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References

Andreasen, T. J., Keller, C. H., LaPorte, D. C., Edelman, A. M., & Storm, D. R. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2782–2785.

Davies, G. E., & Stark, G. R. (1970) *Proc. Natl. Acad. Sci. U.S.A. 66*, 651–656.

Kincaid, R. L., & Vaughan, M. (1983) *Biochemistry 22*, 826–830.

Kincaid, R. L., Manganiello, V. C., & Vaughan, M. (1981) *J. Biol. Chem. 256*, 11345–11350.

Kincaid, R. L., Osborne, J. C., Jr., Vaughan, M., & Tkachuk, V. A. (1982) *J. Biol. Chem. 257*, 10638–10643.

LaPorte, D. C., Toscano, W. A., Jr., & Storm, D. R. (1979) *Biochemistry 18*, 2820–2825.

Peters, K., & Richards, F. M. (1977) *Annu. Rev. Biochem. 46*, 523–551.

Quiocho, F. A., & Richards, F. M. (1966) *Biochemistry 5*, 4062–4076.

Binding of β-Scorpion Toxin: A Physicochemical Study†

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ABSTRACT: The binding to rat brain synaptosomes of a β-scorpion toxin, i.e., toxin II of *Centruroides suffusus suffusus* (Css II), was studied as a function of pH, temperature, and concentration of some monovalent and divalent cations. At 10 °C and pH 6.0, the specific binding of ^{125}I-labeled Css II corresponds to a single class of noninteracting high-affinity binding sites (K_D = 0.18 nM) with a capacity (4.2 pmol/mg of protein) that is almost identical with that generally accepted for saxitoxin. The equilibrium dissociation constant of β-scorpion toxin is pH independent, but the maximum binding capacity is reduced with increasing pH. Li^+, guanidinium, Ca^{2+}, Mg^{2+}, and Mn^{2+} modified the apparent K_D of the ^{125}I-labeled Css II toxin. The equilibrium dissociation constant varies markedly with the temperature. The van't Hoff plot of the data is curvilinear, corresponding to a standard free-energy change associated with an entropy-driven process. The association rate constant also varies considerably with the temperature whereas the Arrhenius plot is linear between 1 and 30 °C. The energy of activation determined from these data is 17.6 kcal/mol. These results support the hypothesis that a cluster of nonpolar amino acid residues present on one face of the molecule is involved in the toxin–receptor interaction.

A large number of neurotoxins of animal or plant origin modify the properties of the sodium channel involved in the action potential of excitable cells. Some have been radiolabeled and their binding properties compared to their pharmacological effect. These investigations lead Catterall (1980, 1982) to suggest the existence of three distinct neurotoxin binding sites associated with the sodium channel. Site "1" binds saxitoxin and tetrodotoxin, which are heterocyclic molecules with a guanidium group. Sodium transport is inhibited when this site, located on the outer side of the membrane, is occupied. Site "2" binds several liposoluble toxins, e.g., grayanotoxin and the alkaloids batrachotoxin, veratridine, and aconitine. These toxins cause persistent activation of sodium channels at the resting membrane potential by blocking channel inactivation and by shifting the voltage dependence of sodium-channel activation to more negative membrane potentials. Site "3" recognizes the scorpion neurotoxins that we have called α-scorpion toxins (Jover et al., 1980) and sea anemone neurotoxins. These neurotoxins slow or block the sodium-channel inactivation phase.

We have identified a fourth site linked to the sodium channel that recognizes neurotoxin II of *Centruroides suffusus suffusus* venom (Css II), a β-scorpion toxin (Jover et al., 1980). Contrary to α-scorpion toxins, the affinity of β-scorpion toxins for their receptor site does not vary with the membrane potential. Css II binding to site 4 modifies sodium-channel activation, as shown by the occurrence of an abnormal sodium current after repolarization of the myelinated frog nerve (Couraud et al., 1982) and by the blocking of sodium conductance activation in the frog skeletal muscle (Jaimovich et al., 1982). Css II toxin inhibits the uptake and stimulates the release of γ-aminobutyric acid by rat brain synaptosomes, an effect that is abolished by tetrodotoxin (Couraud et al., 1982). Two toxins from the venom of the scorpion *Centruroides sculpturatus* Ewing showed similar effects to those of Css II on the node of Ranvier of the myelinated frog nerve (Meves et al., 1982), and a toxin from *Tityus serrulatus* venom competes with Css II at the same binding site (Wheeler et al., 1982). The present paper describes a physicochemical study of a β-scorpion toxin (Css II) interaction with site 4.

The association and dissociation kinetic constants of this toxin have been determined and compared with those of saxitoxin as defined by Weigele & Barchi (1978a) and those of toxin II of *Androctonus australis* Hector, an α-scorpion toxin, which we have measured in the same biological system (Jover et al., 1978). In addition, the thermodynamic parameters in the ligand–receptor reaction were estimated. The results obtained suggest that the interaction of Css II with its receptor involves polar attraction, which may be followed by

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a stabilization of the complex through apolar interactions.

