitive inhibition patterns when tested with a full agonist like batrachotoxin but to give mixed or noncompetitive inhibition patterns when tested with a partial agonist like veratridine (ref. 9 and see Discussion). It was therefore of interest to study the pattern of inhibition of veratridine activation of sodium channels by antiarrhythmic drugs. The results presented in Fig. 4A–D show that all four antiarrhythmic drugs are mixed inhibitors of veratridine activation. Procainamide gives a nearly competitive inhibition pattern, whereas the other three drugs have a substantial effect on both V_{max} and $K_{0.5}$. These results are most consistent with the view that the antiarrhythmic drugs are allosteric inhibitors of alkaloid toxin activation of sodium channels and act at separate receptor site(s).

The competitive inhibition of batrachotoxin activation by antiarrhythmic drugs suggests an experimental approach to test whether procainamide block of scorpion toxin binding (Fig. 2C) results from a specific interaction at the receptor site responsible for inhibition of ion flux. Since procainamide and batrachotoxin interact competitively in ion flux inhibition, the presence of batrachotoxin should increase $K_{0.5}$ for procainamide block of scorpion toxin binding if the same procainamide receptor site is involved in both effects. Comparison of procainamide block of scorpion toxin binding in the presence and absence of batrachotoxin showed that the block was unchanged or slightly enhanced in the presence of batrachotoxin (data not shown). Thus, it seems unlikely that procainamide effects on scorpion toxin binding and ion flux result from interaction at the same receptor site.

FIG. 2. *Inhibition of $^{22}Na^+$ influx, [^{3}H]saxitoxin binding, and [^{125}I]scorpion toxin binding by antiarrhythmic drugs*

$^{22}Na^+$ influx (●): cell cultures were incubated with 1 μM batrachotoxin and the indicated concentrations of antiarrhythmic drugs as described under Experimental Procedure; $^{22}Na^+$ influx was then measured in the presence of the same drug concentrations. [^{3}H]saxitoxin binding (△): cell cultures were incubated with 3 nM [^{3}H]saxitoxin and the indicated concentrations of antiarrhythmic drugs and bound [^{3}H]saxitoxin was determined as described under Experimental Procedure. ^{125}I-labeled scorpion toxin binding (○): cell cultures were incubated with 0.1 nM ^{125}I-labeled scorpion toxin and the indicated concentrations of antiarrhythmic drugs and bound ^{125}I-labeled scorpion toxin was determined as described under Experimental Procedure.

DISCUSSION

Our results show that antiarrhythmic drugs do not bind at neurotoxin receptor site 1, the saxitoxin/tetrodotoxin receptor site, in inhibiting sodium channel ion flux. These results confirm and extend early tetrodotoxin binding experiments which showed that lidocaine did not block toxin binding in unmyelinated nerve (13). Similarly, our results show that antiarrhythmic drugs do not bind at neurotoxin receptor site 3, the polypeptide toxin receptor site, in inhibiting sodium channel ion flux. Thus, only neurotoxin receptor site 2, the alkaloid toxin receptor site, remains a candidate for interaction with antiarrhythmic drugs.

Electrophysiological experiments have provided indirect evidence for competitive interactions between batrachotoxin and certain local anesthetics. Procaine and lidocaine prevent irreversible depolarization of skeletal muscle by batrachotoxin in a manner that suggests block of toxin binding (14). Procaine blocks unmodified sodium channels at lower concentration than batrachotoxin-activated sodium channels suggesting competitive interactions (15). Ion flux studies in neuroblastoma cells have shown that the lidocaine derivative QX572, benzocaine, and the *alpha*-adrenergic blocker yohimbine, which has local anesthetic action at high concentration, are competitive inhibitors of batrachotoxin activation (16, 17). Our results add four local anesthetic-like antiarrhythmic drugs to this list of competitive inhibitors of batrachotoxin activation. It seems that this is a general characteristic of local anesthetic/antiarrhythmic drugs. Therefore, it is of interest to consider the possible mechanisms underlying this competitive interaction.

