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Inhibition of Voltage-Sensitive Sodium Channels in Neuroblastoma Cells and Synaptosomes by the Anticonvulsant Drugs Diphenylhydantoin and Carbamazepine

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SUMMARY

The inhibitory action of a number of clinically effective anticonvulsants on neurotoxin-activated sodium channels in cultured neuroblastoma cells and rat brain synaptosomes has been examined. Diphenylhydantoin (K_I = 35 μM) and carbamazepine (K_I = 41 μM) inhibited batrachotoxin-activated $^{22}Na^+$ influx in N18 cells. Similarly, batrachotoxin-activated $^{22}Na^+$ influx in rat brain synaptosomes was also inhibited by diphenylhydantoin (K_I = 38 μM) and carbamazepine (K_I = 22 μM). Comparison of K_I values with mean brain levels of these drugs achieved during prevention of electroshock seizures indicates that diphenylhydantoin and carbamazepine occupy 35% and 50%, respectively, of their receptor sites associated with sodium channels at mean therapeutic concentrations. Diazepam (K_I = 51 to 63 μM) and phenobarbital (K_I = 1.2 to 1.3 mM) inhibited batrachotoxin-activated $^{22}Na^+$ flux in N18 cells and synaptosomes at concentrations in excess of mean therapeutic central nervous system levels. Carbamazepine, like diphenylhydantoin, acts as a competitive inhibitor of sodium channel activation by the full agonist batrachotoxin, but produces mixed inhibition of veratridine-activated channels. This finding is consistent with the conclusion that both carbamazepine and diphenylhydantoin act as allosteric inhibitors of neurotoxin-activated sodium channels. The dose-response relationships for carbamazepine and diphenylhydantoin inhibition of $^{22}Na^+$ flux in N18 cells are shifted 1.5-fold to higher concentrations when $^{22}Na^+$ flux measurements are made in the presence of physiological concentrations of sodium and calcium ions. These results suggest that anticonvulsant inhibition of neurotoxin-activated $^{22}Na^+$ flux in our standard ion flux media, containing low concentrations of Na^+ and no Ca^{2+}, is likely to reflect an effect of these agents expected *in vivo*. The results of this study provide further evidence to support the hypothesis that diphenylhydantoin and carbamazepine, both of which possess similar therapeutic profiles in the treatment of grand mal and partial seizures, may exert their pharmacological effects by occupany of receptor sites associated with the activation of voltage-sensitive sodium channels in the central nervous system.

INTRODUCTION

There is now considerable evidence that DPH[1] and CBZ may exert their anticonvulsant effects by interacting with voltage-sensitive sodium channels involved in the propagation of action potentials. Electrophysiological investigations have shown that these agents, which are employed in the management of grand mal and partial seizures (1), can inhibit sodium currents in a variety of preparations (2–5). Further information concerning the molecular actions of these anticonvulsants has been provided by characterizing their interaction with radiolabeled neurotoxins which bind to receptor sites associated with the sodium channel in brain (6). In rat brain synaptosomes, there are three distinct classes of neurotoxin receptor sites. Tetrodotoxin and saxitoxin bind to receptor site 1 (7, 8) and block ion transport by the channel. The lipid-soluble toxins grayanotoxin, batrachotoxin, veratridine, and aconitine, which cause prolonged activation of sodium channels, bind to receptor site 2 (9–12). Scorpion toxin from *Leiurus quinquestriatus* binds to receptor site 3 (13–15) and causes a slowing of the rate of channel inactivation in addition to an allosteric enhancement of the persistent activation by neurotoxins acting at receptor site 2 (7, 14). DPH and CBZ act as allosteric competitive inhibitors of BTX-B binding in

[1] The abbreviations used are: DPH, diphenylhydantoin; CBZ, carbamazepine, BTX-B, batrachotoxinin A 20-α-benzoate; Hepes, 4-(2-hydroxylethyl)-1-piperazineethanesulfonic acid; BSA, bovine serum albumin; GABA, γ-aminobutyric acid.

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synaptosomes (6). At concentrations in the central nervous system that protect rats from experimentally induced seizures, DPH and CBZ were calculated to occupy 35% and 25%, respectively, of their receptor sites on brain sodium channels (6). In order to provide evidence that these anticonvulstants block ion flux as a consequence of their binding to the sodium channel, the present study examines the effects of a number of clinically effective anticonvulsants on batrachotoxin- or veratridine-activated $^{22}Na^+$ flux in neuroblastoma cells and synaptosomes.