Materials and Methods

Toxin II from *Centruroides suffusus suffusus* (Css II) was purified in our laboratory according to Garcia (1976). Tris(hydroxymethyl)aminomethane (Tris), lactoperoxidase, bovine serum albumin (fraction V), and *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid (Hepes) were obtained from Sigma Chemical Co. (St. Louis, MO). Carrier-free iodine-125 (13–17 Ci/mg) was obtained from the Radiochemical Center (Amersham, England).

Preparation of Synaptosomes. Synaptosomes were prepared according to the method of Gray & Whittaker (1962) as modified by Tamkun & Catterall (1980). The final suspension of synaptosomes was stored in liquid nitrogen with no appreciable loss of toxin binding (less than 20% after 1 month). Protein concentration was measured by a modified Lowry method (Lowry et al., 1951).

Measurement of Scorpion Toxin Binding. Iodination of Css II (^{125}I-β-ScTx) was carried out following the Rochat et al. (1977) application of the lactoperoxidase method and purified by immunoprecipitation with a monospecific antiserum prepared against the native toxin. The specific radioactivity usually obtained was from 700 to 1000 Ci/mmol. Competition experiments between ^{125}I-β-ScTx and the native toxin were usually carried out in a final volume of 1.5 mL of synaptosomal suspension (30 μg of protein/mL) containing 0.1 nM of ^{125}I-β-ScTx and the required concentration of unlabeled toxin. The reaction was initiated by the addition of synaptosomes to a mixture of labeled and unlabeled toxins and stopped after 30 min by filtration on Whatman GF/C glass-fiber filters. The filters were washed 3 times with the same volume of washing buffer. In order to determine the kinetic association rate, 40 mL of synaptosomal suspension was preincubated for 10 min to the required temperature. The reaction was initiated by addition of 8 mL of ^{125}I-β-ScTx solution (1.5 nM). A 1.5-mL aliquot of synaptosomes was filtered as described above, at the times indicated in figure legends. The kinetic dissociation rate was determined after 30-min incubation of synaptosomal suspension (30 μg of protein/mL) with 0.8 nM ^{125}I-β-ScTx. Then, a small volume of β-ScTx concentrated solution was added (0.5 μM final concentration), and the aliquots were filtered at the times indicated in the figure legends. The incubation medium contained 140 mM choline chloride, 5 mM KCl, 10 mM glucose, 25 mM Hepes, and 0.25% bovine serum albumin adjusted, at 10 °C, with 1 M Tris base to the required pH value. To study the effect of divalent cations, choline was subtracted in an equal-charge equivalent. The washing medium was identical but buffered with 5 mM Hepes.

Results

Binding Constants for β-ScTx at 10 °C. We have previously described β-ScTx binding to the synaptosomal fraction (P2) of rat brain (Jover et al., 1980) and shown that the dissociation constant (K_d) of ^{125}I-β-ScTx at 37 °C was, on average, 3.5 nM for a site capacity of 1.20 pmol/mg of protein. At the same temperature, the K_d for ^{125}I-β-ScTx binding on purified synaptosomes was 3.3 nM and the binding site capacity 2.44 pmol/mg of protein (mean of seven independent experiments), that is, an average site number increment of 100% approximately. Association and dissociation take place too quickly at 37 °C to be measured by using available techniques (Figure 1a). Consequently, we first measured the kinetic constants at 10 °C after determining the equilibrium dissociation constant at this temperature. The Scatchard plot

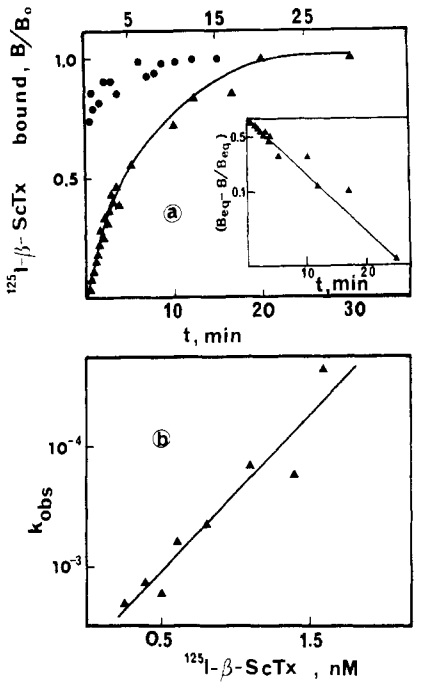

FIGURE 1: Time course of the association of ^{125}I-β-ScTx to synaptosomes. (a) Association kinetics at 10 (▲) and 37 °C (●). (Insert) The data of the experiment at 10 °C were evaluated by a least-squares fit to the semilogarithmic plot to give k_{obsd}. (b) A direct plot of k_{obsd} vs. concentration of ^{125}I-β-ScTx. k_{obsd} was obtained as in (a).