Batrachotoxin is an allosteric activator of sodium channels which acts by binding with high affinity to active state(s) of sodium channels and shifting an equilibrium between active and inactive (nonconducting) states (9). Using the same nomenclature as was previously used (9), the allosteric model of toxin action assumes that an equilibrium between two states or groups of states, R (active) and T (inactive) is characterized by an equilibrium constant M_{RT}, the allosteric constant. Activators (A) like batrachotoxin bind to the two states with dissociation constants K_R and K_T.

$$\begin{array}{ccc} & M_{RT} & \\ A + T & \rightleftharpoons & A + R \\ K_T \Uparrow\Downarrow & & \Uparrow\Downarrow K_R \\ TA & & RA \end{array} \qquad (1)$$

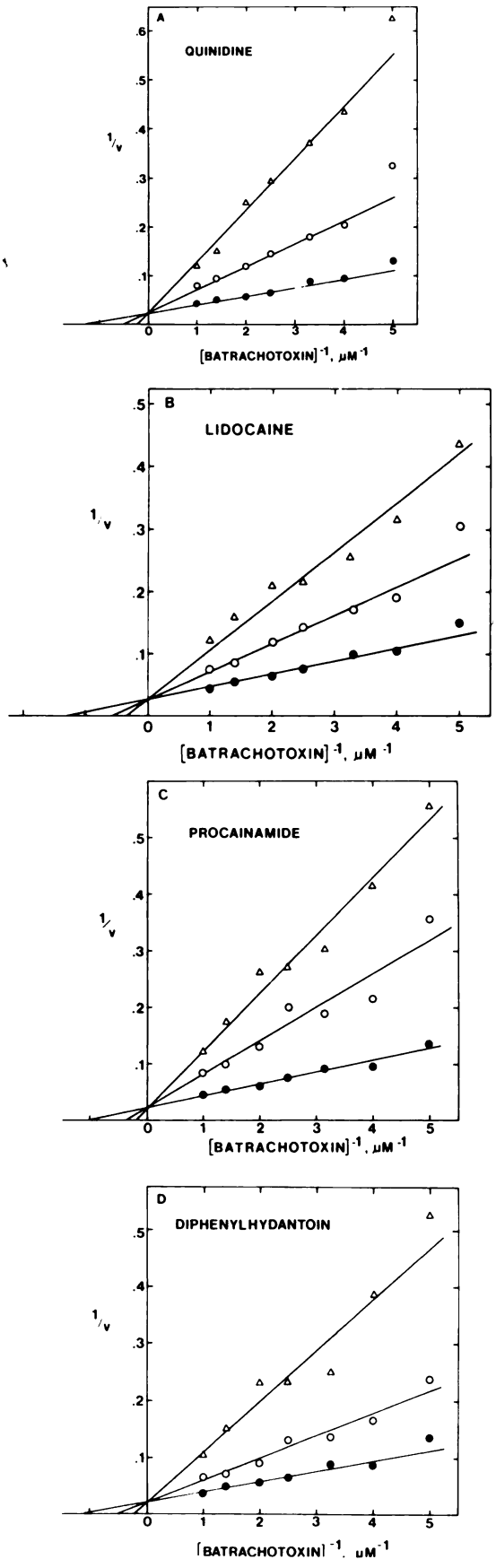

FIG. 3.

The fraction of ion channels activated at equilibrium (P_R) is then a function of activator concentration (9).

$$P_R(A) = \frac{1}{1 + M_{RT}\dfrac{1 + A/K_T}{1 + A/K_R}} \qquad (2)$$

This equation shows that the concentration effect curve for batrachotoxin is controlled by three constants, M_{RT}, K_T, and K_R. Variation in each of these can alter $K_{0.5}$ and V_{max}. The maximal fraction of channels activated [$P_R(\infty)$] is proportional to V_{max} and is given by the following equation (9):

$$P_R(\infty) = \frac{1}{1 + M_{RT}\dfrac{K_R}{K_T}} \qquad (3)$$

For systems like the sodium channel, where M_{RT} is large, $K_{0.5}$ is approximately given by the following equation (9):