EXPERIMENTAL PROCEDURES

Materials. The growth and maintenance of cultured N18 neuroblastoma cells (including commercial sources of serum and media) have been described previously (15). DPH, CBZ, diazepam, phenobarbital, ethosuximide, trimethadione, and sodium valproate were gifts from Drs. A. Camerman and R. H. Levy (University of Washington, Seattle, Wash.). Daily working solutions of DPH and CBZ were prepared by dissolving the drugs in ethanol followed by dilution into aqueous medium with gentle warming. Solutions prepared in this manner were stable for at least 1 hr and were used immediately. Control experiments indicated that the final concentration of ethanol (0.5%) had no effect on alkaloid toxin-induced $^{22}Na^+$ flux in cells or synaptosomes. Batrachotoxin was a gift from Dr. John Daly (Laboratory of Bioorganic Chemistry, National Institutes of Health, Bethesda, Md.). Scorpion (*Leiurus quinquestriatus*) venom and bovine serum albumin were obtained from Sigma Chemical Company (St. Louis, Mo.). Scorpion toxin was purified according to previously described procedures (16). Tetrodotoxin and veratridine were purchased from Calbiochem (San Diego, Calif.) and Aldrich Chemical Company (Milwaukee, Wisc.), respectively. All other standard laboratory reagents were of analytical grade.

Preparation of synaptosomes. Synaptosomes were prepared from whole rat brain according to a modification of the method of Gray and Whittaker (17) as previously described by Tamkun and Catterall (11).

Measurement of batrachotoxin-activated $^{22}Na^+$ flux in rat brain synaptosomes. Prior to use, synaptosomes were thawed at 36° for 5 min and then stored on ice. Synaptosomes (0.62 mg of protein) were incubated with batrachotoxin (1–2 μM) for 20 min at 36° in a 50-μl solution containing 5.4 mM KCl, 0.8 mM $MgSO_4$, 5.5 mM glucose, 50 mM Hepes-Tris (pH 7.4), 130 mM choline chloride, and BSA (1 mg/ml). Previous studies have shown batrachotoxin activation of $^{22}Na^+$ flux is maximal within this period of incubation (11). After 20 min, 150 μl of the $^{22}Na^+$ uptake solution were added and mixed rapidly by vortexing. The uptake solution contained the same toxin concentration used in the preincubation plus 5.4 mM KCl, 0.8 mM $MgSO_4$, 5.5 mM glucose, 50 mM Hepes-Tris (pH 7.4), 128 mM choline chloride, 2.66 mM NaCl, 5 mM ouabain, BSA (1 mg/ml), and carrier-free $^{22}NaCl$ (0.3 μCi/ml). The synaptosomes were incubated with this assay solution for 5 sec at 36°, and the reaction was terminated by the addition of 3 ml of an ice-cold wash solution containing 163 mM choline chloride, 0.8 mM $MgSO_4$, 1.8 mM $CaCl_2$, 5 mM Hepes-Tris (pH 7.4), and BSA (1 mg/ml). The mixture was rapidly filtered under vacuum through an Amicon cellulose filter disc (0.45 μm), and the filter disc and trapped synaptosomes were washed twice with 3 ml of the wash solution. Radioactivity was determined by liquid scintillation spectrometry. Specific $^{22}Na^+$ influx attributable to activation of the voltage-sensitive sodium channel was determined as the difference in $^{22}Na^+$ uptake in the absence and presence of 1 μM tetrodotoxin added to both preincubation and uptake solutions. The data presented are corrected for nonspecific uptake and nonspecific binding of $^{22}Na^+$ to filter discs.

Measurement of batrachotoxin- and veratridine-activated $^{22}Na^+$ flux in cultured N18 neuroblastoma cells. In experiments in which batrachotoxin (0.02–1.25 μM) was used to activate sodium channels, neuroblastoma cell cultures were incubated with the alkaloid and varying concentrations of anticonvulsants as indicated in the figures for 30 min at 36° in 0.25 ml of medium containing 135 mM KCl, 50 mM Hepes-Tris (pH 7.4), 5.5 mM glucose, 0.8 mM $MgSO_4$, and BSA (1 mg/ml). After 30 min, the medium was removed by aspiration, and the cells were incubated for 30 sec in assay medium containing the same concentrations of batrachotoxin and anticonvulsants, and 5.4 mM KCl, 120 mM choline chloride, 10 mM NaCl, 5 mM ouabain, 50 mM Hepes-Tris (pH 7.4), 5.5 mM glucose, 0.8 mM $MgSO_4$, and $^{22}NaCl$ (1.0 μCi/ml). The assay was terminated by washing the cells three times with ice-cold wash medium identical with that used in the synaptosomal flux procedures described above. In some experiments, scorpion toxin (0.3 μM) was included in the incubation and assay media.

