

Influence of negative surface charge on toxin binding to canine heart Na channels in planar bilayers

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ABSTRACT The presence of negative surface charge near the tetrodotoxin/saxitoxin binding site of canine heart Na channels was revealed by analysis of the kinetics of toxin block of single batrachotoxin-activated Na channels in planar bilayers as a function of [NaCl]. The voltage-dependence of toxin binding and the toxin dissociation rate are nearly constant as [NaCl] is varied from 0.05 to 3 M. In contrast, the association rate constant of the toxins is inversely dependent on [NaCl], with the rate for

the divalent toxin, saxitoxin²⁺, affected more steeply than that of the monovalent toxin, tetrodotoxin¹⁺. These results for toxin-insensitive Na channels from canine heart parallel previous findings for toxin-sensitive Na channels from canine brain. The model of Green et al. (Green, W. N., L. B. Weiss, and O. S. Anderson. 1987. *J. Gen. Physiol.* 89:873-903), which includes Na⁺ competition and Gouy-Chapman screening of surface charge, provided an excellent fit to the data. The results suggest

that the two canine Na channel subtypes have a similar density of negative surface charge ($1 e^-/400 \text{ \AA}^2$) and a similar dissociation constant for Na⁺ competition (0.5 M) at the toxin binding site. Thus, negative surface charge is a conserved feature of channel function of these two subtypes. The difference in toxin binding affinities arises from small differences in intrinsic association and dissociation rates.

INTRODUCTION

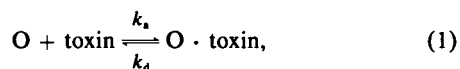
The guanidinium toxins, tetrodotoxin (TTX) and saxitoxin (STX), are useful ligands for discriminating subtypes of voltage-dependent Na channels that differ in binding affinity for these toxins at a common extracellular site (Barchi, 1987). For example, canine heart Na channels have 48-fold lower affinity for STX and 94-fold lower affinity for TTX than canine brain Na channels at 0.5 M NaCl (Green et al., 1987, Guo et al., 1987). Previous work established a role for negatively charged functional groups in the binding of these toxins. This evidence includes inhibition of toxin binding by carboxyl modifying reagents (Baker and Robinson, 1975; Spalding, 1980; Worley et al., 1986), monovalent alkali cations, divalent metal ions, and protons (Henderson et al., 1974). By varying the concentration of alkali cations at fixed ionic strength, it has been shown that binding of [³H]TTX and [³H]STX is inhibited competitively by alkali cations with a selectivity sequence similar to that of the channel's ion selectivity, $\text{Li}^+ > \text{Na}^+ > \text{K}^+ > \text{Rb}^+ > \text{Cs}^+$ (Reed and Raftery, 1976; Weigele and Barchi, 1978; Barchi and Weigele, 1979). By varying ionic strength with CaCl_2 or MgCl_2 , a greater reduction in affinity is observed for the divalent toxin, STX^{2+} , relative to the monovalent toxin, TTX^{1+} (Henderson et al., 1974; Hille et al., 1975; Grissmer, 1984; Strichartz et al., 1986). This observation implies that a negative surface potential must

also affect toxin binding. In a recent study, Green et al. (1987) dissected the separate effects of Na⁺ competition and surface potential by measuring rate constants for STX and TTX block of single batrachotoxin-activated Na channels from canine brain as a function of NaCl concentration. These authors used the Gouy-Chapman theory of surface charge to estimate an apparent surface charge density of $1 e^-/300 \text{ \AA}^2$ in the vicinity of the toxin binding site. Our laboratory also reported suggestive evidence of a negative surface potential for rat muscle and canine heart Na channels in a study of the blocking rates of STX derivatives with different net charges (Guo et al., 1987). These results led us to conclude that differences in negative surface charge do not account for the slower toxin association rates observed for heart vs. muscle and brain Na channels. Here we extend these observations by analyzing the effect of NaCl concentration on the blocking kinetics of STX and TTX for canine heart Na channels. By using the same experimental conditions and methods as Green et al. (1987) for canine brain Na channels, we find that two distinct Na channel subtypes from the same species have very similar intrinsic binding constants for Na⁺ competition and a similar density of negative surface charge near the toxin binding site. However, brain and heart Na channels exhibit 2- to 10-fold differences in intrinsic association and dissociation rate constants for STX and TTX, pointing to other conformational and chemical differences in the toxin binding reactions.