$$\frac{1}{K_{0.5}} = \frac{1}{M_{RT}K_R} + \frac{1}{K_T} \qquad (4)$$

For a full agonist like batrachotoxin, $P_R(\infty) = 1$, $M_{RT}(K_R/K_T) \ll 1$ (from Eq. 3), and $M_{RT}K_R \leqq K_T$. Thus, from Eq. 4, $K_{0.5} \sim M_{RT}K_R$. Compounds or conditions which increase M_{RT} or K_R will increase $K_{0.5}$ but have little or no effect on $P_R(\infty)$ or V_{max}. Apparently competitive inhibition of batrachotoxin action can occur if either the allosteric constant for the $T \rightleftarrows R$ transition (M_{RT}) or the dissociation constant for binding to the R state (K_R) is increased. This type of inhibition would be termed allosteric competitive inhibition (18) to distinguish it from simple competitive inhibition due to steric interaction at a common site. Our data on inhibition of batrachotoxin activation by antiarrhythmic drugs do not distinguish between direct and allosteric competitive inhibition and we therefore cannot conclude from the competitive inhibition patterns observed in those experiments that the antiarrhythmic drugs studied bind at receptor site 2.

Studies of inhibition of veratridine activation provide additional information in this regard. Since veratridine is a partial agonist (9), any effect on M_{RT} or K_R must be accompanied by an effect on $P_R(\infty)$ and V_{max} (Eq. 3). Thus, inhibitors which appear strictly competitive with respect to partial agonists are likely to be direct competitive inhibitors. Our data show that the antiarrhythmic drugs have effects on both $K_{0.5}$ and V_{max} for veratridine action. They must, therefore, have effects on M_{RT} and K_R. These effects are characteristic of all allosteric com-

FIG. 3. *Mechanism of inhibition of batrachotoxin activation of sodium channels by antiarrhythmic drugs*

Cell cultures were incubated with the indicated concentrations of batrachotoxin and different fixed concentrations of antiarrhythmic drugs, and $^{22}Na^+$ influx was measured as described under Experimental Procedure.

A. 0 (●), 2×10^{-5} M (○), and 6×10^{-5} M (△) quinidine.
B. 0 (●), 3×10^{-4} M (○), and 9×10^{-4} M (△) lidocaine.
C. 0 (●), 2×10^{-3} M (○), and 6×10^{-3} M (△) procainamide.
D. 0 (●), 6×10^{-5} M (○), and 2×10^{-4} M (△) diphenylhydantoin.

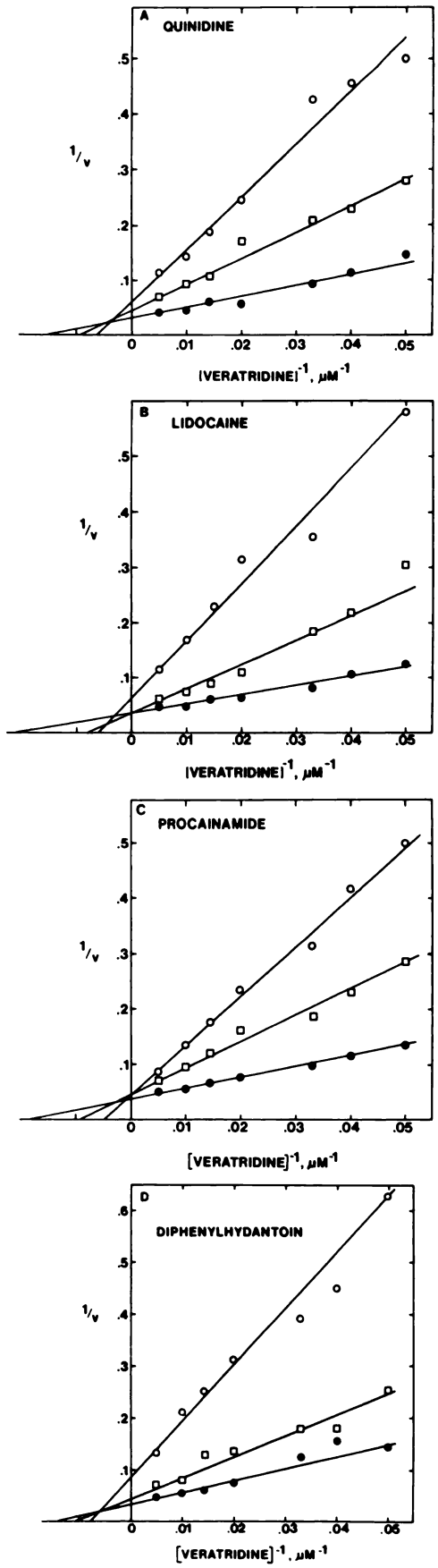

FIG. 4.