In experiments in which sodium channels were activated with veratridine (0.02–0.2 mM), cell cultures were incubated for 30 min at 36° with varying concentrations of anticonvulsants and 130 mM choline chloride, 50 mM Hepes-Tris (pH 7.4), 5.5 mM glucose, 0.8 mM $MgSO_4$, and BSA (1 mg/ml). The $^{22}Na^+$ flux solution contained varying concentrations of veratridine and anticonvulsants. In all other respects, the assay and wash procedures and composition of media were identical with those used in the batrachotoxin experiments described above. Previous experiments in this laboratory have shown that the procedures described above provide an accurate estimate of sodium ion permeability at constant membrane potential (15, 18).

Other methods. Synaptosomal and cellular protein concentration was measured according to the method of Peterson (19). Except where mentioned, the data presented are the results of a single experiment that are representative of three to four similar experiments. Each data point represents the mean of three to six replicate determinations. In all experiments, standard errors of the mean were less than 10% of the mean value observed. Double reciprocal plots of neurotoxin activation of sodium channels were fitted by computer-assisted least-squares regression analysis. IC_{50} determinations were calculated by log-probit analysis according to the method of Finney (20). For anticonvulsants which act as competitive inhibitors of batrachotoxin action, IC_{50} values were converted to K_I values according to the relationship

$$K_I = \frac{IC_{50}}{1 + [L]/K_L}$$

where K_L is the equilibrium dissociation constant for batrachotoxin, $[L]$ is the concentration of batrachotoxin used, and IC_{50} is the concentration of drug which produces 50% inhibition of batrachotoxin-activated $^{22}Na^+$ flux.

RESULTS

Anticonvulsant inhibition of batrachotoxin-activated $^{22}Na^+$ influx in neuroblastoma cells and rat brain synaptosomes. In a recent study examining the effects of various anticonvulsants on the binding of BTX-B to sodium channel receptor sites in synaptosomes, it was found that DPH and CBZ act as allosteric competitive inhibitors of BTX-B binding at therapeutically relevant brain concentrations (6). On the other hand, these agents did not alter the binding of radiolabeled saxitoxin or scorpion toxin to receptor sites 1 and 3, respectively, suggesting a degree of specificity for the alkaloid neurotoxin binding site (receptor site 2). These results predict that DPH and CBZ should exert inhibitory effects on batrachotoxin-activated $^{22}Na^+$ flux at comparable concentrations which produce inhibition of [^{3}H]BTX-B binding. DPH and CBZ inhibited batrachotoxin-activated $^{22}Na^+$ flux in N18 cells in a concentration-dependent manner, with K_I values of 35 ± 2.1 μM (n = 4) and 41 ± 2.6 μM (n = 4), respectively (Fig. 1A). Trimethadione, ethosuximide, and sodium valproate did not affect batrachotoxin activated flux at concentrations up to 1 mM (Fig. 1A), in agreement