METHODS

Procedures for preparing plasma membrane vesicles from canine heart and methods for incorporating and recording single batrachotoxin-activated Na channels in planar bilayers are detailed in a previous publication (Guo et al., 1987). Here, planar bilayers were formed exclusively from neutral lipids, 80% bovine brain phosphatidylethanolamine and 20% 1,2-diphytanoylphosphatidylcholine (Avanti Polar Lipids, Inc., Birmingham, AL). Single channel experiments were carried out at ambient temperature (21°–24°C). The buffer solution on both sides of the bilayer contained 10 mM MOPS-NaOH, 0.2 mM EDTA, pH 7.4 and NaCl concentrations ranging from 0.05 to 3 M. TTX and STX were purchased from Calbiochem Behring Corp. (La Jolla, CA). Stock solutions of the toxins were prepared in 1 mM citrate, pH 5.0.

TTX and STX blocking kinetics of single batrachotoxin-activated Na channels follow a simple one-site binding reaction:



where O is an unblocked channel, $O \cdot \text{toxin}$ is a blocked channel, and k_a and k_d are respective association and dissociation rate constants for toxin binding (French et al., 1984; Moczydlowski et al., 1984). Rate constants for TTX and STX block of single heart Na channels were measured by statistical analysis of exponential dwell-time populations of at least 100 blocked and unblocked events at a given voltage, as described previously (Guo et al., 1987).

Data in Figs. 2 and 3 were fit to Eqs. 9–11 by an interactive method using an Indec Systems LSI-11/73 computer (Sunnyvale, CA) with an HP1345A digital display. A two-parameter fit of data plotted according to Eq. 11 was first performed to obtain best-fit values for σ and $k_{a,0,STX}/k_{a,0,TTX}$ in Fig. 3. These values were then used to fit data plotted according to Eq. 9 (Fig. 2) to obtain best fit values for K_{Na} and $k_{a,0,TTX}$ or $k_{a,0,STX}$. The goodness of fit was evaluated both visually and by computation of a χ^2 parameter:

$$\chi^2 = \sum_{i=1}^n \frac{(o_i - e_i)^2}{e_i}, \quad (2)$$

where o_i are observed data, and e_i are theoretical values calculated from Eqs. 9–11. The fitted parameters were varied over a wide range until a visually acceptable fit and minimum value of χ^2 was observed. The values of the best-fit parameters were refined until less than a 10% adjustment in the values was needed to improve the fit.

RESULTS

In previous studies, the measured rate constants for TTX and STX block of batrachotoxin-modified Na channels in planar bilayers have been found to exhibit an exponential dependence on holding voltage in the range of -60 to $+60$ mV (French et al., 1984; Moczydlowski et al., 1984; Green et al., 1984, 1987; Guo et al., 1987). A similar observation has been made in voltage-clamped nodes of Ranvier (Rando and Strichartz, 1986), but not in patch-clamped cardiac cells of neonatal rats, where state-dependent toxin binding has been found (Huang et al., 1987). Whether the disagreement posed by the latter

study is due to differences in techniques or preparations is not known at present. However, in agreement with previous bilayer studies, we find a similar voltage-dependence of toxin block for canine heart Na-channels at symmetrical NaCl concentrations ranging from 0.05 to 3 M. This behavior can be described by