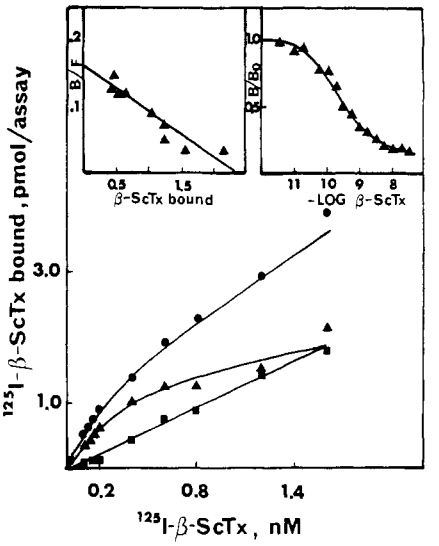

FIGURE 2: Constant equilibrium binding of ^{125}I-β-ScTx. (a) Synaptosomes (30 μg of protein/mL) were incubated 30 min at 10 °C with the indicated concentrations of ^{125}I-labeled Css II in the presence (■) or in the absence (●) of 0.3 μM native Css II. Specific binding (▲) was the difference between the two curves. (Left insert) Scatchard plot of specific binding. (Right insert) Displacement of ^{125}I-labeled Css II bound to the synaptosomes by native Css II.

of the saturation curve shows a single class of noninteracting binding sites with K_d equal to 0.56 nM and a site capacity of 2.02 pmol/mg of protein (mean of five experiments) (Figure 2). Since experiments at 37 °C were carried out with fresh synaptosomes whereas synaptosomes that had been frozen were used in experiments at 10 °C, it was not surprising to find a loss of about 20% in the binding capacity resulting from freezing and thawing of the sample. Competition experiments between ^{125}I-β-ScTx and the native toxin show a K_d = 0.2 nM with a Hill number very close to 1.

The kinetic studies measuring the rate of ^{125}I-β-ScTx association were performed under pseudo-first-order conditions

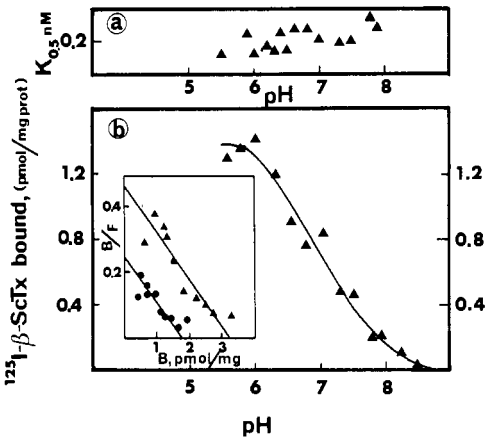

FIGURE 3: Effect of pH on ^{125}I-β-ScTx binding to synaptosomes. (a) $K_{0.5}$ values obtained by displacement experiments at different pH values. (b) Variation in the binding capacity at a constant concentration of ^{125}I-β-ScTx (0.2 nM). (Insert) Scatchard plot of isotherm saturation curves carried out at pH 7.2 (●) and pH 6.0 (▲).

$[^{125}\text{I-}\beta\text{-ScTx}]_0 \gg [\text{binding sites}]_0$. As can be seen from Figure 1a, only a single relaxation time was observed. The data (Figure 1a, insert), plotted in semilogarithmic form, as $[B_{eq} - B/B_{eq}]$ vs. time extrapolate to an ordinate intersect of 0.98, which shows that all the specifically bound ^{125}I-β-ScTx can be accounted for by the single kinetic phase. When the slope of the obtained straight line (k_{obsd}) was plotted vs. ^{125}I-β-ScTx concentration (Figure 1b), a linear relationship was found showing a simple reversible equilibrium. The equation of this slope, $k_{obsd} = k_1[^{125}\text{I-}\beta\text{-ScTx}] + k_{-1}$ enables one to obtain the rate constants of association (k_1) and dissociation (k_{-1}) according to Strickland et al. (1975), which are respectively $k_1 = 6.37\ 10^6\ M^{-1}\ s^{-1}$ and $k_{-1} = 1.57\ 10^{-3}\ s^{-1}$. The same association rate constant was found in other experiments performed under non-pseudo-first-order conditions in which k_1 was calculated by using the integrated second-order rate equation [see Weiland & Molinoff (1981)]. The average of 14 independent experiments was $8.70\ 10^6\ M^{-1}\ s^{-1}$. The kinetic dissociation constant was also confirmed: $k_{-1} = 2.50\ 10^{-3}\ s^{-1}$ (mean of seven experiments). The dissociation equilibrium constant calculated from the rate constants (0.3 nM) is near to the dissociation constant obtained by equilibrium experiments.