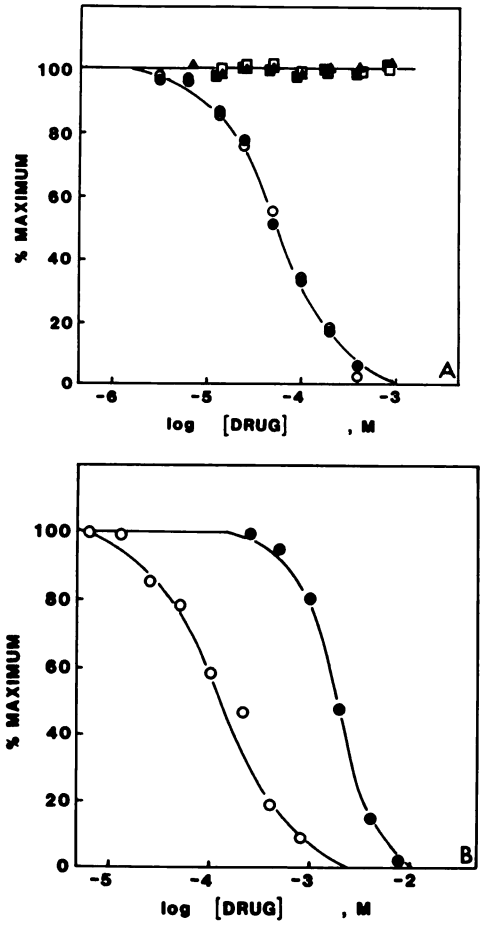

FIG. 1. *Effect of anticonvulsants on batrachotoxin-activated $^{22}Na^+$ flux in N18 cells*

A. Effect of DPH, CBZ, trimethadione, ethosuximide, and sodium valproate. Cell cultures were incubated in the presence of batrachotoxin (1 μM) and varying concentrations of DPH (●), CBZ (○), trimethadione (▲), ethosuximide (■), and sodium valproate (□) for 30 min. $^{22}Na^+$ flux was then measured as described under Experimental Procedures.

B. Effect of diazepam and phenobarbital. Cell cultures were incubated in the presence of batrachotoxin (1 μM) and varying concentrations of diazepam (○) and phenobarbital (●), $^{22}Na^+$ flux was the measured as described under Experimental Procedures.

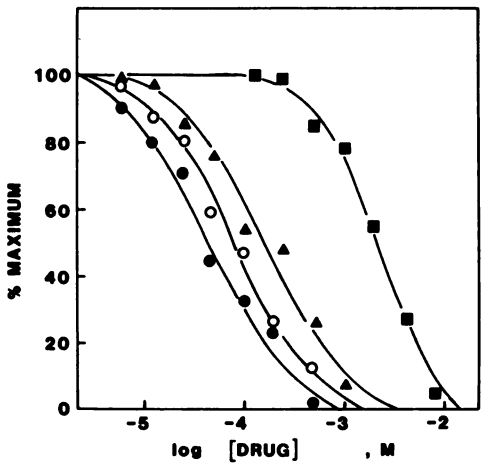

FIG. 2. *Effect of anticonvulsants on batrachotoxin-activated $^{22}Na^+$ flux in synaptosomes*

Effect of CBZ, DPH, diazepam, and phenobarbital. Synaptosomes were incubated in the presence of batrachotoxin (1.0 μM) and varying concentrations of CBZ (●), DPH (○), diazepam (▲), and phenobarbital (■) for 20 min. $^{22}Na^+$ flux was then measured as described under Experimental Procedures.

with the lack of effect of these agents on the binding of [^{3}H]BTX-B to intact synaptosomes (6). Diazepam (K_I = 51 ± 3 μM, n = 3) and phenobarbital (K_I = 1.2 ± 0.05 mM, n = 3) are capable of inhibiting batrachotoxin-activated $^{22}Na^+$ flux in N18 cells (Fig. 1B), which is in agreement with their effects on [^{3}H]BTX-B binding in synaptosomes. However, the relatively high concentrations required to elicit these effects would suggest that inhibition of sodium channels is not of primary importance in the action of these drugs (Table 1) (6).

A similar pattern of anticonvulsant action on batrachotoxin-activated $^{22}Na^+$ flux in synaptosomes is shown in Fig. 2. CBZ (K_I = 22 ± 1.0 μM, n = 3) and DPH (K_I = 38 ± 1.6 μM, n = 3) inhibit flux at concentrations in the central nervous system which protect laboratory animals against electroshock seizures. Diazepam (K_I = 63 ± 3 μM, n = 3) and phenobarbital (K_I = 1.3 ± 0.06 mM, n = 3) are, by comparison, relatively poor inhibitors of synaptosomal $^{22}Na^+$ flux at therapeutically relevant concen-

TABLE 1

Effects of anticonvulsants on sodium channel function

Anticonvulsant	K_I BTX-activated $^{22}Na^+$ influx (cells)	K_I BTX-activated $^{22}Na^+$ influx (synaptosomes)	IC_{50} BTX-B binding (ref. 6)	Brain levels[a]	% Inhibition at mean therapeutic level[b]
	μM	*μM*	*μM*	*μmoles/kg*	
DPH	35	38	40	10–20	35–40
CBZ	41	22	131	10–50	50–60
Phenobarbital	1200	1300	2600	10–80	0
Diazepam	51	63	152	0.5–1.0	0
Sodium valproate	NA[c]	NA	NA	300–600	0
Trimethadione	NA	NA	NA	≥1000	0
Ethosuximide	NA	NA	NA	600–700	0

[a] Brain levels achieved after an effective dose in rats (except trimethadione for mice) as reported in refs. 21–25.