$$k_d(V) = k_d(0) \exp(z'_d FV/RT) \quad (3)$$

$$k_a(V) = k_a(0) \exp(-z'_a FV/RT) \quad (4)$$

$$K_D(V) = K_D(0) \exp(z' FV/RT), \quad (5)$$

where $k_d(0)$, $k_a(0)$, and $K_D(0)$ are rate and equilibrium constants at 0 mV and z'_d , z'_a , and z' are effective valences according to a widely used model of voltage-dependent binding (Woodhull, 1973). In such expressions, F is the Faraday constant, V is applied voltage, R is the gas constant, and T is absolute temperature. Table 1 summarizes the results of nonlinear least square fits of data to Eqs. 3–5 for TTX and STX block of canine heart Na channels at various NaCl concentrations. As observed for other Na channel subtypes (Moczydlowski et al., 1984; Green et al., 1987), the voltage dependence of the binding rate constants is insensitive to NaCl concentration. The mean value of z' for all 14 measurements in Table 1 is 0.59 ± 0.08 (SD). This value of z' is not significantly different for similar experiments reported for TTX block of rat muscle Na-channels ($z' = 0.61 \pm 0.06$, $n = 6$) (Moczydlowski et al., 1984) or for TTX and STX block of canine brain Na-channels ($z' = 0.63 \pm 0.12$, $n = 12$) (Green et al., 1987). Thus, neither changes in NaCl concentration nor the tested variations in Na channel subtype, measurably affect the voltage-dependence of toxin binding.

The zero-voltage dissociation rate constant, $k_d(0)$, is also insensitive to NaCl concentration. Fig. 1 shows that $k_d(0)$ for TTX block of the heart channel only increases by a factor of 2 as $[NaCl]$ is increased from 0.1 to 3 M. However, $k_d(0)$ for STX is relatively constant in this range of $[NaCl]$. Fig. 1 also compares our data for heart Na channels with that of Green et al. (1987) for brain Na channels. This comparison shows that both toxins have quite similar dissociation rates for a given subtype, but the heart subtype exhibits 10-fold faster dissociation rates than the brain subtype under the same conditions.

In contrast to the lack of effect of NaCl on the dissociation rate and voltage dependence of toxin binding, Fig. 2B shows that the zero-voltage association rate constant for TTX decreases by 15-fold and that for STX by 60-fold as NaCl concentration is raised from 0.1 to 3 M for heart Na channels. Fig. 2A shows similar results for brain Na-channels that were previously reported by Green et al. (1987). The specific effect of NaCl on toxin association rate constants is indicative of Na^+ competition with toxin binding and screening of negative surface

TABLE 1 Effect of NaCl on toxin blocking kinetics of canine heart Na channels*

[Na ⁺] (n)	Association rate		Dissociation rate		Equilibrium constant	
	k_a (0)	z'_a	k_d (0)	z'_d	K_D (0)	z'
<i>M</i>	$10^6 \text{ s}^{-1} \text{ M}^{-1}$		s^{-1}		μM	
TTX						
0.106 (15)	0.67 ± 0.03	0.22 ± 0.03	0.53 ± 0.03	0.31 ± 0.03	0.80 ± 0.05	0.53 ± 0.06
0.206 (16)	0.46 ± 0.04	0.19 ± 0.05	0.52 ± 0.03	0.32 ± 0.03	1.1 ± 0.1	0.51 ± 0.13
0.506 (6)	0.20 ± 0.01	0.19 ± 0.02	0.65 ± 0.07	0.31 ± 0.06	3.3 ± 0.3	0.50 ± 0.10
1.006 (8)	0.13 ± 0.01	0.22 ± 0.05	0.76 ± 0.08	0.33 ± 0.06	5.8 ± 0.6	0.55 ± 0.12
2.006 (10)	0.068 ± 0.002	0.18 ± 0.02	0.83 ± 0.12	0.47 ± 0.08	12.0 ± 1.7	0.65 ± 0.11
3.006 (6)	0.046 ± 0.001	0.27 ± 0.02	1.12 ± 0.07	0.39 ± 0.04	24.0 ± 1.6	0.67 ± 0.07
STX						
0.056 (7)	39 ± 1	0.34 ± 0.03	0.49 ± 0.01	0.24 ± 0.01	0.012 ± 0.0003	0.57 ± 0.05
0.106 (5)	13 ± 1	0.30 ± 0.07	0.55 ± 0.10	0.24 ± 0.10	0.042 ± 0.007	0.55 ± 0.22
0.206 (6)	4.4 ± 0.3	0.40 ± 0.04	0.45 ± 0.01	0.21 ± 0.01	0.10 ± 0.007	0.59 ± 0.06
0.506 (11)	1.6 ± 0.2	0.35 ± 0.08	0.65 ± 0.03	0.25 ± 0.03	0.40 ± 0.06	0.60 ± 0.14
1.006 (6)	0.88 ± 0.08	0.32 ± 0.05	0.65 ± 0.07	0.25 ± 0.06	0.74 ± 0.08	0.57 ± 0.14
2.006 (5)	0.46 ± 0.06	0.35 ± 0.08	0.64 ± 0.03	0.33 ± 0.03	1.4 ± 0.2	0.67 ± 0.15
3.006 (11)	0.22 ± 0.01	0.35 ± 0.03	0.49 ± 0.05	0.43 ± 0.06	2.2 ± 0.2	0.78 ± 0.11