Effect of pH on ^{125}I-β-ScTx II Binding. Specific binding of ^{125}I-β-ScTx at a constant ligand concentration decreased as the pH increased from 6 to 8.5 and was virtually undetectable above pH 8.5 (Figure 3b). We have also characterized the effect of pH by competition experiments between ^{125}I-β-ScTx and the native toxin. The $K_{0.5}$ value varies, independently of the pH, from 0.14 to 0.35 nM (Figure 3a). This variation is probably due to experimental error and cannot account for the inhibition of specific binding. To confirm that the equilibrium dissociation constant is pH independent, we plotted isothermal saturation curves at pH 6 and 7.2 (Figure 3b, insert); the equilibrium dissociation constant for both pH values was 0.18 nM, but the maximum binding capacity at pH 6 was twice that at pH 7.2; it was 4.2 pmol/mg of protein (mean of four experiments).

Equilibrium Constant and Free-Energy Changes. The equilibrium dissociation constant determined by competition experiments between ^{125}I-β-ScTx and the native toxin varies markedly with the temperature. A van't Hoff plot of the data (Figure 4a) is curvilinear with a maximum around 17 °C. The standard free-energy change is

$$\Delta G^\circ = -RT \ln K_A \qquad (1)$$

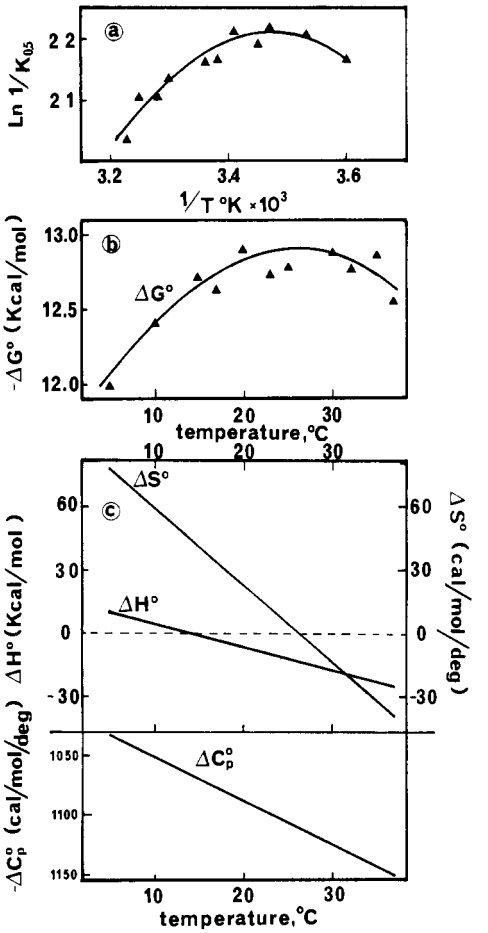

FIGURE 4: van't Hoff plot of β-ScTx binding and the variation of thermodynamic parameters with temperature. (a) $K_{0.5}$ values (nM) were determined at different temperatures by competition experiments between ^{125}I-β-ScTx and native toxin. Each point is the average of three independent experiments. (b) Variation of the free-energy change (ΔG°) with temperature. ΔG° was calculated from the $K_{0.5}$ values according to eq 1. (c) Variation of the enthalpy change (ΔH°), entropy change (ΔS°), and heat capacity (ΔC°_p) with temperature, calculated from eq 4–6.

where R is the gas constant (1.99) and T the absolute temperature. A plot of ΔG° against the temperature (Figure 4b) also shows a marked curvilinearity. The enthalpy change, ΔH°, is usually determined from the slope of the van't Hoff plot at various temperatures, but this procedure would have yielded inaccurate values in this case due to the curvature of the plot. Therefore, we used the procedure of Edelhoch & Osborne (1976) and carried out a regression analysis of the free-energy change by using

$$\Delta G^\circ = -RT \ln K_A = A + BT + CT^2 \qquad (2)$$

The best fit of the experimental data is represented by the solid line in Figure 4b and the coefficients shown in

$$\Delta G^\circ = 153\,260.7 - 1110.06T + 1.85T^2 \qquad (3)$$

which gives

$$\Delta H^\circ = \delta(\Delta G^\circ/T)/\delta(1/T) = 153\,260.7 - 1.85T^2 \qquad (4)$$

$$\Delta S^\circ = \delta\Delta G^\circ/\delta T = 1110.06 - 3.708T \qquad (5)$$

where ΔS° is the entropy change of the reaction. The heat capacity change ΔC°_p can also derived:

