A fluorescent N-methylanthraniloyl derivative of the potent depolarizing agent batrachotoxin has been used to probe the structural and conformational properties of the neurotoxin receptor site on the voltage-dependent sodium channel. Batrachotoxinin A 20-α-N-methylanthranilato (BTX-NMA) retains high affinity for its receptor site on the synaptosomal sodium channel with a $K_d$ between 78 and 91 nM and an average site capacity of 2 pmol/mg of synaptosomal protein in the presence of Leiurus quinquestriatus quinquestriatus α-scorpion toxin. The fluorescence emission of BTX-NMA upon binding to synaptosomes indicates a hydrophobic environment. Toxin V from *L. quinquestriatus*, an allosteric activator, effects a 20-nm red shift in the spectrum of bound BTX-NMA and a 4-fold enhancement in the fluorescence quantum yield disclosing a conformational change into a hydrophilic environment. Fluorescence resonance energy transfer measurements show that the distance separating the receptor sites is 37 ± 10 Å. Thus, the binding of α-scorpion toxin must involve conformational changes that extend over large distances from the batrachotoxin-binding locus. This information together with the distance measurements between the tetrodotoxin and α-scorpion toxin receptors and the conformational transition associated with this distance upon batrachotoxin addition indicate a conformationally flexible channel with coupling of sites through the polyatomic framework of individual subunits or through extensive alterations in subunit/subunit interactions.

In recent years, a number of neurotoxins have been used to biochemically and pharmacologically dissect the ionic mechanisms of nerve and muscle impulse propagation. The voltage-dependent sodium channel appears to be the primary site of action of these naturally occurring neurotoxins. Toxins that are specific for this channel include TTX and STX which block ion flux through the Na$^+$ channel, veratridine, BTX, aconitine, and grayanotoxin, all of which stabilize the open form of the channel and elicit persistent depolarization, and the polypeptide toxins such as those from the scorpions *Centruroides suffusus suffusus* (β-scorpion toxins) and Leiurus quinquestriatus quinquestriatus (α-scorpion toxins) which specifically prolong the kinetics of channel opening or closing, respectively.

Combined biochemical and electrophysiological studies have revealed the existence of at least four distinct binding sites for these toxins (for a review, see Ref. 1), together with at least four characteristically different ways of altering sodium channel function. Externally applied TTX selectively eliminates the transient current, while not affecting the kinetics of channel opening or closing.

The binding and action of the Leiurus scorpius toxins are highly voltage-dependent and markedly enhanced in the presence of batrachotoxin (2). The $K_d$ for α-scorpion toxin binding to its receptor increases 10-fold for each 31-mV depolarization. The enhancement of α-scorpion toxin binding by batrachotoxin is accompanied by a shift in the voltage dependence of α-scorpion toxin binding. The enhancement by BTX and effects of depolarization on α-scorpion toxin binding suggest that α-scorpion toxin binds to a voltage-sensitive component of the sodium channel which undergoes a conformational change on depolarization. The enhancement by BTX reflects an allosteric interaction between the receptor sites.

Recently, Brown et al. (10) have prepared a radiolabeled derivative of BTX. The availability of radiolabeled BTX has permitted thermodynamic and kinetic studies of the channel-toxin interaction in synaptosomes (3) and in brain microsacs (26). Specific binding of BTX is markedly increased in the presence of α-scorpion toxin.

Because of the high affinity and specificity of these neurotoxins for the sodium channel, we have sought structural information on the arrangement of these important sites by fluorescence resonance energy transfer measurements. Fluorescent and photoactivatable fluorescent derivatives of tetro...
dotoxin, Lqq V, and Css II, have been prepared and shown by equilibrium binding, electrophysiology, and spectroscopic measurements to interact selectively and with high affinity with the Na+ channel (4-7). The fluorescence properties indicate that the TTX-binding site is highly polar (4) whereas the α and β-scorpion toxin receptor sites are buried in more hydrophobic domains (6, 29). Using these fluorescent neurotoxins, the average distance from the TTX site to the Leiwurus toxin V receptor is 35 Å (6) and from the TTX site to the Css II receptor is 34 Å (8). In the presence of BTX, the TTX to Lqq V distance changes to 42 Å, indicating a neurotoxin-induced conformational change in one subunit of the channel or a change in the interaction between two subunits coupled to the batrachotoxin-binding site. Further evidence for a conformational change was provided by the observation that the fluorescence properties of fluorescent Css II are markedly altered in the presence of Lqq V (6, 7).

In this study, a fluorescent congenere of batrachotoxin, BTX-NMA, has been synthesized and shown to bind with high affinity to the BTX site of voltage-dependent sodium channels. The derivative has a fluorescence emission spectrum which overlaps those of previously synthesized Lqq V and Css II derivatives. The present study reports on the utility of BTX-NMA to determine the molecular environment of NTX receptors. The present study reports on the spatial relationship between the BTX and Lqq V α-scorpion toxin receptors of the voltage-dependent sodium channel.

**EXPERIMENTAL PROCEDURES**

**Materials**

Chemicals were obtained from the following sources: tetrodotoxin (citrate free) from Calbiochem, scorpion venom (L. quinquestratus quinquestratus) from Sigma, Lqq V was purified, labeled with 3H by lactoperoxidase-catalyzed iodination, and repurified by immunoprecipitation as described (5). Saxitoxin was obtained from Dr. S. Schantz, University of Wisconsin, labeled with tritium by the method of Peterson (13) with bovine serum albumin as a standard.

**Synthesis of Batrachotoxinin A 20-a-N-Methylanthraniloyl-BTX—Batrachotoxinin A 20-a-N-methylanthraniloyl-** was prepared by esterification of the aliphatic 20α-hydroxyl group of BTX-A, a nonotoxin alkaloid component from skins of Phylobates australis (18, 10). To 1.0 mg of BTX-A (2.4 mM) dissolved in 0.6 ml of dry benzene containing 12 μmol of triethylamine were added 12 μmol (2.12 mg) of N-methylisatoic anhydride. The mixture was allowed to react for 1 h at 65 °C and then placed in a water bath at 50 °C for 2 additional h. The formation of product was followed by the disappearance of BTX-A (Rf = 0.1) and the appearance of a fluorescent spot (λmax = 385 nm) (Rf = 0.4) on Merck Silica Gel G thin layer chromatoplates developed in chloroform/methanol, 20:1 (v/v). The product, batrachotoxinin A 20-a-N-methylanthraniloyl, was isolated in 80% yield by preparative thin layer chromatography in the same solvent system by eluting with five 3.0-ml portions of absolute methanol.