*Blocking rate constants measured in the range of -60 to $+60$ mV were fit to exponential functions of voltage according to Eqs. 3 and 4. n refers to the number of points used in the fit at a given Na⁺ concentration. The uncertainty of the values refers to the standard deviation derived from the fitting procedure. Parameters for the equilibrium constant were calculated from the ratio of the rate constants.

charge by Na⁺. Green et al. (1987) previously presented a quantitative model for the dependence of k_a (0) on NaCl concentration. We used this model as described below in a comparative analysis of the data in Fig. 2 for canine brain and heart.

For a simple competitive interaction between Na⁺ and toxin binding at a single site, the observed association rate

constant for the toxin is predicted to decrease with increasing [Na⁺] according to

$$k_a = k_{a,0} / \{1 + [\text{Na}^+] / K_{\text{Na}}\}, \quad (6)$$

where $k_{a,0}$ is the toxin association rate constant at [Na⁺] = 0 and K_{Na} is the equilibrium dissociation constant for Na⁺. If there is also a negative surface potential at the toxin binding site, then both charged toxin and Na⁺ will be more concentrated near the binding site relative to bulk solution. The effect of surface potential on the observed toxin association rate constant and on the local concentration of Na⁺ can be described by Boltzmann distributions:

$$k_a = k_{a,0} \exp(-zF\psi_s/RT) \quad (7)$$

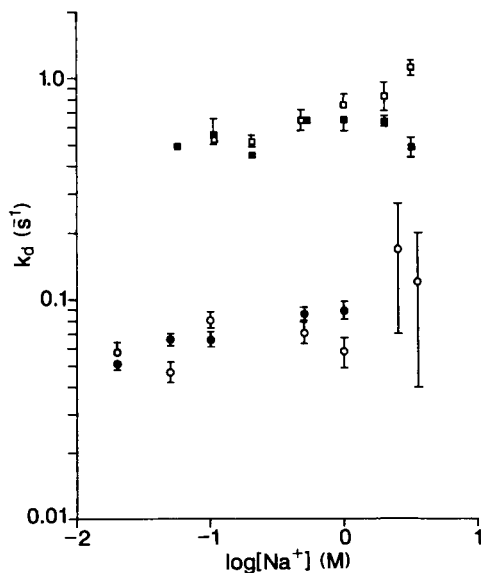


FIGURE 1 Effect of [Na⁺] on zero-voltage dissociation rate constants for TTX (□, ○) and STX (■, ●). Data obtained for canine heart Na channels (□, ■) are compared with previously published results of Green et al. (1987) for canine brain Na channels (○, ●).

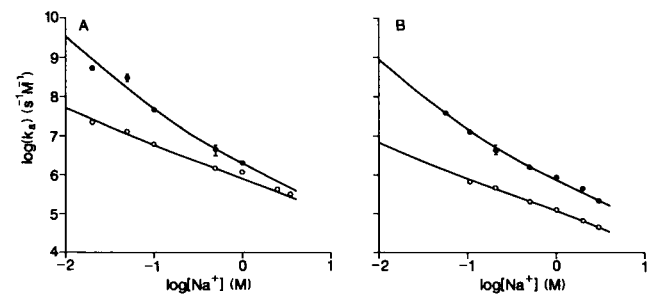


FIGURE 2 Effect of [Na⁺] on zero-voltage association rate constants for TTX (○) and STX (●). Our data obtained in *B* for canine heart Na channels are compared with previously published data (Green et al., 1987) for canine brain Na channels in *A*. Solid lines are calculated best fit curves using Eqs. 9 and 10 and parameters for this work listed in Table 2.