$$\Delta C^\circ_p = \delta\Delta H^\circ/\delta T = -3.708T \qquad (6)$$

As shown in Figure 4c, the changes in both enthalpy and

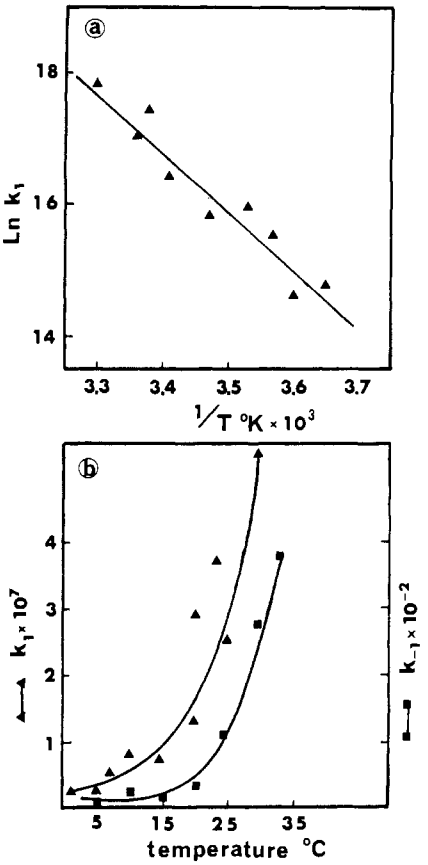

FIGURE 5: Variation of kinetic constants with temperature. (a) Arrhenius plot of rate association constants calculated according to the integrated second-order rate equation (Weiland & Molinoff, 1981). (b) Variation of rate association constant (▲) and rate dissociation constant (■).

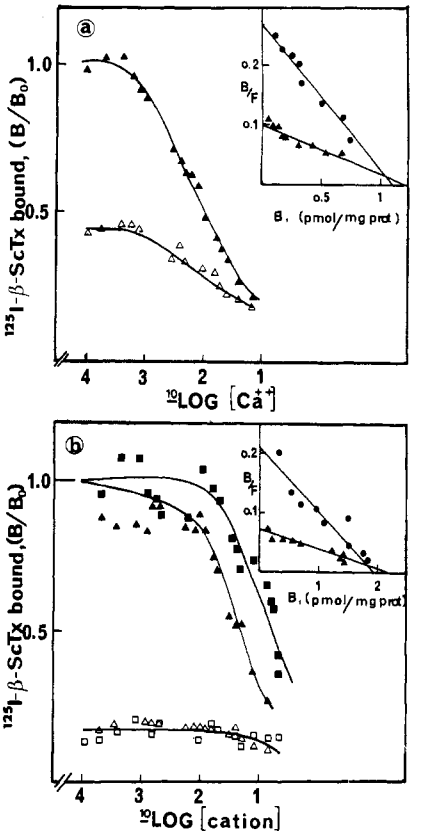

FIGURE 6: Effect of monovalent and divalent cations and ionic strength on ^{125}I-β-ScTx binding. (a) Synaptosomes were incubated 30 min at 37 °C with 0.2 nM ^{125}I-β-ScTx in a medium containing Ca^{2+} in place of choline. The binding was measured in the presence (△) or in the absence (▲) of 0.3 μM native toxin. (Insert) Scatchard plots of isotherm saturation curves carried out in the absence (●) or in the presence (▲) of 4 mM Ca^{2+}. (b) Synaptosomes were incubated 30 min at 37 °C with 0.2 nM ^{125}I-β-ScTx in a medium containing guanidinium (▲) or Li^+ (■) in place of choline. The binding was measured in the presence (△, □) or in the absence (▲, ■) of 0.3 μM native toxin. (Insert) Scatchard plots of isotherm saturation curves carried out in 140 mM choline (●) and 140 mM choline plus 80 mM Li^+ (▲).

entropy for the β-ScTx receptor decrease continuously and vary directly with the temperature, within the limits studied. The thermodynamic stability of the complex ($\delta\Delta G^\circ/\delta T = \Delta S^\circ = 0$) is maximum at 26 °C. The change in heat capacity, that is, the slope of the ΔH° curve, is negative, suggesting that the heat capacity for β-ScTx and receptor molecules is greater than for the toxin–receptor complex. Although the plot of ΔH° against the temperature appears linear given the experimental error and the range of temperatures studied, it is represented by a second-degree equation (eq 4) of which the first derivative, ΔC°_p, also varied with temperature (Figure 4c). The absolute value of the variation, however, is the second derivative of the initial data plot and thus relatively inaccurate. The value of ΔC°_p is traditionally expressed at 25 °C and, in this case, it was found to be −1105 cal/(mol deg).