The identity and purity of the product were determined by mass spectral analysis using a Hewlett-Packard 5985A mass spectrometer operated in the electron impact mode at an ionizing voltage of 70 eV, and the sample was introduced by a direct insertion probe which was heated from 50 to 70 °C at 30 °C/min. The fragmentation pattern shows fragment ions at m/z 151, 133, 105, 91, and 77 corresponding to the N-methylanthraniloyl moiety and a fragment at m/z 483 formed from the loss of the C4H8NO moiety of the homomorpholinocring from the mother ion, m/z 465, which is not seen under our analysis conditions. The other major peaks in the mass spectrum result from fragmentations in the steroidal skeleton previously identified in the mass spectra of a number of BTX derivatives (8). Furthermore, BTX-NMA was toxic upon intraperitoneal injection in Swiss Webster mice with an LD50 of approximately 15 μg/kg, and the symptoms of intoxication mimicked those produced by similar injections of native BTX. Since the starting material, BTX-A, is not toxic and produces no symptoms at these concentrations, these results may be taken as further evidence that the desired product was obtained. Further details will be given in a subsequent publication.

**N-Methylanthraniloyl-TTX, anthraniloyl-TTX, and the five chromophoric derivatives of Lqq V were prepared as previously described (4, 5). All other reagents were of the highest commercial grade available.

**Methods**

**Preparation of Synaptosomes—**Synaptosomes and synaptic plasma membranes were prepared by a combination of differential and density gradient centrifugation as described by Catterall et al. (11). The 1.0 M fraction was used for all experiments based upon STX receptor density, ability to retain a membrane potential by the fluorescence dye method (12), and high field electron micrograph profiles. Protein content was determined by the method of Peterson (13) with bovine serum albumin as a standard.

**Binding Measurements—**[3H]STX and [3H]Lqq V binding to synaptosomes was measured by a rapid filtration assay as described (5). The binding medium consisted of 130 mM choline chloride, 50 mM Hepes adjusted to pH 7.4 with Tris base, 5.5 mM glucose, 0.8 mM MgSO4, 5.4 mM KCl, and 1.8 mM CaCl2 (standard binding medium), containing 1 mg/ml of bovine serum albumin. Samples were mixed and incubated for 1 h at 27 °C for [3H]STX and 2 h for [3H]Lqq V. Counting efficiency for tritium and iodine was determined by the method of internal standards. Nonspecific binding was measured in the presence of a saturating concentration of TTX (1 μM) or Lqq V (200 nM) and was subtracted from the results. Binding measurements were done at least in triplicate at each ligand concentration.

**Binding of [3H]BTX-B to synaptosomal fractions—**collected from the 1.0-1.2 mg sucrose interface was also measured by a rapid filtration assay. The assay tubes contained 5.3 μg of synaptosomal protein/ml, 7 nM [3H]BTX-B, 50 μg of crude L. quinquestratus quinquestratus scorpion venom, and in certain experiments varying concentrations of BTX-NMA in a total volume of 35 μl of standard binding medium. Samples were incubated for 40 min at room temperature, diluted with 3 ml of ice-cold wash buffer consisting of 183 mM choline chloride, 50 mM Hepes-Tris (pH 7.4), 1.8 mM CaCl2, and 0.8 mM MgSO4, and immediately collected under vacuum onto GF/C or GF/B filters. Filters were washed three times with 3 ml of ice-cold wash buffer and counted by scintillation counting. Counting efficiency for tritium was determined by the method of internal standards. Nonspecific binding was determined in parallel assay tubes containing 235 μM veratridine, and subtracted from the data. All data points were determined in duplicate.

**Electrophysiological Measurements—**The biological activity of BTX-B and BTX-NMA was assessed by the ability of the toxins to depolarize surface fibers of frog sartorius muscle. Frog Ringer’s I solution which was composed of 112 mM NaCl, 3.2 mM KCl, 2.7 mM CaCl2, 1 mM MgSO4, and 0.7 mM NaHCO3 was bubbled with 5% CO2 and 95% O2 to make pH 7.4. The muscle was allowed to equilibrate at room temperature in this solution for 30 to 45 min before the experiments began. Intracellular recordings from surface muscle fibers were obtained with a Dagan 8500 two-electrode preamp-clamp system and displayed on a Nicolet digital oscilloscope. Resting membrane potentials and action potentials were recorded by inserting a glass capillary microelectrode (5-15 Mohm) filled with a 3 M KCl solution. Membrane potentials were monitored continually during toxin application. When stimulated, direct electrical pulses were applied by either a pair of silver wire electrodes placed underneath or by inserting a second microelectrode into the same muscle fiber about 150 μm from the recording electrode to pass current. End-plate potentials were monitored by an intracellularly placed microelectrode in a frog sartorius nerve-muscle preparation which was indirectly stimulated by applying pulses at a rate of 1 Hz to nerve draped across the silver wire electrodes. The Ringer’s solution was modified by reducing the Ca++ to 0.05 mM and adding 5 mM Mg++ to decrease transmitter release. BTX-B and BTX-NMA were added to the solution from a stock solution in absolute ethanol, so that the final ethanol concentration was less than 0.5%. Spectroscopic Measurements—UV-visible spectra were recorded with a Cary 210 spectrophotometer equipped with a thermostated cell

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All steady state fluorescence measurements were performed in a SLM 4800 subnanosecond spectrofluorimeter interfaced to a LS1-11/2 computer (Digital Equipment Corporation) using 0.3 x 0.3 cm microcuvettes with a capacity of 150 µl. The excitation slits were 2 nm to minimize chromophore bleaching, and shutters were closed between measurements. Measurements were performed at 25 °C unless otherwise stated. Fluorescence spectra were corrected for wavelength-dependent variation in light source output, phototube response, and monochromator efficiency as described before (4, 5).

The quantum yield, φ, of the bound fluorophore was determined by comparison with the quantum yield of quinine sulfate (0.70 in 0.1 N H₂SO₄ (14)). Equation 1 describes the ratio of quantum yields, φ₁, as a function of the area under the emission spectra, F₁, and the absorbances at the exciting wavelength, A₁, for two fluorophores.

\[
φ₁ = \frac{F₁ A₂}{F₂ A₁} \left(1 - \frac{R}{4}\right)
\]

(1)

The term 1 - R/4 corrects for polarized emission (15) and R is the anisotropy of the fluorophore. The absorbances of the samples were kept below 0.05 to minimize inner filter effects.