[b] Calculated from the mean effective brain level and the $K_{0.5}$ measured in ion flux experiments.

[c] NA denotes no effect at concentrations up to 1 mM.

trations, in agreement with neurotoxin binding experiments (6) and the effects of these agents on batrachotoxin-activated $^{22}Na^+$ flux in N18 cells (Fig. 1B).

Table 1 summarizes the K_I values for inhibition of batrachotoxin-activated sodium channels by anticonvulsant drugs in neuroblastoma cells and rat brain synaptosomes. Comparison of these values with the brain levels of anticonvulsants that are effective in preventing seizures in mice and rats leads to the conclusion that 35–40% of sodium channels are blocked during DPH action and 50–60% during CBZ action. An insignificant fraction of channels is blocked by the other agents tested. Thus, these ion flux data are consistent with previous neurotoxin binding results indicating that DPH and CBZ are sodium channel-selective anticonvulsants.

Effect of scorpion toxin on the anticonvulsant inhibition of batrachotoxin-activated $^{22}Na^+$ influx. While there is good general agreement between the effects of anticonvulsants on ion flux and neurotoxin binding, CBZ appears to be less potent in inhibiting [^{3}H]BTX-B binding (6) compared with its effects on $^{22}Na^+$ flux. In experiments examining the effects of anticonvulsants on [^{3}H] BTX-B binding (6), scorpion toxin (0.3 μM) is included in the assay mixture to decrease the K_D for BTX-B from approximately 1 μM to 70 nM via the allosteric interaction between neurotoxin receptor sites 2 and 3 (26). It was suggested that the shift in the IC_{50} for CBZ inhibition of BTX-B binding from 131 μM at 0.3 μM scorpion toxin to 394 μM at 2 μM scorpion toxin (compare Fig. 1A with fig. 2 in ref. 6) was due to a reduction in the affinity of CBZ for its receptor in the presence of scorpion toxin. In the present experiments examining the effects of anticonvulsants on neurotoxin-activated ion flux, scorpion toxin is not routinely included in the assay medium. If neuroblastoma cells are incubated with 20 nM batrachotoxin in the presence of 0.3 μM scorpion toxin, a similar fraction of sodium channels is activated compared with incubating cells in the presence of 400 μM batrachotoxin alone (18). Under these conditions the K_I values for CBZ are 70 μM and 35 μM, respectively (Fig. 3). These results show that scorpion toxin does reduce the affinity of CBZ binding to a receptor site associated with the voltage-sensitive sodium channel and provide an explanation for the differences in affinity of CBZ observed previously (6).

Inhibitory effects of DPH and CBZ on neurotoxin-activated ion flux in "physiological" assay media. In experiments involving the activation of sodium channels by batrachotoxin, low concentrations of NaCl (10 mM) are used in the assay medium to ensure that membrane potential is unaffected by the increased Na^+ permeability caused by neurotoxin treatment. Under these conditions, sodium ion influx is linearly proportional to Na^+ permeability (18). In addition, Ca^{2+} is routinely omitted from the assay medium since the presence of this ion produces a competitive inhibition of alkaloid activation of the sodium channel (27). While it is clear that DPH and CBZ exert inhibitory effects on batrachotoxin-activated $^{22}Na^+$ flux in low-sodium, calcium-free media, it was important to establish the effects of these agents on sodium channel function using conditions which approximate more closely the physiological ionic environment. For this purpose, veratridine (0.2 mM) was used instead of batrachotoxin, since it activates only approximately 8% of the voltage-sensitive sodium channels in N18 cells (18) and therefore, in the presence of 130 mM NaCl, a smaller depolarization is expected. The relationship between $^{22}Na^+$ influx and Na^+ concentration is linear up to 30 mM external Na^+ but reaches a plateau by 50 mM. Thus, at 130 mM Na^+, the measured $^{22}Na^+$ influx underestimates Na^+ permeability, even when only 8% of the channels are activated by veratridine (data not shown). In the presence of 130 mM NaCl and 1.8 mM $CaCl_2$, DPH and CBZ inhibit veratridine-activated $^{22}Na^+$ flux with IC_{50} values of 93 μM and 193 μM, respectively (Fig. 4A and B). This represents 1.69- and 1.53-fold increases in the IC_{50} values for DPH (55 μM) and CBZ (126 μM) inhibition of flux in low-sodium, calcium-free media (Fig. 4A and B). Thus, these drugs also are effective in blocking sodium channels at therapeutic concentrations under more physiological conditions.

