Ionic Channels and Nerve Membrane Constituents

Tetrodotoxin-like interaction of saxitoxin with cholesterol monolayers

RAIMUNDO VILLEGAS and FLOR V. BARNOLA
From the Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela

ABSTRACT Saxitoxin (STX) and tetrodotoxin (TTX) have the same striking property of blocking the Na⁺ channels in the axolemma. Experiments with nerve plasma membrane components of the squid Dosidicus gigas have shown that TTX interacts with cholesterol monolayers. Similar experiments were carried out with STX. The effect of STX on the surface pressure–area diagrams of lipid monolayers and on the fluorescence emission spectra of sonicated nerve membranes was studied. The results indicate a TTX-like interaction of STX with cholesterol monolayers. The expansion of the monolayers caused by 10⁻⁴ M STX was 2.2 Å²/cholesterol molecule at 25°C. From surface pressure measurements at constant cholesterol area (39 Å²/molecule) in media with various STX concentrations, it was calculated that the STX/cholesterol surface concentration ratio is 0.54. The apparent dissociation constant of the STX-cholesterol monolayer complex is 4.0 × 10⁻⁷ M. The STX/cholesterol ratio and the apparent dissociation constant are similar to those determined for TTX. The presence of other lipids in the monolayers affects the STX-cholesterol association. The interactions of STX and TTX with cholesterol monolayers suggest (a) that cholesterol molecules may be part of the nerve membrane Na⁺ channels, or (b) that the toxin receptor at the nerve membrane shares similar chemical features with the cholesterol monolayers.

Following observation of the specific interaction between tetrodotoxin (TTX) and cholesterol monolayers (1), Dr. C. Y. Kao kindly suggested that we carry out similar experiments with saxitoxin (STX). STX and TTX are produced by quite different organisms and have different chemical structures, sharing only the presence of a guanidinium group (2–4). In spite of these facts, both molecules have the same striking property of blocking the Na⁺ channels in the axolemma (5–10). Na⁺ channels are the pathways normally used by this
ion to move through the excitable membrane during the nervous impulse and the transient early phase of conductance increase in voltage-clamped axons. We are grateful to Dr. E. J. Schantz, who kindly provided the sample of STX employed in this work.

The experiments to be described were carried out to explore the possible interaction of cholesterol and other constituents of squid nerve fiber plasma membranes with STX. The interactions were measured by the effect of STX on the surface pressure–area (π–A) diagrams of lipid monolayers and on the fluorescence emission spectra of sonicated nerve membrane suspensions.

The results indicate a specific interaction between cholesterol monolayers and STX, similar to that observed with TTX (1).

EXPERIMENTAL METHOD

Material

(a) Nerve fiber plasma membranes and their components, obtained from stellar nerves of the squid Dosidicus gigas, were used. The method of isolation of the plasma membranes as well as those of extraction and fractioning of their lipids have been described in detail (see reference 11). Two plasma membrane fractions were obtained at the end of the isolation procedure. Evidence has been presented to indicate that membrane fractions I and II correspond to plasma membranes of different cells: membrane fraction I to the axolemma, and membrane fraction II to the plasma membrane of the periaxonal cells, mainly of the Schwann cells. Membrane fraction I contains approximately 30% proteins and 70% lipids; membrane fraction II is made up of 48% proteins and 52% lipids. Membrane fraction I was used for the spectrophotofluorometric measurements, and the lipids extracted together from both membrane fractions were utilized in the surface pressure experiments. For some experiments the total lipids were separated into polar and nonpolar fractions. For a detailed description of the procedures and chemical composition of the plasma membranes, see reference 11.

(b) In some experiments cholesterol, cholesteryl acetate, cholesterol methyl ether, cholestanol, and purified egg lecithin (Sigma Chemical Co., St. Louis, Mo.) were used.

Surface Pressure Measurements

The surface pressure–area (π–A) diagrams of cholesterol, cholesterol derivatives, nerve membrane lipids, and egg lecithin–cholesterol mixtures were obtained using the Wilhelmy method (12). The surface pressure was continuously monitored by means of a sand-blasted platinum plate with a perimeter of 2.07 cm suspended from a Cahn RG automatic recording balance (Cahn Instrument Co., Paramount, Calif.). A Teflon trough of 40 ml capacity was used. As liquid subphase, a 0.15 M NaCl solution adjusted to pH 7.4 with a 7 × 10⁻³ M phosphate buffer was employed. The water used to prepare the solution was tridistilled in an all-glass apparatus in the presence of KMnO₄. The salts were analytical reagent grade. The area of the liquid-air interface was 54.97 cm². The temperature of the solution was kept constant at 25°C by circulating water around the trough.
The lipids, dissolved at concentrations of 0.44–1.64 mg/ml in chloroform-methanol (85:15 v/v), were spread in successive 0.5 or 1.0 μl additions to the liquid surface by means of a 25 or 50 μl Hamilton microsyringe (Hamilton Co., Whittier, Calif.) with a Hamilton PB600 dispenser attachment. The concentrations of the solutions of total lipids, polar lipids, nonpolar lipids, and of the individual lipids were established by dry weight determinations. Appropriate samples were placed in tared aluminum pans, the solvent was evaporated by placing the pans on a hot plate at 60°C, and the pans were then weighed in the Cahn RG electric recording balance with a sensitivity of 4 μg.

In order to investigate the effect of STX, the toxin was added to the subphase before spreading the lipids. STX was dissolved in distilled water at pH 2–3, containing 20% ethanol. An equivalent amount of ethanol was added to the subphase in the STX-free experiments.