Fluorescence anisotropies were measured in the T format of the spectrofluorimeter which simultaneously measures the ratio of the vertical and horizontal components of the emitted light with either the exciting light vertically polarized, (V/H)ᵥ, or horizontally polarized, (V/H)ₕ. The anisotropy, r, is defined by Equation 2 where

\[
r = \frac{(V/H)ᵥ - (V/H)ₕ}{(V/H)ᵥ + 2(V/H)ₕ}
\]

(2)

In solution, the fluorescence anisotropy, r₀, of the bound fluorophores in the absence of macromolecular rotation was evaluated by extrapolating the linear portion of a Perrin plot, 1/r versus (7/r) (F₀/F), at relatively low viscosity (η) to infinite viscosity as described by Dale and Elsinger (16). The viscosity was varied by increasing the sucrose concentration at constant temperature, and the excited state lifetime was assumed to be proportional to the fluorescence intensity, F. The reported anisotropies are corrected for the depolarization due to light scattering, and the anisotropies of the specifically bound toxins were calculated as described before (5-8). The r₀ values for fluorescent Lqq V were taken from Ref. 5.

Since batrachotoxinin A 20-α-N-methylanthranilates fluoresces in solution as well when bound to the channel, the quantum yield of the bound toxin was computed taking into consideration the known dissociation constant. The concentration of bound BTX-NMA was determined from

\[
L₀ = 0.5(B₀ + L₀ + K₀ - \sqrt{(B₀ + L₀ + K₀)^2 - 4(B₀L₀)})
\]

(3)

where B₀ and L₀ are the total concentrations of the sodium channel BTX receptor sites and BTX-NMA, respectively, and K₀ is the dissociation constant. The quantum yield then of the bound toxins was determined from

\[
\phi_{obs} = \chi₁ φ₁ + \chi₂ φ₂
\]

where χ₁ and χ₂ are the mole fractions of free and bound ligand. The BTX-V, Lqq V, and BTX-binding site densities of freshly prepared membranes were determined immediately prior to spectroscopic measurements. Schott KV cut-off filters were sometimes used instead of the emission monochromator to increase the detected signal.

Energy Transfer from BTX-NMA to Fluorescent Lqq V—For any given donor-acceptor pair, the Förster critical distance, R₀, for which energy transfer is 50% efficient is described as

\[
R₀ = \frac{9.79 \times 10^{11} (Q₀K²)ν_{\lambda\lambda}^{-3/2}}{ελ²}
\]

(4)

where K² is the orientation factor, Q₀ is the quantum yield of the donor in the absence of the acceptor, and n is the refractive index of the intervening medium, taken to be 1.4 as for water. The term J is an overlap integral between the emission spectrum of the donor and the absorption spectrum of the acceptor and is expressed as

\[
J = \sum \frac{F₀λ(λa)Aλ}{Δλ} = \frac{S}{Aλ}
\]

(5)

where λ is the wavelength, F₀(λ) is the corrected fluorescence emission of the donor as a function of λ, and ε is the molar extinction coefficient of the acceptor as a function of λ. Numerical values for J were obtained by digitizing the steady state fluorescence emission spectrum for channel-bound BTX-NMA and the absorption spectrum for each acceptor.

The orientation factor, K², depends on the relative transition dipole moments of the donor and acceptor and the vector joining them. When donor and acceptor dipoles are freely reorienting during the excited state lifetime, K² has a value of 4π. In principle, K² can range from 0 to 4. However, measurement of the emission anisotropy of the donor and/or acceptor places limits on the value of the orientation factor. The range can be narrowed by evaluating fluorophores that have some degree of rotation and/or transitions of mixed polarization. The distances calculated here between BTX-NMA and fluorescent α-scorpion toxins used K² = 3.5, and the estimates of the most probable ranges of distances from the fluorescence polarizations of the ligands and the values at the half-heights of the probability density function for R²(%) given in Table III of Haas et al. (17). Previous measurements of the polarizations of fluorescent Leurisus toxin V derivatives indicate that the polarizations increase toward the red edge and point to the existence of transitions of mixed polarizations.

The actual distance separating the donor from the acceptor (R) can be calculated according to Equation 6.

\[
R = R₀(1/E - 1)^{-1/6}
\]

(6)

The efficiency of energy transfer (E) between donor and acceptor was measured by both donor quenching and the sensitized emission of the acceptor. Equation 7 gives the efficiency of energy transfer by donor quenching where Q₀ and Q₀ are the quantum yields of the donor in the presence and absence of the acceptor.

\[
E = 1 - Q₀/Q₀
\]

(7)

In order to demonstrate that the observed donor quenching in the presence of acceptor is due to dipolar energy transfer, steady state efficiencies were measured by the fluorescence enhancement of fluorescent Lqq V. Ideally, the wavelength of excitation should be where only the donor absorbs and that of the emission should be where only the acceptor emits. However, this has not been possible with the BTX-NMA/Lqq V pairs used here. The absorption spectra of DACA-Lqq V and CPM-SH-Lqq V overlap considerably with that of BTX-NMA. Accordingly, this problem was resolved by excitation at two wavelengths; both the donor and acceptor absorb at one wavelength whereas only the acceptor absorbs at the other wavelength. For the BTX-NMA/DACA-Lqq V pair, excitation was set at 340 nm (both BTX-NMA and DACA-Lqq V absorb) and 400 nm (only DACA-Lqq V absorbs), and emission was scanned from 440 to 520 nm. The apparent efficiency of energy transfer can be expressed as

\[
E = \frac{(F₀D₀ₐₐ)₁₆₀}{(F₀Dₐₐ)₁₆₀}
\]

(8)

where F₀D₀ₐₐ is the enhancement of fluorescence emission of the acceptor at 480 nm due to energy transfer from the donor excited at 340 nm; F₀Dₐₐ is the fluorescence intensity of the acceptor at 480 nm using 400 nm excitation; fₐₐ and fₐₐ are the fractions of light absorbed at 400 nm by the bound acceptor and at 340 nm by the donor, respectively. Using synaptosomes with BTX-NMA bound and partially saturated with FL-Lqq V, fₐₐ and fₐₐ can be determined from known values of the concentrations of bound ligands and extinction coefficients of donor and acceptor, the fraction of acceptor bound to the channel, and the cuvette light path. Fₐₐ is determined by direct measurement according to

\[
Fₐₐ = Fₐₐ₀ - Fₐₐ₀ - Fₐₐₐ
\]

(9)

where total, D₁ and A are the total fluorescence observed, the remaining donor emission, and the acceptor emission due to direct excitation, respectively, all with excitation at 340 nm and emission measured at 480 nm. Fₐₐ₀ is determined using synaptosomes with bound BTX-NMA (and unlabeled Lqq V) in the absence of the acceptor, and Fₐₐ can then be expressed as

\[
Fₐₐ = \frac{(Fₐₐ₀/fₐₐ₀)₀}{(fₐₐ)₀}
\]

where fₐₐ₀ is the fraction of light absorbed at 340 nm by the acceptor.