petitive inhibition mechanisms. On the basis of these results, the antiarrhythmic drugs are most likely to be allosteric inhibitors of alkaloid toxin action which bind at separate site(s) and inhibit alkaloid toxin action by increasing M_{RT} and/or increasing K_R. The results do not provide evidence for direct interaction of the antiarrhythmic drugs with any of the three neurotoxin sites described in earlier work.

Our results and similar data with yohimbine, QX572, and benzocaine (16, 17) are not consistent with the view that antiarrhythmic drugs and/or local anesthetics act as a plug occluding the ion conducting pore of the sodium channel. Such a mechanism without allosteric interactions would yield noncompetitive inhibition patterns in which V_{max} is reduced with no change in $K_{0.5}$. Our results are consistent with two models. In the first, antiarrhythmic drugs and local anesthetics, at equilibrium, bind selectively with high affinity to nonconducting state(s) of sodium channels and thereby increase the value of the allosteric constant M_{RT} (Eq. 2). This model is consistent with the proposal of Courtney (4) and Hille (5) that local anesthetics bind with high affinity to the inactivated state of sodium channels. High-affinity binding to the inactivated sodium channel is sufficient to explain the results described here.

In the second model, antiarrhythmic drugs and local anesthetics, at equilibrium, bind to both nonconducting and conducting states of sodium channels but selectively increase K_R (eq. 2), the dissociation constant for alkaloid toxin binding to active states. A decrease in K_R would imply alteration of the conformation of the conducting state of the sodium channel so that alkaloid toxins would bind with lower affinity. This second model is less attractive because it does not derive support from the published electrophysiological data. However, it does provide an equally valid interpretation of the data presented here.

We have discussed our results with these four antiarrhythmic drugs from the viewpoint that all four have generally similar actions in this experimental system. Although the similarity is striking, procainamide has some characteristically different effects that are worthy of consideration. Procainamide inhibits scorpion toxin binding in the same concentration range at which it blocks $^{22}Na^+$ uptake. We have presented evidence suggesting that these two effects are unrelated. Since procainamide acts at higher concentration than the other drugs studied, the similarity in concentration dependence may represent a fortuitous overlap of a specific effect on ion flux and a nonspecific membrane effect on scorpion toxin binding or, alternatively, might be due to multiple

FIG. 4. *Mechanism of inhibition of veratridine activation of sodium channels by antiarrhythmic drugs*

Cell cultures were incubated with the indicated concentrations of veratridine and different fixed concentrations of antiarrhythmic drugs, and $^{22}Na^+$ influx was measured as described under Experimental Procedure.

A. 0 (●), 2×10^{-5} M (□), and 5×10^{-5} M (○) quinidine.
B. 0 (●), 1×10^{-4} M (□), and 3×10^{-4} M (○) lidocaine.
C. 0 (●), 2×10^{-3} M (□), and 6×10^{-3} M (○) procainamide.
D. 0 (●), 6×10^{-5} M (□), and 2×10^{-4} M (○) diphenylhydantoin.

sites of procainamide action. Procainamide is more nearly competitive in its inhibition of veratridine activation than are the other antiarrhythmic drugs (Fig. 4). This result can be accommodated within the framework of the allosteric model if procainamide increases both M_{RT} and K_T, since M_{RT} and K_T have opposite effects on V_{max} (Eq. 3) but have the same effect on $K_{0.5}$ (Eq. 4). However, the nearly competitive inhibition of veratridine activation may also indicate direct steric interactions between procainamide and the alkaloid toxins that are not observed with the other antiarrhythmic drugs studied. Thus, although we wish to emphasize the considerable similarities among the effects of the four antiarrhythmic drugs studied in this experimental system, we cannot exclude the possibility that additional sites and mechanisms of procainamide action also contribute to the observed effects.

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