Rate Constants and Energy of Activation. The association rate constant varied markedly with temperature, ranging from 2.64 10^6 M^{-1} s^{-1} at 1 °C to 54.37 10^6 M^{-1} s^{-1} at 30 °C. Above 30 °C, association took place so fast that multiple points could not be accurately analyzed even when a filtration assay was used. By use of these measured on-rates and the measured equilibrium binding constants at each temperature, dissociation rate constants were calculated and ranged from 0.86 10^{-3} s^{-1} at 5 °C to 28.5 10^{-3} s^{-1} at 30 °C. The variations of these two rate constants with the temperature are not linear but follow an exponential curve ($y = ax^b$) (Figure 5b). However, the Arrhenius plot of ln k_1 vs. $1/T$ between 1 and 30 °C was linear (Figure 5a), and the energy of activation, E_a, determined from these values was 17.6 kcal/mol.

Effect of Monovalent and Divalent Cations. We have previously shown (Jover et al., 1980) that K^+ (below 140 mM) has no influence on the binding of Css II to synaptosomes. However, the substitution of choline by Na^+, K^+, and Rb^+ is without effect whereas substitution by Li^+ or guanidinium inhibits binding. Half-maximum inhibition is observed with 140 mM Li^+ or 25 mM guanidinium (Figure 6b). Increasing the concentration of monovalent cations above 145 mM (i.e., increasing ionic strength) was accompanied by a decrease of the specific and nonspecific binding. Specific and nonspecific binding were also lowered by increasing the concentration of divalent cations Ca^{2+}, Mg^{2+}, and Mn^{2+} (Figure 6a), giving a half-maximum inhibition of binding at 4.4, 5, and 7 mM, respectively. This effect is due to decreased affinity as the binding site capacity remained constant. In the same way, the apparent K_D for the toxin was modified by the presence of Li^+ or guanidinium or by increasing the ionic strength. This is shown in the insert to Figure 6b, both for a high (Li^+) concentration (80 mM) and an increased ionic strength (220 mM of monovalent cation).

Discussion

The ^{125}I-β-ScTx receptor capacity of purified rat brain synaptosomes is very close to that reported for [^{3}H]saxitoxin in the same type of preparation (Catterall et al., 1979). These authors also demonstrated a 100% increase in the saxitoxin receptor number in purified synaptosomes, when compared to a synaptosomal fraction.

Table I: Comparison of STx and β-ScTx

	temp (°C)	K_d (nM)	k_1 (M^{-1} s^{-1})	k_{-1} (s^{-1})	$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta G^{\ddagger}$ (kcal/mol)
STx[a]	0	0.5	6.67×10^6	3.03×10^{-3}	12.3	7.38
β-ScTx	10	0.25	6.37×10^6	1.57×10^{-3}	17.09	7.26

[a] Data from Weigele & Barchi (1978a).

When the pH of the incubation medium was changed, the maximum binding capacity of ^{125}I-β-ScTx was modified but not the affinity of the toxin for its site, contrary to what has been found for saxitoxin (STx) (Weigele & Barchi, 1978b). Consequently, the maximum number of receptor sites for β-ScTx (≃4 pmol/mg of protein) was measured at pH 5.5–6.0, whereas (Figure 3, insert) at a physiological pH, only half as many receptor sites can be detected. Catterall et al. (1979) on a comparable synaptosome preparation obtained a very similar maximum binding capacity for STx. It seems therefore that the stoichiometry between β-Sctx and STx binding sites must be equal to one when quantitative determinations are done at optimum pH values for each toxin. A next important step would be to ascertain whether α-ScTx and β-ScTx binding sites numbers could be modified by pH in the same way.

The binding constants for STx at 0 °C (Weigele & Barchi, 1978a) and β-ScTx at 10 °C are comparable (Table I). The complete study of binding constants for saxitoxin as a function of the temperature carried out by Weigele and Barchi makes it possible to calculate the free energy ($\Delta G^{\ddagger}$) and enthalpy ($\Delta H^{\ddagger}$) in the transition phase and to compare them with our own results (Table I). In both cases, $\Delta G^{\ddagger}$ is larger than zero, which reflects the presence of a significant energy barrier ($\Delta H^{\ddagger} > 0$), which is true for both toxins. The energy barrier, however, is greater for β-ScTx than for STx. This, perhaps, accounts for the binding and release velocities, which are faster for STx than for β-ScTx at these temperatures. The kinetic association constant for α-ScTx, at 37 °C, was 4.5 10^7 M^{-1} s^{-1} (Jover et al., 1978), which suggests that the energy barrier for α-ScTx binding is larger than the energy barrier for the other two toxins. The above data supply information on the nature of the reaction between these three types of receptor sites associated with the sodium channel and their respective ligands.