All the parameters needed to calculate the apparent energy transfer are described above. Finally the observed efficiencies obtained with channels partially saturated with fluorescent Lqq V acceptors, are normalized to actual transfer efficiencies per acceptor as described (6-8, 18).
RESULTS

Preparation, Purification, and Biological Properties of BTX-NMA—Batrachotoxinin A 20-α-N-methylantranilate is prepared by selective esterification at the allylic, 20α-hydroxyl group of BTX-A. BTX-A is a minor alkaloid component from the skins of *P. aurotania* which lacks the pyrrole ring of BTX and as a result is a biologically inactive derivative (8). Replacement of the pyrrole ring by [³H]benzozoate (10) or by N-methylantranilate restores potent biological activity.

When exposed to either BTX-B or BTX-NMA, the surface fibers from frog sartorius muscle are depolarized at similar rates and there is no observable difference in the ability of either toxin to depolarize these fibers. Within 20 min of application of a solution of 80 nM BTX-NMA, the resting membrane potential rose from -78 to -31 mV (Fig. 1A, d) with a reduction in the spike amplitude and rate of rise of the action potential (Fig. 1A, a-c). After 15 min, the membrane potential was at -37 mV and the ability to generate an action potential was completely lost despite polarization of the membrane to -78 mV, although end-plate potentials could still be recorded upon indirect stimulation of BTX-NMA-depolarized muscle fibers. In addition after 15 min, the frequency of miniature end-plate potentials increased from about 10–20 Hz to more than 400 Hz, and finally disappeared at a membrane potential of -33 mV as the muscle fiber continued to depolarize. The effect of BTX-NMA was partially reversible upon perfusing the tissue with frog Ringer's I for 20 min.

Displacement of bound batrachotoxinin A 20-α-[³H]benzozoate by batrachotoxinin A 20-α-N-methylantranilate on rat brain synaptosomes is shown in Fig. 1B. The shape of the inhibition curve is hyperbolic and is consistent with a com-

![Fig. 1](http://www.jbc.org/)

**Fig. 1. Biological properties of BTX-NMA.** *A*, effects of 80 nM BTX-NMA on frog muscle action potentials. Experiments were carried out at 25 °C in frog Ringer's I solution. The toxin was diluted into the experimental buffer, and a random sampling of surface fibers were examined. a, control action potential recorded in normal Ringer's solution before toxin addition. Stimulation at 1 Hz after 5 (b) and 15 (c) min of exposure to 80 nM BTX-NMA. d, time course of 80 nM BTX-NMA (○) and 80 nM BTX-B (●) induced depolarization at 25 °C of surface fibers of frog sartorius muscle. B, displacement of 7 nM [³H]BTX-B by BTX-B (●) and BTX-NMA (■) on 5.3 mg/ml of rat brain synaptosomes in the presence of 50 μg of crude *Leiurus* venom. The inset shows a log-log plot of the specific binding of BTX-NMA. The *K₀* for BTX-B with the synaptosomal membrane preparation used here was 44 nM with a BTX-B-binding site capacity of 0.75 pmol/mg of protein.
petitive interaction of BTX-NMA at the BTX-B receptor site of the sodium channel. From a logit-log plot, the \( K_d \) is 90 nM and the \( K_d \) for BTX-NMA binding can be calculated from \( K_d = K_{d,0}/(1 + [{^3}H]BTX-B/K_{d,0}) \) where \( K_{d,0} = 44 \) nM under these conditions. This calculation yields a \( K_d \) value for BTX-NMA binding to synaptosomes of 78 nM, which is approximately twice that of native BTX-B. A Scatchard plot of the specific binding of \([^3]H\)BTX-B in this experiment reveals a single class of noninteracting binding sites with a \( K_d \) of 44 nM and a synaptosomal site capacity of 0.75 pmol/mg of protein. From a number of independent experiments on different synaptosomal preparations, the \( K_d \) for BTX-B was between 35 and 44 nM with site capacities between 0.75 and 2.2 pmol/mg of protein.

**Spectroscopic Properties of BTX-NMA Free and Bound to the Sodium Channel**—Fig. 2 shows the absorption and fluorescence spectra of BTX-NMA. The absorption maximum of the toxin is 350 nm and the fluorescence emission of the free toxin is 425 nm. The fluorescence emission spectrum shares some spectral overlap with DNP-Lqq V, DACA-Lqq V, and CPM-SH-Lqq V (5, 6). When BTX-NMA is added to synaptosomes, a 20-nm blue shift in the fluorescence emission maximum and a 4-fold enhancement is observed (Fig. 2B). The emission spectrum of the N-methylanthraniloyl moiety is highly dependent upon its microenvironment, and a 30-nm shift to higher energy is seen upon decreasing the solvent polarity from water to dioxane (4). This blue shift and spectral enhancement indicates that BTX-NMA is located in a very hydrophobic environment.

To determine whether these fluorescence changes were due to specific binding at the BTX-binding site on the sodium channel, similar experiments were made with membranes which were pre-equilibrated with 1.8 \( \mu \)M BTX-B to displace bound BTX-NMA. Under these conditions, the fluorescence emission was reduced by 30%. This indicates that the fluorescence spectrum and enhancement of BTX-NMA bound to synaptosomes presented in Fig. 2B reflect mostly nonspecific binding. This observation finds support in the \([^3]H\)BTX-B binding data on lysed and washed membrane preparations from mouse brain (10). Under these conditions, specific binding of \([^3]H\)BTX-B was observed but the specific binding was only about 10% of the total binding, probably due to the low affinity and high lipid solubility of batrachotoxin. In order to be able to use BTX-NMA as a specific energy transfer ligand, it is necessary to increase the specific binding component. We have similarly taken advantage of the positive cooperativity between batrachotoxin and Lqq V to enhance the affinity of BTX-NMA for the receptor and to thus increase the ratio of specific to nonspecific binding. Using \([^3]H\)BTX-B, Catterall et al. (3) have shown that the specific binding of this toxin is increased 10- to 20-fold by the polypeptide scorpion toxin from L. quinquestriatus venom (3).