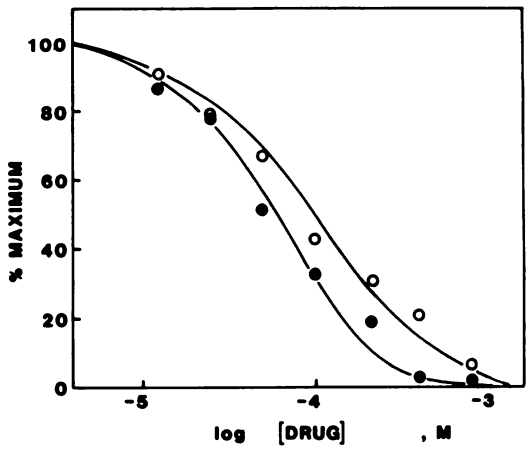

FIG. 3. *Effect of scorpion toxin on the inhibition of batrachotoxin activation of sodium channels in N18 cells by CBZ*

Cell cultures were incubated in the presence of batrachotoxin (400 μM) alone (●) or batrachotoxin (20 μM) and 0.3 μM scorpion toxin (○) in the presence of the indicated concentrations of CBZ. $^{22}Na^+$ flux was then measured as described under Experimental Procedures.

Nature of the inhibition of neurotoxin-activated $^{22}Na^+$ flux by carbamazepine. Batrachotoxin and other alkaloid toxins act as allosteric activators of voltage-sensitive sodium channels (18), and previous ion flux studies have demonstrated that diphenylhydantoin and other antiarrhythmic drugs act as allosteric inhibitors of alkaloid toxin action (28). On the basis of an allosteric model of alkaloid toxin action described in an earlier study (18), allosteric inhibitors would be expected to produce competitive inhibition of activation in the presence of a full agonist such as batrachotoxin but mixed or noncompetitive inhibition in the presence of a partial agonist such as veratridine. The results presented in Fig. 5A and B show that carbamazepine also acts as a competitive inhibitor of batrachotoxin action and as a mixed inhibitor of veratridine activation. This suggests that DPH and CBZ act similarly to produce an allosteric inhibition of alkaloid neurotoxin-activated $^{22}Na^+$ influx.

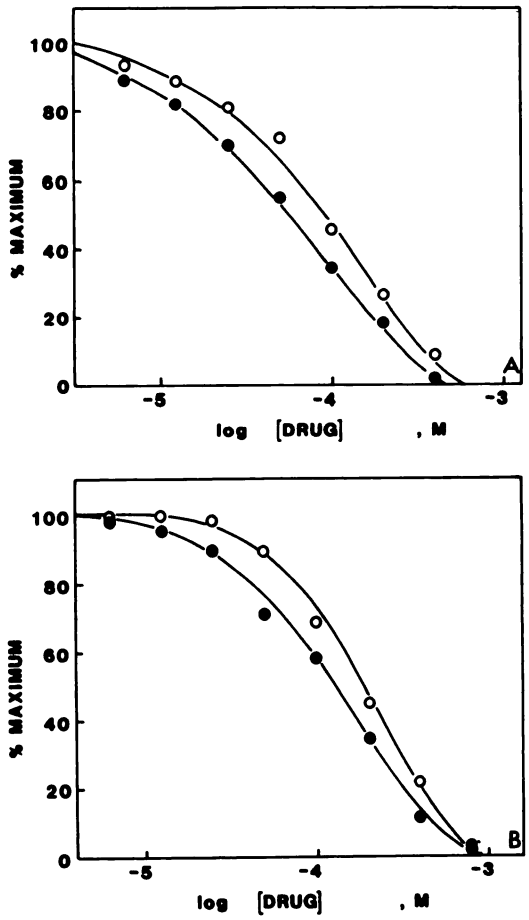

FIG. 4. *Effect of Na^+ and Ca^{2+} on the inhibition of veratridine activation of sodium channels in N18 cells by anticonvulsant drugs*

A. Cell cultures were incubated with 0.2 mM veratridine for 30 min in Na^+ (10 mM), Ca^{2+}-free medium (●) or Na^+ (130 mM), Ca^{2+} (1.8 mM) medium (○) in the presence of the indicated concentrations of DPH. $^{22}Na^+$ flux was measured as described under Experimental Procedures.