$$[\text{Na}^+]_i = [\text{Na}^+]_b \exp(-F\psi_s/RT). \quad (8)$$

Eq. 7 relates the observed toxin association rate constant, k_a , to the rate constant at zero surface potential, $k_{a,0}$, the net charge of the toxin molecule, z , and the electrostatic potential at the site, ψ_s . Eq. 8 is a similar expression relating local Na^+ concentration at the receptor, $[\text{Na}^+]_i$, to bulk Na^+ concentration, $[\text{Na}^+]_b$. In a case where both Na^+ competition and surface charge influence toxin binding, Eqs. 6–8 can be combined to give

$$k_a = k_{a,0} \exp(-zF\psi_s/RT) / \{1 + [\text{Na}^+]_b \exp(-F\psi_s/RT)/K_{\text{Na}}\}. \quad (9)$$

In Eq. 9, $k_{a,0}$ is now redefined as an intrinsic association rate constant at $[\text{Na}^+] = 0$ and $\psi_s = 0$. K_{Na} is also redefined as an intrinsic equilibrium constant for Na^+ at $\psi_s = 0$. In order to apply Eq. 9 to the data of Fig. 2, Green et al. (1987) used the Gouy-Chapman theory to relate electrostatic potential at the site, ψ_s , to apparent surface charge density, σ , and the bulk Na^+ concentration, $[\text{Na}^+]_b$:

$$\psi_s = (2N_0 kT/F) \operatorname{arcsinh} \{\sigma / (8[\text{Na}^+]_b \epsilon_0 \epsilon_r kT)^{0.5}\}, \quad (10)$$

where ϵ_0 is the permittivity constant, ϵ_r is the dielectric constant of water, N_0 is the Avogadro constant, and k is the Boltzmann constant. Eqs. 9 and 10 can be used to fit the data of Fig. 2 to three adjustable parameters, σ , $k_{a,0}$, and K_{Na} . However, since STX and TTX compete for binding at a common site, the competitive binding interaction of Na^+ can be eliminated by taking the ratio of Eq. 9 for STX ($z = +2$) to that of TTX ($z = +1$):

$$k_{a,\text{STX}}/k_{a,\text{TTX}} = \{k_{a,0,\text{STX}}/k_{a,0,\text{TTX}}\} \exp(-F\psi_s/RT). \quad (11)$$

Eqs. 10 and 11 now contain only two adjustable parameters, σ , and the ratio of intrinsic association constants, $k_{a,0,\text{STX}}/k_{a,0,\text{TTX}}$.

Fig. 3 plots the ratio of the observed association rate constants for STX/TTX at various bulk Na^+ concentrations for heart Na channels and brain Na channels. For both channel subtypes, the ratio of $k_{a,\text{STX}}/k_{a,\text{TTX}}$ steeply increases at low Na^+ concentration, as predicted for a negative surface charge. At high Na^+ concentrations, where the surface charge is screened, it appears that the ordinate parameter of Fig. 3 asymptotes at a distinctly higher value for the heart than the brain subtype. This behavior is reflected in the curve fitting results listed in Table 2.

The results of Table 2 show that the best-fit charge densities for heart ($1 e^-/450 \text{ \AA}^2$) and brain ($1 e^-/380 \text{ \AA}^2$) only differ by 20%, while best-fit values of $k_{a,0,\text{STX}}/k_{a,0,\text{TTX}}$ for heart (1.2) and brain (3.6) differ by a factor of 3. The best fit values of σ obtained by analysis of data in Fig. 3 were used in fitting the data in Fig. 2 to Eqs. 9 and 10. For

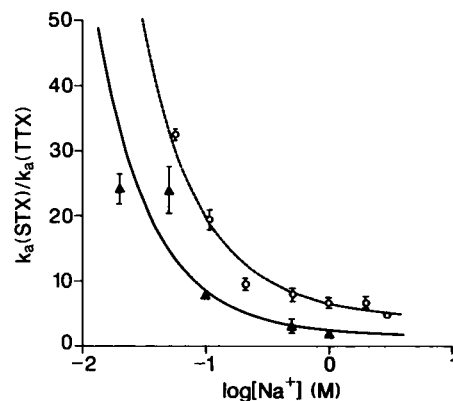


FIGURE 3 Effect of $[\text{Na}^+]$ on the ratio of zero-voltage association rate constants for STX to that of TTX. Data obtained in this study for canine heart Na channels (O) are compared to previously published results for canine brain Na channels (\blacktriangle) (Green et al., 1987). Solid lines are calculated best fit curves using Eqs. 10 and 11 and parameters for this work listed in Table 2.

both subtypes, we found that $K_{\text{Na}} = 0.5 \text{ M}$ provided an optimum fit. The corresponding best fit values of $k_{a,0}$ for TTX and STX from the fit of Fig. 2 were also consistent with $k_{a,0,\text{STX}}/k_{a,0,\text{TTX}}$ ratios obtained from the analysis of Fig. 3 (see Table 2). Table 2 also shows that our particular fitting procedure resulted in parameters very similar to those reported by Green et al. (1987) for their brain data.