When BTX-NMA was added to synaptosomes which were pre-equilibrated with 1 \( \mu \)M Lqq V, an 8-fold enhancement of the fluorescence emission and a spectral shift of 10 nm to the red from the emission spectrum of the free BTX-NMA was observed. Of this, more than 70% could be displaced by 1.8 \( \mu \)M BTX-B in contrast to that observed with the allosteric activator Lqq V (Fig. 2B). In the presence of Lqq V, the fluorescence emission maximum of specifically bound BTX-

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**Fig. 2. UV-visible absorption and fluorescence emission spectra of BTX-NMA. A, molar extinction coefficient, \( \epsilon \) (——), of BTX-NMA. The lower trace is the buffer base-line under these instrumental settings (0.01 absorbance range). B, corrected fluorescence emission spectra (excitation 345 nm, 4-nm band passes) of 6 nM BTX-NMA free (——) and bound (———) to synaptosomal membranes (8.5 mg of protein/ml). The 
(\([^3]H\)BTX-B)-binding capacity of these membranes was 1.9 pmol/mg of synaptosomal protein. Fluorescence emission spectra of bound BTX-NMA in the presence of 1 \( \mu \)M Lqq V (———), and with 1.8 \( \mu \)M BTX-B and 1 \( \mu \)M Lqq V (———) to displace specifically bound BTX-NMA.**
NMA is 435 nm. Therefore, to increase the specific signal of the specifically bound BTX-NMA relative to nonspecifically bound to and light scattering, all subsequent measurements of the bound BTX-NMA (donor) were done in the presence of 1 μM Lqq V.

The enhancement of specific binding and of the binding constant for BTX-NMA and [3H]BTX-B in the presence of α-scorpion toxin can be partly attributed to the rate of dissociation of the toxin-receptor complex (3). At 36 °C, the toxin-receptor complex dissociates with a half-time of approximately 60 min with 200 μM veratridine to displace BTX-B, whereas at 0 °C, the dissociation is greatly slowed (3). Even after 50 min, no loss of specifically bound [3H]BTX-B is observed (3). For comparison in the absence of α-scorpion toxin, all radioactivity is removed within 1.5 min at 36 °C in mouse cerebral cortex microsomes. This property was utilized in the subsequent energy transfer experiments in which saponinsomes with bound BTX-NMA and either unlabeled Lqq V or acceptor chromophoric Lqq V were incubated at 37 °C for 45 min, cooled to 4 °C, then washed three times with cold synaptosome buffer. When BTX-NMA and membranes were treated in this fashion for preparation of donor samples, the buffer washes contained 1 μM Lqq V and 1 μM TTX. In this manner, all available channel sites could be saturated with donor to maximize the fluorescence emission signal, followed by extensive washing at 4 °C to remove the unbound probe.

Previous experiments with [125I]-DACA or [125I]-NBD-Lqq V bound to synaptosomes showed that only 15% of the toxin dissociates in the first 10 min (5) after extensive centrifugation and washing at 4 °C. In the presence of 200 nM BTX-B, experiments conducted for this study show that less than 10% of the fluorescent Lqq V-receptor complex dissociates. Therefore, we have made extensive use of these properties in order to increase the Na+ channel receptor site occupancies for BTX-NMA and fluorescent Lqq V on the synaptosomes and to remove the remaining unbonded ligands. The half-time of dissociation at 25 °C of the specific BTX-NMA-receptor complex in the presence of 1 μM Lqq V was determined by displacement with 2 μM BTX-B and the decrease in the fluorescence emission intensity at 435 nm. The slow rate of toxin dissociation (~35 min) correlates closely with the slow rate of dissociation of [3H]BTX-B and the slow rate of reversal of BTX activation of sodium channels at 36 °C in neuroblastoma cells (3).

In order to gain some insight into the mobility of BTX-NMA bound to the channel, which is potentially important information for the evaluation of the orientation factor K2, measurements of the steady state emission anisotropy and excited state lifetime were made. The lifetime at 30-MHz modulation of BTX-NMA is 4.2 ns. The anisotropy of free BTX-NMA was very low and compared to the limiting anisotropy indicates unrestricted reorientation of the probe. This is not surprising in view of the fact that batrachotoxinin A 20-α,N-methylanlanilate is a small molecule (550 molecular weight) and because the chromophore is attached through an orientationally free ester linkage. The anisotropy of bound BTX-NMA was determined to assess the dipole orientation factor, to investigate any conformational restrictions upon probe rotation, and to examine conformational dynamics of the batrachotoxin receptor from the interaction with other sodium channel activators. The anisotropy was corrected for the depolarization due to light scattering and the anisotropy of the specifically bound toxin was calculated as described before (4-7, 29), considering the change in quantum yield of BTX-NMA upon binding to the channel. The emission anisotropy of specifically bound BTX-NMA was determined in the absence and presence of 1 μM Lqq V, and after extensive washing at 4 °C of the synaptosomes with bound BTX-NMA and 1 μM Lqq V. In the former, the emission anisotropy is 0.032 and the emission anisotropy is 0.041, under conditions in which the LG/LF ratio is 4.3. The anisotropy of bound BTX-NMA, in which all available channel sites could be occupied, is 0.062, which indicates that the fluorescent moiety of BTX-NMA has some rotational mobility when associated with its neurotoxin receptor site on the sodium channel. A summary of the optical properties of free and bound BTX-NMA is given in Table I.

The fluorescence emission properties of BTX-NMA have also been examined with the addition of other sodium channel ligands. No change in the quantum yield or emission maximum of BTX-NMA was observed with the addition of 1 μM TTX or toxin II from C. suffusus suffusus.

Distance between the BTX and Lqq V Receptors—Energy transfer measurements were done in which the N-methylanlanlanilate ligand at the 20 position of BTX served as the donor and in which the DACA, DNP, and CPM chromophores linked to Lqq V served as the acceptors. The absorption spectra of DACA-Lqq V, CPM-SH-Lqq V, and DNP-Lqq V overlap the fluorescence spectrum of BTX-NMA bound to the sodium channel. The spectral relationships for the donor-acceptor pairs are shown in Fig. 3 and the calculated overlap integrals are presented in Table II. The spectral overlap, quantum yield in the absence of acceptor, and consequently the critical transfer distance Ro characterizing the N-methylanlanlanilate and DACA, CPM, and DNP chromophores exhibit distinct differences. Hence, the use of three acceptors of different spectroscopic character should serve as independent measures of distance. Efficiencies of energy transfer between excited state dipole were determined by measuring the quenching of donor fluorescence and sensitization of fluorescence of the acceptors, DACA-Lqq V and CPM-SH-Lqq V.