B. Cell cultures were incubated with 0.2 mM veratridine for 30 min in Na^+ (10 mM), Ca^{2+}-free medium (●) or Na^+ (130 mM), Ca^{2+} (1.8 mM) medium (○) in the presence of the indicated concentrations of CBZ. $^{22}Na^+$ flux was measured as described under Experimental Procedures.

DISCUSSION

The present study provides further evidence, from ion flux measurements, that DPH and CBZ exert inhibitory actions on voltage-sensitive sodium channels in mammalian brain at concentrations which are pharmacologically relevant to their anticonvulsant action. In a previous study, it was suggested, on the basis of the inhibitory effects of these agents on [^{3}H]BTX-B binding, that DPH and CBZ could inhibit 35% and 25%, respectively, of brain sodium channels during anticonvulsant therapy (6). The data for CBZ inhibition of [^{3}H]BTX-B binding may represent an underestimate of the potency of this drug on sodium currents. It seems likely that the presence of scorpion toxin in the [^{3}H]BTX-B binding assay may have reduced the potency of CBZ, since scorpion toxin increased the K_I for CBZ inhibition of batrachotoxin-activated ion flux by 2-fold (Fig. 5). The data summarized in Table 1 indicate that DPH blocks 35–40% of sodium channels and CBZ blocks 50–60% at therapeutic concentrations. The excellent agreement between the inhibitory effects of DPH and CBZ on ion flux and neurotoxin binding (6) and the inhibitory effects observed on Na^+ currents in a variety of electrophysiological studies (2–5) provide strong evidence that DPH and CBZ block voltage-sensitive sodium channels during their anticonvulsant action. Furthermore, batrachotoxin-activated $^{22}Na^+$ flux and/or [^{3}H]BTX-B binding may provide a useful *in vitro* method for screening potential anticonvulsant agents which have been shown to protect laboratory animals against electroshock seizures (21).

DPH and CBZ appear to be relatively selective in their effects on voltage-sensitive sodium channels, since a number of other clinically effective anticonvulsants have no action or inhibit batrachotoxin-activated $^{22}Na^+$ flux at concentrations in excess of therapeutic levels in the central nervous system (Fig. 1A and B). Thus, the results obtained in this study provide further support of a pre-

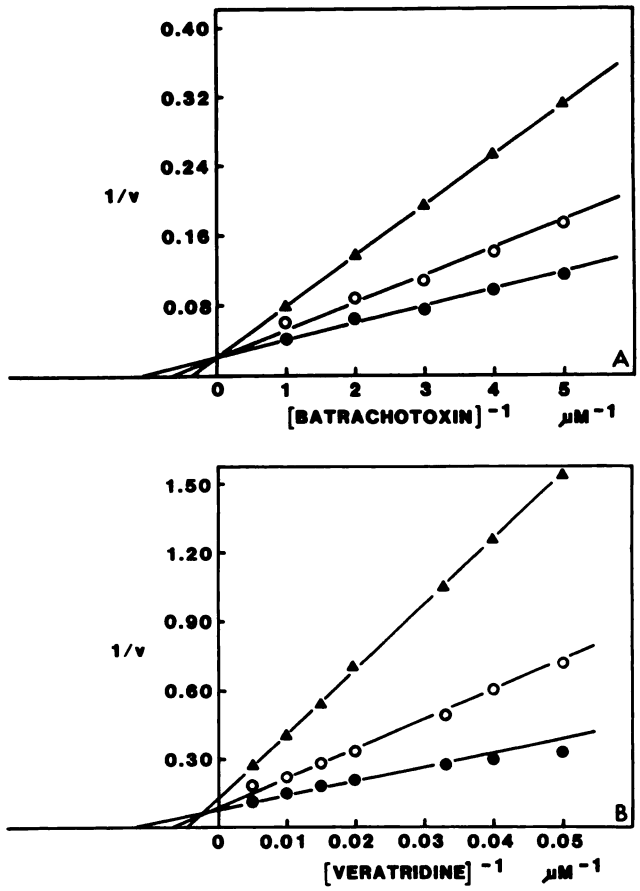

FIG. 5. *Mechanism of inhibition of neurotoxin activation of sodium channels in N18 cells by carbamazepine*

A. Cell cultures were incubated with the indicated concentrations of batrachotoxin in the absence (●) and presence of 100 μM (○) and 200 μM (▲) carbamazepine. $^{22}Na^+$ flux was measured as described under Experimental Procedures.