The results of the study of the variation of the value of the equilibrium dissociation constant vs. temperature (van't Hoff curvilinear plot) may originate from three different phenomena: (1) a transition phase of membrane lipids (Fourcans & Jain, 1974); (2) a toxin–receptor reaction comprising more than one stage, e.g., $T + R \rightleftarrows TR \rightleftarrows TR'$ (Waelbroeck et al., 1979); (3) a heat capacity difference for reagents and product (Edelhoch & Osborne, 1976). The first assumption may be discarded since the variation, with temperature, of the kinetic association constant (Arrhenius plot), between 1 and 30 °C, is linear. We have tested the second hypothesis by evaluating the association kinetics over a wide range of ^{125}I-β-ScTx (0.2–2 nM) concentrations, in conditions in which a pseudo-first-order reaction must prevail. A reaction including more than one stage should have given a nonlinear plot of K_{obsd} vs. ^{125}I-β-ScTx concentration (Strikland et al., 1975) and not the straight line we found (Figure 1b). This confirms a single-stage reaction of the $T + R \rightleftarrows TR$ type.

The thermodynamic parameters calculated on the basis of our results seem consistent with the third hypothesis, namely, that the heat capacity of the reagents and product are different. They are strikingly similar to those obtained by Osborne et al. (1976) in a thermodynamic study of the self-association of the reduced and carboxymethylated form of the apo-A-II protein from the human high-density lipoprotein complex. They also fit with the more recent data obtained by Waelbroeck et al. (1979) in a thermodynamic study of the interaction of insulin with its receptor. These reactions showed a similar shape in the temperature dependence of the free-energy change, with a maximum near 28 °C for apo-A-II protein and 37 °C for insulin (26 °C in our study). They also showed the same inverted relationship between $\Delta H°$ or $\Delta S°$ and the temperature and a comparable variation in the heat capacity change between the unassociated and associated molecule: −1250 cal/(mol deg) for apo-A-II protein, −766 cal/(mol deg) for insulin and −1105 cal/(mol deg) in our own study. This type of behavior is typical of the interactions with water of the nonpolar moieties of a variety of compounds such as acids, bases, alcohols, amino acids, and surfactants and contrasts with the interaction of polar moieties with water. This "hydrophobic effect" and its consequence on the thermodynamics of various model systems has been analyzed by Edelhoch & Osborne (1976), and understanding its basis may prove crucial in many ligand–receptor interactions. The most useful thermodynamic parameter that expresses the interaction of various groups with water is the heat capacity change (Edelhoch & Osborne, 1976; Osborne et al., 1976). From the large negative heat capacity change observed in our study, it appears that the β-ScTx receptor interaction is probably assisted by the removal of water from the less polar moieties (probably surface residues of both proteins) and the resulting changes in the cooperative organization of hydrogen binding between water molecules, which is an entropic-driven process.

The structure of the scorpion toxin is sufficiently well-known to allow speculation as to which part of the molecule interacts with the receptor. Recently, Fontecilla-Camps et al. (1980) have determined the three-dimensional structure of variant 3 present in the venom of the scorpion *Centruroides sculpturatus* Ewing. Out of 66 residues, 40 are common to both variant 3 and Css II (Rochat et al., 1979). One of the faces of the molecule of variant 3 includes a surface strip of nonpolar amino acid residues (Fontecilla-Camps et al., 1981). When the peptidic chain of the Css II toxin is superimposed on the tridimensional structure determined by Fontecilla-Camps et al., one finds a nonpolar surface, composed of Ala-45, Leu-17, Leu-19, Trp-47, Trp-58, Tyr-4, Tyr-40, and Tyr-42 residues, which are in contact with the solvent (Fontecilla-Camps, personal communication). This surface also contains a number of highly conserved residues among scorpion toxins including in particular Tyr-4 and Trp-47. It is possible to calculate, using Tanford's (1970) estimate, how these residues contribute to the heat capacity change of the binding reaction: the obtained value [−717 cal/(mol deg)] may represent the contribution of the toxin.

Polar interactions are also present in β-ScTx receptor recognition since protonation of a moiety is necessary for toxin binding to take place. Some monovalent (Li^+, guanidinium) and divalent cations, as well as increased ionic strength, inhibit β-ScTx binding by modifying its affinity for the receptor. This provides an additional argument in favor of the participation of polar interactions during recognition. Similarly, the modification of a least one lysine residue of the β-ScTx by succinimidyl [(4-azido-2-nitrophenyl)amino]acetate, which removes a positive charge, leads to a loss of affinity for the receptor (Darbon et al., 1983). In conclusion, these results show that the interaction of the β-ScTx with its receptor, thought to be a gating component of the sodium channel,

involves both hydrophobic and polar regions of the scorpion toxin.

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Registry No. Li, 7439-93-2; Ca, 7440-70-2; Mg, 7439-95-4; Mn, 7439-96-5; guanidinium, 25215-10-5.