In all energy transfer experiments the following synaptosome samples were prepared: 1) BTX-NMA and 1 μM Lqq V; 2) BTX-NMA, 1.8 μM BTX-B, and 1 μM Lqq V; 3) BTX-NMA, DACA-Lqq V, CPM-SH-Lqq V, or DNP-Lqq V; 4) BTX-NMA, DACA-Lqq V, CPM-SH-Lqq V, or DNP-Lqq V, and 1 μM Lqq V; 5) 1.8 μM BTX-B, DACA-Lqq V or CPM-SH-Lqq V; 6) 1.8 μM BTX-B, DACA-Lqq V or CPM-SH-Lqq V plus 1 μM Lqq V; 7) 1.8 μM BTX-B and 1 μM Lqq V; and 8) BTX-NMA plus 1.8 μM BTX-B, DACA-Lqq V, CPM-SH-Lqq V, or DNP-Lqq V plus 1 μM Lqq V. Measurements were performed by the addition of the appropriate concentration and combination of ligands to a suspension of synaptosomal membranes, whose receptor site capacities for [3H]BTX-B, 125I-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Notation</th>
<th>Free</th>
<th>Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption maximum, nm</td>
<td>λabs</td>
<td>350</td>
<td>4.70</td>
</tr>
<tr>
<td>Emission maximum, nm</td>
<td>λem</td>
<td>425</td>
<td>405^*</td>
</tr>
<tr>
<td>Quantum yield</td>
<td>φ</td>
<td>0.17</td>
<td>0.67</td>
</tr>
<tr>
<td>Anisotropy</td>
<td>r</td>
<td>0.008</td>
<td>0.032</td>
</tr>
</tbody>
</table>

* G. B. Brown, unpublished observations.

^ In the presence of 1 μM Lqq V.
Energy Transfer on the Na⁺ Channel

FIG. 3. Spectral relationships characterizing BTX-NMA: Lqq V acceptor energy transfer pairs on the sodium channel. The relationships for the BTX-NMA/DACA-Lqq V (A) and BTX-NMA/CPM-SH-Lqq V (B) and BTX-NMA/DNP-Lqq V conjugates (C). The absorption (— — —) and emission (—) spectra for the donor are drawn with heavier lines than those for the acceptor. In addition, the darkest tone serves to emphasize the region of overlap between the donor emission and acceptor absorption (light tone) spectra. The absorption spectra are presented as extinction coefficients, $c$ (M⁻¹ cm⁻¹), while the corrected emission spectra are presented in arbitrary intensity units and are not shown in proportion to the relative quantum yields of donor and acceptor.

Lqq V, and [³H]STX were determined just prior to spectros copy. Accumulation and processing of spectra were done as described previously (4–7). Controls for membrane scattering upon toxin addition were also performed to verify that no precipitation of membranes or ligands occurred upon toxin addition.

Using these procedures, a representative energy transfer experiment between BTX-NMA and DNP-Lqq V is shown in Fig. 4. It is clear that the fluorescence emission of the donor BTX-NMA is reduced in the presence of the energy acceptor, DNP-Lqq V. The observed efficiency for this experiment is 0.32. The fractional occupancy of the Lqq V sites, under these conditions ($K_{D_{Lqq}} = 1.3$ nM (in the presence of BTX), receptor site concentration = 32 nM, $L_{T} = 32$ nM, $L_{B}$ = 29 nM DNP-Lqq V) is 0.91. Therefore, the normalized efficiency is 0.35 by donor quenching. The observed and corrected efficiencies measured by both donor quenching and the sensitized emission of DACA-Lqq V and CPM-SH-Lqq V and relevant energy transfer parameters are given in Table II. The good agreement between efficiencies measured by donor quenching and sensitized emission indicates that the observed quenching of donor fluorescence is through dipolar energy transfer to chromophoric Lqq V.

The BTX-NMA and Lqq V intersite distance may now be calculated from values of $R_0$ and the energy transfer efficiencies. From both donor quenching and acceptor sensitization measurements, the average distance between these sites is calculated to be 37 Å. This assumes that energy transfer occurs between a single donor-accepted pair, and that the value of $K^2$ is $\frac{1}{2}$.

Based upon equilibrium binding measurements to synaptosomes, the site capacities for BTX and Lqq V appear to be equal. If however, one BTX transfers to two equidistant Lqq V sites, then the calculated distance given in Table II for the single donor-acceptor pair would be 12% longer. Because there is no indication of a different stoichiometry at this time, the calculation of a distance between a single donor-acceptor pair appears to be reasonable.

The anisotropy of BTX-NMA, the anisotropy of acceptors DACA-Lqq V and CPM-SH-Lqq V, and the anisotropy of the

FIG. 4. Typical result of an energy transfer experiment. The donor is BTX-NMA (29 nM) and the acceptor is DNP-Lqq V (32 nM). Synaptosomes at 7.5 mg of protein/ml. [³H]BTX-B and [±H]-Lqq V binding to these membranes indicated BTX-B and Lqq V site concentrations of 31 and 32 nM, respectively. The fractional saturation of the acceptor is 0.9. Excitation was set at 345 nm (4-nm band passes) and the spectra were obtained at 25°C in standard binding medium, pH 7.4. The resultant spectra (one accumulation at 2-nm resolution, 10 samples/step) represent specifically bound donor, donor in the presence of the acceptor, donor and acceptor + 1 μM Lqq V to displace the acceptor. The contributions from membrane light scattering and nonspecifically bound BTX-NMA with 345-nm excitation have been subtracted.
transferred excitation are well below the theoretical limit of 6.4. The anisotropy measurements indicate that the probes reorient freely during their excited state lifetime and during the transfer event. The dynamic properties of the fluorescent α-scorpion toxins and the evaluation of their axial depolarization factors indicate rapid rotation about the single bonds between chromophore and toxin. Furthermore, the polarization of the coumarin chromophore covalently bound to the scorpion toxins increases across the emission band (5, 6, 29), and indicates mixed polarizations and transitions. Thus, the treatment of the orientation factor, \( K^2 \), can be extended to those probes showing depolarization due to local rotation during the excited state lifetime and/or due to a combination of mixed polarization and local rotation. We derive a range of most probable distance of 33 to 40 Å, and an uncertainty of ±10% using Table III of Haas et al. (17) when \( K^2 = \frac{1}{3} \) (Table II).

**DISCUSSION**

Batrachotoxin and α-scorpion toxin bind specifically to receptor sites associated with voltage-dependent sodium channels in neuroblastoma cells (19, 20) and synaptosomes (2, 3). The binding of the α-scorpion toxins is voltage-dependent and the dissociation constant is markedly reduced in the presence of batrachotoxin. Similarly, in the presence of α-scorpion toxin, binding of [\(^{3}H\)]BTX-B is enhanced 10- to 20-fold so that approximately 75% of the toxin binding is specific (3). Based upon these observations and others, an allosteric model which describes this heterotropic cooperative interaction has been described (3, 20). The activation of sodium channels by batrachotoxin is assumed to result from the preferential binding to an active conducting state of the sodium channel. α-Scorpion toxin enhances BTX action by reducing the equilibrium constant for the transition from inactive to active states.