B. Cell cultures were incubated with the indicated concentrations of veratridine in the absence (●) and presence of 100 μM (○) and 200 μM (▲) carbamazepine. $^{22}Na^+$ flux was measured as described under Experimental Procedures.

vious classification of clinically employed anticonvulsants according to their mechanism of action (6). On the basis of this classification, it is proposed that DPH and CBZ, which have a similar spectrum of action in the management of partial seizures and grand mal seizures and in protection of experimental animals against electroshock seizures, act primarily by modulating sodium channel function. A second class of anticonvulsants, including phenobarbital, benzodiazepines, and sodium valproate, appears to enhance inhibitory synaptic transmission at synapses which release GABA (reviewed in ref. 6), and it is likely that this action is of greater importance than the relatively weak effects of these agents on sodium channels in explaining their anticonvulsant properties. A third class of anticonvulsants, including the succinimides and oxazolidinediones, affects neither sodium channel function nor GABA receptors at clinically effective concentrations. Further work is necessary to elucidate the mechanism of action of these agents, which are used primarily in the management of petit mal disorders.

It appears likely that DPH and CBZ act through similar mechanisms to inhibit sodium channel function. Two lines of evidence support this hypothesis. In the present study, CBZ acts as a competitive inhibitor of batrachotoxin activation and as a mixed inhibitor of veratridine activation (Fig. 3A and B). Additional experiments (figures not shown) indicated that DPH produced a similar pattern of neurotoxin inhibition, in agreement with previously published findings (28). These results suggest that DPH and CBZ act as allosteric competitive inhibitors of alkaloid toxin action (20). Previous observations are also in support of this viewpoint. (a) Both DPH and CBZ increase the K_D of the alkaloid neurotoxin receptor site for [^{3}H]BTX-B without altering maximal binding capacity (6). (b) Both DPH and CBZ accelerate the rate of dissociation of the [^{3}H]BTX-B-receptor complex following the addition of excess veratridine at equilibrium. This effect would not be expected if these drugs acted as simple competitive inhibitors of alkaloid neurotoxins. While these findings suggest that DPH and CBZ modulate sodium channel function by a similar mechanism, the development of a suitable radioligand to characterize the anticonvulsant receptor site associated with the sodium channel directly will be required to determine whether DPH and CBZ bind to the same site(s) or separate site(s).

In order to study the activation of voltage-sensitive sodium channels by neurotoxins under conditions where sodium permeability is directly proportional to ion flux, experiments are normally performed in media containing low concentrations of sodium ions. This is particularly important in examining the responses to batrachotoxin, which acts as a full agonist at neurotoxin receptor site 2 to produce a persistent state of depolarization in excitable tissue. On the other hand, it is equally important to determine the effects of anticonvulsants under conditions which resemble most closely that encountered *in situ*. For these purposes, we have used veratridine, which activates only a small fraction of sodium channels in N18 cells (18). Under these conditions, DPH and CBZ inhibit veratridine-activated $^{22}Na^+$ flux in a concentration-dependent manner, although in each case the potency is slightly reduced (approximately 1.6-fold). The results are not directly comparable to the effects on batrachotoxin-activated $^{22}Na^+$ flux (in low Na^+, Ca^{2+}-free media) because some depolarization does occur and linearity of ion flux with sodium concentration is not maintained (18). Since a nonlinear relationship between ion flux and sodium permeability will cause an apparent increase in K_I, the 1.6-fold increase we observe may be mainly due to these artifacts. In any case, a reduction in potency of approximately 1.6-fold would still result in an inhibition of a significant fraction of Na^+ channels by DPH and CBZ at therapeutic concentrations.

While it appears likely that hydantoin and iminostilbene derivatives act at a receptor site associated with the activation of the voltage-sensitive sodium channel, further work is now in progress to establish the molecular characteristics of this site of anticonvulsant action.

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