DISCUSSION

The results of this study confirm the presence of a negative surface potential near the guanidinium toxin

TABLE 2 Results of curve fitting*

Subtype	Reference	σ	$k_{a,\text{STX}}/k_{a,\text{TTX}}$
Brain	Green et al. (1987)	$e^-/\text{\AA}^2$	
Brain	This work	1/300	0.8
Brain	This work	1/380	1.2
Heart	This work	1/450	3.6

Subtype	Toxin	Reference	σ	K_{Na}	$k_{a,0}$
Brain	TTX	Green et al. (1987)	$e^-/\text{\AA}^2$	M	$s^{-1}M^{-1}$
Brain	TTX	This work	1/300	0.3	3×10^6
Brain	TTX	This work	1/380	0.5	2×10^6
Brain	STX	Green et al. (1987)	1/300	0.3	3×10^6
Brain	STX	This work	1/380	0.5	2.4×10^6
Heart	TTX	This work	1/450	0.5	3×10^5
Heart	STX	This work	1/450	0.5	10.4×10^5

*The top part of the table lists results of fitting the data in Fig. 3 to Eqs. 10 and 11. The bottom part gives the results of fitting data in Fig. 2 to Eqs. 9 and 10.

binding site of canine heart Na channels. The density of surface charge ($1 e^-/450 \text{ \AA}^2$) measured here by varying $[\text{NaCl}]$ is close to our previous estimate ($1 e^-/410 \text{ \AA}^2$) for the heart subtype that was obtained at a fixed NaCl concentration of 0.2 M (Guo et al., 1987). In the latter study, we used STX derivatives with different net charges ($-1, 0, +1, +2$) and observed an approximately exponential dependence of the toxin association rate on the net charge as predicted by Eq. 7.

However, this latter approach is fraught with more assumptions than the present experiments and its validity could be questioned. For example, since the exact pK_a 's of the two guanidinium groups and the N-1 hydroxyl group of all the various STX derivatives had not been measured by direct titration, the assumed values of the net charges were based on the known pK_a 's of only two of the derivatives, saxitoxin and neosaxitoxin. Also, Guo et al. (1987) assumed that STX derivatives containing up to 5 charges, 3 negative and 2 positive functional groups separated by as much as 8 \AA , could be approximated as a point charge with a magnitude equal to the sum of the individual charges. These approximations are much less severe in the present experiments because the active species of TTX and STX are known to be monovalent and divalent cations, respectively, under physiological conditions (Kao, 1986). Also, the carbon to carbon distance of the C-2 to C-8 guanidinium groups in STX is only 3.8 \AA . For these reasons, one could argue that Na^+ titration of the STX/TTX association rate is a sounder approach for quantitation of the surface potential.

In retrospect, the similar results of the two types of experiments might be expected from other data in the literature. This includes the fact that the various sulfated STX derivatives behave as net charges of 0, +1, and +2 at acidic pH in chromatographic separations (Hall, 1982). The work of Carnie and McLaughlin (1983) and Alvarez et al. (1983) previously suggested that fairly rigid divalent molecules whose charges are separated by distances less than the Debye length behave as an approximate point charge in electrostatic interactions at a membrane/water interface. The clear correlation of the association rate with the net charge of the STX derivatives suggests that the charge distribution in these molecules is close to a point charge approximation.

The present study also provides firmer support for a second conclusion of Guo et al. (1987). This concerns the hypothesis that surface charge is a conserved feature of function among different Na-channel subtypes. While Guo et al. (1987) observed a similar negative surface charge density for rat muscle and canine heart Na channels at a fixed Na^+ concentration, it could be argued that this coincidence was the result of fortuitous canceling of species-dependent differences and differences in the affinity of Na^+ competition for two channel subtypes.