Development of specific fluorescent probes for the tetrodotoxin, α-scorpion, and β-scorpion toxin receptor sites on the sodium channel has led to the characterization of the molecular environment, conformational dynamics, and intersite spatial relationships. Structural mapping of the receptors for TTX and Lqq V has shown that these receptors are separated by 35 Å (6). Tetrodotoxin binds to a very polar region of the channel and has limited conformational mobility, while fluorescent Lqq V moves into a hydrophobic environment upon binding to its receptor. Previous energy transfer distance measurements indicate that the Lqq V site is sensitive to voltage- and neurotoxin-dependent conformational changes. When transfer efficiencies between TTX and Lqq V sites were measured by both donor quenching and sensitized emission, the efficiencies upon BTX addition were lower, indicating an increase in the distance of separation of these sites by \( \sim 7 \) Å (6). None of the other sodium channel ligands, e.g. toxin II from *Centruroides*, effected these changes. These observations are the first indications of direct or indirect molecular interactions between these sites, in which a conformational change induced by BTX through one subunit or through alterations in subunit/subunit interactions is observed.

It thus becomes important not only to identify these molecular interactions but also to elucidate the spatial relationships of these receptor sites which are allosterically coupled.

When BTX-NMA binds to synaptosomes in the absence of Lqq V, changes in the fluorescence emission intensity and wavelength are observed. From a comparison of the spectral behavior of the N-methylanthraniloyl chromophore in various solvents, it appears that the BTX-binding environment under these conditions is quite hydrophobic. This reflects the nonspecific binding component, which is probably associated with the membrane lipid, due in part to the high lipid solubility of batrachotoxin. In the presence of α-scorpion toxin, however, the fluorescence maximum of specifically bound BTX-NMA is shifted 10 nm to lower energy relative to BTX-NMA free in aqueous solution. Thus, in contrast to the nonspecific binding environment, the environment of specifically bound toxin appears to be relatively hydrophilic. This assessment is supported by another line of evidence as well. Recent studies have shown that at physiological pH batrachotoxin and its derivatives exist as mixtures of positively charged and neutral species, reflecting titration of the homomorpholinor nitrogen, and that it is the charged form which interacts preferentially with the sodium channel site (25). For a charged ligand, a relatively hydrophilic binding environment would be energetically preferable to a hydrophobic environment and our fluorescent measurements support this interpretation.

There is evidence from these experiments that the BTX and Lqq V receptor sites are directly conformationally coupled. When 1 μM Lqq V is added to synaptosomes with BTX-NMA, increases in both the fluorescence intensity and wavelength maximum are observed. The enhancement of fluorescence emission can be reduced by 70% with the addition of 1 μM BTX-B, resulting in a 4-fold enhancement of the specific over the nonspecific binding component. This enhancement is not as large as that seen for binding of [\(^{3}H\)]BTX-B, although it is not reasonable to expect the fluorescence emission to directly parallel the increase in specific site capacity as seen with the tritiated ligand. Nevertheless, the fluorescent chromatophore reports on subtle changes of macromolecular conformation and environment, and with the addition of 1 μM Lqq V a dramatic 30-nm red shift from the signal of nonspecifically bound BTX-NMA is seen. There are two possible explanations for this observation. The first reason is that the spectral signal derived from the 405-nm maximum of BTX-NMA represents 80-90% nonspecific lipid-associated BTX-NMA. Addition of α-scorpion toxin alters the partitioning of BTX-NMA such that the enhancement observed reflects a redistribution of the bound BTX-NMA from lipid to protein domains. Alternatively, it is possible that BTX-NMA binds peripherally to protein domains with major exposure to

---

**Table II**

<table>
<thead>
<tr>
<th>Donor:Acceptor</th>
<th>( J^\circ ) (× 10^(-24) cm^3 M^(-1))</th>
<th>( R_0 ) Å</th>
<th>( R'/\text{acceptor} )</th>
<th>( R'/\text{acceptor} ) range</th>
<th>Distance range</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTX-NMA:DNQ-Lqq V</td>
<td>1.33</td>
<td>32.9</td>
<td>0.35</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>BTX-NMA:DDCA-Lqq V</td>
<td>1.44</td>
<td>33.8</td>
<td>0.35</td>
<td>37</td>
<td>33-40</td>
</tr>
<tr>
<td>BTX-NMA:CPM-SH-Lqq V</td>
<td>11.1</td>
<td>47.4</td>
<td>0.76</td>
<td>0.79</td>
<td>38</td>
</tr>
</tbody>
</table>

*\( \ast \) Calculated from Fig. 3.*  
*\( \ast \) Calculated by donor quenching.*  
*\( \ast \) Calculated by sensitized emission of the acceptor.*
lipid hydrocarbon. In the presence of α-scorpion toxin, a
conformational change is effected in this domain such that the
microenvironment is altered. This latter suggestion is
consistent with the idea that α-scorpion toxin alters the
population of inactive and active channel conformers and, by
stabilizing a particular active conformation, enhances the binding
of batrachotoxin. Our spectral measurements provide direct
confirmation of the allosteric model used to account for the
enhancement of alkaloid toxin action by the polypeptide α-
scorpion toxins and provide some insight on the nature and
microenvironments of the conformational transitions.

With the characterization of the molecular components of
the sodium channel and the description of the mode of inter-
action of the neurotoxins with their receptor sites, we have
sought structural information on the organization of the al-
losterically coupled BTX and Lqq V sites.

The distance between the BTX and Lqq V receptors deter-
mind in this work by fluorescence resonance energy transfer is
about 37 Å. In our experiments, energy transfer was mea-
 sured by both donor quenching and acceptor sensitization in
order to control for possible energy transfer artifacts. The
agreement of efficiencies measured by both methods indicates
that transfer is by the dipole-dipole mechanism. Unfortu-
nately, we were unable to carry out energy transfer experi-
ments in which the signal of BTX-NMA bound to synapto-
 somes (in the absence of α-scorpion toxin) was utilized, in
order to examine possible discrepancies in donor quenching
and sensitized emission. However, the spectral shift in the
 emission maximum does support the notion of a conforma-
tional change upon Lqq V binding. In spite of the sensitivity
of the fluorescence to ligand binding, the energy transfer
measurements summarized in Table II indicate that the BTX
and Lqq V sites are quite far apart. Thus, the binding of Lqq
V must involve conformational changes that extend over large
distances from the BTX-binding locus. This information to-
gether with the distance measurement of TTX to Lqq V and
the conformational transition associated with this distance
upon BTX addition indicate a conformationally flexible chan-
el with coupling of sites through the polyatomic framework
of individual subunits or through extensive alterations in
 subunit/subunit interactions.