References

Catterall, W. A. (1980) *Annu. Rev. Pharmacol. Toxicol. 20*, 15–43.
Catterall, W. A. (1982) *Cell (Cambridge, Mass.) 30*, 672–674.
Catterall, W. A., Morrow, C. S., & Hartshorne, R. P. (1979) *J. Biol. Chem. 254*, 11379–11387.
Couraud, F., Jover, E., Dubois, J. M., & Rochat, H. (1982) *Toxicon 20*, 9–16.
Darbon, H., Jover, E., Couraud, F., & Rochat, H. (1983) *Biochem. Biophys. Res. Commun. 115*, 415–422.
Edelhoch, M., & Osborne, J. C. (1976) *Adv. Protein Chem. 30*, 183–250.
Fontecilla-Camps, J. C., Almassy, R. J., Suddath, F. L., Watt, D. D., & Bugg, C. E. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 6496–6500.
Fontecilla-Camps, J. C., Almassy, R. J., Ealick, S. E., Suddath, F. L., Watt, D. D., Feldmann, R. J., & Bugg, C. E. (1981) *Trends Biochem. Sci. (Pers. Ed.) 6*, 291–296.
Fourcans, B., & Jain, M. K. (1974) *Adv. Lipid Res. 12*, 147–226.
Garcia, L. G. (1976) Thèse d'Université, Faculté des Sciences, Nice, France.
Gray, E. G., & Whittaker, V. P. (1962) *J. Anat. 96*, 79–88.
Jaimovich, E., Ildefonse, M., Barhanin, J., Rougier, O., & Lazdunski, M. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 3896–3900.
Jover, E., Martin-Moutot, N., Couraud, F., & Rochat, H. (1978) *Biochem. Biophys. Res. Commun. 85*, 377–382.
Jover, E., Couraud, F., & Rochat, H. (1980) *Biochem. Biophys. Res. Commun. 95*, 1607–1614.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. G. (1951) *J. Biol. Chem. 193*, 265–275.
Meves, H., Rubly, N., & Watt, D. (1982) *Pflügers Arch. 393*, 56–62.
Osborne, J. C., Palumbo, G., Brewer, H. F., & Edelhoch, M. (1976) *Biochemistry 15*, 317–320.
Rochat, H., Tessier, M., Miranda, F., & Lissitzky, S. (1977) *Anal. Biochem. 82*, 532–548.
Rochat, H., Bernard, P., & Couraud, C. (1979) *Adv. Cytopharmacol. 3*, 325–334.
Strickland, S., Palmer, G., & Massey, V. (1975) *J. Biol. Chem. 250*, 4048–4052.
Tamkun, M. M., & Catterall, W. A. (1981) *Mol. Pharmacol. 19*, 78–86.
Tanford, C. (1970) *Adv. Protein Chem. 24*, 1–95.
Waelbroeck, M., Van Oblerghen, E., & De Meyts (1979) *J. Biol. Chem. 254*, 7736–7740.
Weigele, J. B., & Barchi, R. L. (1978a) *FEBS Lett. 91*, 310–314.
Weigele, J. B., & Barchi, R. L. (1978b) *FEBS Lett. 95*, 49–53.
Weiland, G. A., & Molinoff, P. B. (1981) *Life Sci. 29*, 313–330.
Wheeler, K. P., Barhanin, J., & Lazdunski, M. (1982) *Biochemistry 21*, 5628–5634.

Properties of Curaremimetic Neurotoxin Binding Sites in the Rat Central Nervous System†

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ABSTRACT: Properties of mammalian central nervous system binding sites for curaremimetic neurotoxins are investigated with the Simonsen-Albino rat and ^{125}I-labeled α-bungarotoxin or the principal neurotoxin from *Naja naja siamensis*. Evidence is presented that high-affinity toxin binding sites are distributed as expected for a synaptic neurotransmitter receptor, display distinct nicotinic cholinergic pharmacology, and are sensitive to preincubation with nicotinic agonists. Affinity of toxin sites for agonists is altered by specific sulfhydryl/disulfide modification and by Ca^{2+}, and sites may be labeled with the nicotinic acetylcholine receptor affinity reagent bromoacetylcholine. New data are also presented indicating that toxin binding sites with $\bar{K}'$ values of ~1 nM and ~100 nM may be detected on rat brain crude mitochondrial fractions. Evidence is also reported suggesting the existence of two classes of toxin binding site disulfides/sulfhydryls, which interact with affinity reagents and nonspecific alkylating agents and are located proximal and distal, respectively, to the acetylcholine binding site. The results indicate that central nervous system (CNS) toxin binding sites share significant biochemical homology with nicotinic receptors from the periphery and provide a foundation for further study of toxin binding site biochemistry and the relationship between toxin sites and authentic CNS nicotinic acetylcholine receptors.

The introduction of curaremimetic neurotoxins as neuromuscular junction nicotinic receptor ligands was predicated upon demonstration of their antagonistic potency at that locus (Lee, 1972). Subsequent molecular characterization of nicotinic receptors from the electric organ of ray and eel relied heavily on application of snake neurotoxins (Karlin, 1980; Conti-Tronconi & Raftery, 1982).

Since the identification of acetylcholine as a neurotransmitter substance at the Renshaw cell [see Curtis & Eccles

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