Our confirmation of the results of Green et al. (1987) rules out such speculation because K_{Na} for Na^+ competition is quite similar for both heart and brain subtypes.

The major quantitative difficulty of the present experiments is the use of the Gouy-Chapman model, which assumes a planar and uniformly smeared charge distribution. We know that this situation is far removed from the actual features of Na channel structure, an integral membrane protein with an approximate protein diameter of 80 \AA based on the reported protein mass (Noma and Noda, 1986). The possibility that negatively charged phospholipids contribute to the surface charge effects on toxin binding is ruled out by the use of neutral lipids in the present study and previous experiments that found insignificant effects of exogenously added phosphatidylserine (Guo et al., 1987). Thus, it is believed that the surface charge influence on TTX/STX binding must originate from acidic amino acid residues on the Na channel protein itself or sialic acid groups contained on the carbohydrate portion (Miller et al., 1983) of the channel protein.

Recent work on the structure of the sialylated carbohydrate demonstrated the presence of long unbranched homopolymers of polysialic acid on *Electrophorus* electroplex Na channels (James and Agnew, 1987). These authors suggested that the *Electrophorus* Na channel contains five polymeric carbohydrate groups with core units of 8–10 neutral sugars extruding from NH_2 -linked glycosylation sites on the protein. Each of these neutral sugar chains appears to be capped with 20 consecutive sialic acids. Such a hypothetical carbohydrate structure places some limitations on the possible contribution of the sialic acid to surface charge effects. At low or moderate ionic strength, such strings of negative charge would be repulsive and could extend greater than 100 \AA in linear distance from the protein surface. Depending on the ionic strength and Debye length, little contribution from the sialic acid groups would be felt at the level of the protein surface for such a large distance. This situation might be similar to the relative effects of acidic phospholipids and sialogangliosides on the conductance of Tetanus toxin channels in planar bilayers. Phosphatidylserine and phosphatidylinositol, with negatively charged groups at the membrane surface, have been shown to increase the unit conductance of single Tetanus toxin channels much more than the sialogangliosides which have their charged groups at a distance of 10 \AA from the membrane surface (Gambale and Montal, 1988). Based on such considerations and assuming that Na channels from other species have a carbohydrate structure similar to that of *Electrophorus*, it seems likely that the surface charge effects we observe on TTX/STX binding are due to the presence of local aspartate and glutamate groups near the binding site itself.

Irrespective of the origin of negative charge, the simple Gouy-Chapman model is remarkably successful in fitting the data of Figs. 2 and 3. Previous work has already established that this model accurately predicts various surface charge phenomena in model membrane systems (McLaughlin et al., 1981; Winiski et al., 1986). This success and the usefulness of the model demonstrated by Green et al. (1987) implies that the Gouy-Chapman theory provides a reasonable description of the influence of local fixed charges on superficial ion binding sites in membrane proteins (e.g., see Dani, 1986).

Finally, we would like to discuss our results in the context of current research on Na-channel subtypes. The available sequence data suggest that muscle- and neuronal-type Na channels as sequenced from eel electroplax and rat brain are highly homologous proteins with substitutions of individual amino acid residues interspersed throughout the 208–228 kD sequence (Numa and Noda, 1986). A mammalian heart Na channel has not yet been cloned, so it is not known whether this TTX-insensitive subtype exhibits any unusual structural features. The available functional comparisons of Na-channel subtypes suggest that the amino acid substitutions result in highly specific and localized functional differences rather than a generalized perturbation of channel behavior. For example, the eightfold faster off rate of TTX and STX from heart vs. brain Na channels can be explained by the absence of a single hydrogen bond between a toxin hydroxyl group and a protein residue (Guo et al., 1987). The differences in the association rates of these toxins appear not to result from gross differences in surface charge or Na⁺ competition, but small (2- to 10-fold) differences in intrinsic rate constants that might be explained by specific local differences in conformational accessibility of a few complexing ligands in the binding site. Other channel properties that we are beginning to compare (conductance and divalent cation block) also show small and specific differences in functional detail. From our admittedly limited perspective, it appears the evolution of Na channel subtypes preserved key aspects of function and altered certain local chemical affinities that may be advantageous in a developmental or tissue-specific context.

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