The range of distance calculated between these two sites
arises from the orientation factor K2 used to calculate R0. K2
enters into the calculation of R0 as a function raised to the ½
power. The limits of the relative orientation of the fluoro-
 phores have been established by using the procedure of Haas
et al. (17), using the measured polarizations of donor, acceptor,
and sensitized acceptor emission. The polarizations of all the
ligands used are quite low and support the idea that the
fluorophores dipoles reorient during the excited state lifetime.
Furthermore, previous dynamic depolarization measurements
of fluorescent Lqq V derivatives confirm that a rapid compo-
 nent of less than 0.5 ns, probably corresponding to rapid
reorientation about the fluorophore-toxin linkage, is detected
(5). The limits due to the orientation factor of the fluoroph-
 ores used have been tabulated in Table II, and show that the
range of distance is quite narrow.

Other considerations used to evaluate the measured dis-
 tance include 1) the fact that the dimodified derivative, CPM-
SH-Lqq V, will behave as two independent acceptors of ab-
sorptivity 59 mM−1 cm−1 rather than a single acceptor of
absorptivity 29.5 mM−1 cm−1 (5), and 2) the emission transi-
dipole moment of the probe may be displaced from the
binding site.

The position of the CPM moiety on Lqq V has been
determined to be on residues 58 and 80 (5, 7). These chro-

mophores are separated by ~6 Å. In the calculation of R0
for this derivative, the two acceptors were therefore treated as
a single acceptor with twice the extinction coefficient. Calcu-
lation of the critical distance for the BTX-NMA/CPM-Lqq
V pair using a single absorptivity decreases R0 by only 12%.
Therefore, we believe any error in treating CPM as a single
acceptor is quite small. In any case, the measured distance
agrees very well with those obtained with monomodified de-
rivatives, a further indication that the two CPM chromo-
phores on Lqq V can be treated as a single acceptor with twice
the extinction coefficient.

It is unlikely that the dimensions of the fluorescent α-
scorpion toxin will seriously affect the measured BTX to Lqq
V intersite distance. This has been discussed before (6, 7).
Considering the dimensions of α-scorpion toxin (diameter
~ 10 Å) and BTX-NMA (length ~ 10 Å), the minimum and
maximum distances of separation between the receptor sites
are 27 and 47 Å, respectively. It is apparent from this work
that these sites are not overlapping, and that the conforma-
tional transitions of the channel observed by our fluorescence
measurements still take place over significant distances.

The distance reported here is of primary concern for the
elucidation of the conformational transitions and structural
features of the voltage-dependent sodium channel. We have
shown that the distance between the TTX and voltage-de-
pendent Lqq V receptor sites is 55 Å and increases to 42 Å
upon batrachotoxin binding, and that the TTX to Centru-
roides II site is 34 Å. Together with the available polypeptide
composition (21-23) and α-scorpion toxin photosbeling pat-
ttern (23, 24), we have tentatively suggested that three TTX
sites located at the extracellular synaptosomal surface reside
on the 250,000-Da α-polypeptide and are equidistant from a
single Lqq V site receptor on a 39,000-Da unit. This latter
receptor site is placed ~15 Å below the lipid head groups (6,
7) of the membrane bilayer. Similarly, identification of a
39,000-Da polypeptide by a photoreactive Css II derivative
indicates that the receptors for Css II and Lqq V may be on
the same polypeptide (28) separated by ~22-33 Å across4
and that this polypeptide expresses two distinct domains.
This polypeptide could indeed represent both the activation
and inactivation gating system coupled to discrete regions on
the large subunit in a regulatory arrangement. Therefore, the
binding domains of these scorpion toxins and environments
of the receptor sites would be expected to be different, as
observed by the sensitive fluorescence technique, and as a
result of the heterogeneity different conformational transi-
tions would be expressed depending on their loci. In fact,
direct evidence of receptor site coupling between Lqq V and
Css II has been observed spectrscopically (7, 29), and
electrophysiologically (27) to support the suggestion of intrasub-
unit communication.

Batrachotoxin is coupled to the Lqq V site, but is physically
removed from the Lqq V site by 37 Å. No interaction between
the Css II and BTX receptors has been reported. It is possible
that BTX exerts its effect by an alteration in subunit/subunit
interaction through the TTX units (6) rather than direct
conformational changes on the Lqq V receptor site. We sug-
gest that the BTX receptor is either 1) a domain of the
250,000-Da α unit, 2) located on a polypeptide not purified
with the STX-binding component of the channel, or 3) is
located on the β1 subunit of the channel. Recent radiation
inactivation studies of the synaptosomal BTX receptor indi-
cates a functional binding unit size of 110,000 ± 5,000 Da.5

K. J. Angelides and H. Darbon, unpublished observations.
K. J. Angelides, T. J. Nutter, L. W. Elmer, and E. S. Kempner,
manuscript submitted.
is unlikely that this indeed represents a stable domain of the α-subunit of the channel since ionizing energy completely destroys its target. Reconstitution studies show that the BTX-binding component containing purified α, β1, and β2 units is capable of BTX-mediated ion flux (22), and argues against the absence of the BTX receptor in these preparations. The most likely possibility, in view of the receptor site stoichiometries, radiation inactivation, and energy transfer, is that the BTX receptor is primarily associated with the α-polypeptide changes with α-scorpion toxin binding. The β1 component would be imbedded in the hydrophobic interior of the cell membrane which modulates inactivation and channel opening through remote interactions to the LqV site which are communicated to the large subunit. Unfortunately, the photolabeling of the BTX receptor is not yet available and these suggestions cannot be fully evaluated until such information becomes available.

In conclusion, these energy transfer measurements have served in locating important neurotoxin/channel modulator sites on the sodium channel, and together with available biochemical information, a more defined structural map of the organization of the molecular components and mechanism of action of the channel can be formulated. Future experiments will be aimed at elucidating the relationship of the LqV and Css II sites, the BTX and Css II sites, and the polypeptide gating systems.

Acknowledgment—We thank Tom Nutter for electrophysiological measurements.

REFERENCES
Fluorescence resonance energy transfer on the voltage-dependent sodium channel. Spatial relationship and site coupling between the batrachotoxin and Leiurus quinquestriatus quinquestriatus alpha-scorpion toxin receptors.

K J Angelides and G B Brown


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