**Spectrophotofluorometric Measurements**

The uncorrected fluorescence emission spectra of the nerve membrane proteins were obtained with an Aminco-Bowman spectrophotofluorometer (American Instrument Co., Inc., Silver Spring, Md.). The spectra were measured between 200 and 700 nm, using an excitation light source of 270 nm. Nerve membrane fraction I, isolated as described previously (11), was suspended in 0.15 M NaCl solution buffered at pH 7.4 with a 5 mM tris(hydroxymethyl)aminomethane (Tris)-HCl buffer. The ice-cold membrane suspension was sonicated with a Branson sonifier (model W185D, Heat Systems-Ultrasonics, Inc., Plainview, N.Y.) for 3 min. The spectra were measured using 1 ml of sonicated membrane suspension containing 10 μg of protein to which 2 μg of STX had been added. The protein content was measured by the method of Lowry et al. (13). STX was initially dissolved at a concentration of 100 μg/ml in distilled water, adjusted to pH 2–3 with HCl, containing 20% ethanol. An equivalent amount of ethanol was added in the STX-free experiments.

**RESULTS**

**Interaction of STX with Cholesterol Monolayers**

Fig. 1 shows the π-A diagrams of cholesterol monolayers spread on 10^{-6} M STX and STX-free subphases at 25°C. They reveal that STX produces expansion of the cholesterol monolayers. The expansion caused by STX at this concentration is 2.2 A²/molecule. The cholesterol areas used to calculate the expansion were those obtained by extending the linear portion of the π-A diagrams to intersect the x-axis.

**Dose-Response Experiments**

Fig. 2 shows the expansion of the cholesterol monolayers at various subphase concentrations of STX. The cholesterol areas used to calculate the expansion caused by each STX concentration were determined as described in the preceding paragraph. The data are approximated by a rectangular hyperbola.
calculated by assuming that the expansion is due to the association of individual STX molecules with a single type of site in the cholesterol monolayers. The sigmoid curve shown in Fig. 2 is a semilogarithmic plot of the rectangular hyperbolic function. The apparent dissociation constant of the cholesterol-STX complex used to calculate the curve was $4.0 \times 10^{-7}$ M.

**Figure 1.** Surface pressure–area diagrams of cholesterol monolayers spread on $10^{-4}$ M STX (solid circles) and STX-free subphases (open circles). Each value is the mean ± standard error of six (open circles) and four (solid circles) experimental measurements. The standard errors are covered by the circles. Subphase, 0.15 M NaCl solution; pH 7.4; temperature 25°C.

**Figure 2.** Dosage-response of cholesterol monolayers to STX. The expansion of cholesterol in square Ångstroms per molecule is plotted as a function of the molar concentration of STX in the liquid subphase. The apparent dissociation constant used to calculate the curve is $4.0 \times 10^{-7}$ M. The values represented were obtained from 22 STX experiments. Subphase, 0.15 M NaCl solution; pH 7.4; temperature 25°C.

### Surface Concentrations of STX and Cholesterol

Information on the surface concentration of STX at a cholesterol monolayer may be obtained by measuring the changes in surface pressure produced by various STX concentrations in the subphase. The approach followed is similar to that used by Guggenheim (14) to deal with the liquid-vapor interface. It is assumed (a) that the interfacial region contains cholesterol, STX, and water; (b) that each cholesterol area corresponds to a fixed cholesterol concentration at the interface; and (c) that at a constant cholesterol area a change in surface pressure produced by addition of STX to the subphase is due to a variation of the independent variable components (STX and water) in the interfacial region.

Following Guggenheim (14):

$$\left(\frac{1}{RT}\right)\frac{d\pi}{d\chi} = \left(\frac{\Gamma_s}{\chi} - \frac{\Gamma_l}{1 - \chi}\right) = \frac{I}{\chi(1 - \chi)}$$

(1)
in which \( R \) is the gas constant (8.3 \( \times \) 10\(^7\) erg (g mole\(^{-1}\) deg\(^{-1}\)); \( T \), the temperature in absolute degrees; \( d\pi \), the change in surface pressure due to change in mole fraction of STX \((d)\chi\); \( \chi \), the mole fraction of STX; \( A \), the area of cholesterol which is held constant; and \( \Gamma_1 \) and \( \Gamma_2 \) are the surface concentrations of water and STX, respectively, in moles per square centimeter. \( I \), which is defined by the expression \( I = (1 - \chi)\Gamma_3 - \chi\Gamma_1 \), may be replaced by \( \Gamma_3 \) when \( \chi \) is as small as 5.4 \( \times \) 10\(^{-8}\), the largest mole fraction of STX used in the present experiments. Thus, under the present experimental conditions, \( \Gamma_2 \), the surface concentration of STX corresponding to a fixed surface concentration or area of cholesterol, may be calculated from the following equation:

\[
\Gamma_2 = (\chi/RT)[(d\pi)/(d\chi)]_A
\]

which may be written as:

\[
\Gamma_2 \approx (\Delta\pi/RT \Delta \ln \chi)_A.
\]

It follows from equation 3 that the plot of \( \pi \) against \( \ln \chi \) should give a straight line. As shown in Fig. 3, the experimental values of \( \pi \) measured at 39 A\(^2\)/molecule and 25\(^\circ\)C plotted against \( \ln \chi \) indicate that in the present system a straight line is obtained only up to an STX mole fraction of 1.80 \( \times \) 10\(^{-8}\) (10\(^{-4}\) m STX concentration). At higher mole fractions of STX the line deviates markedly from linearity, perhaps due to the fact that completion of STX-cholesterol monolayer complex formation is being reached. The first part of the curve may be applied to calculate \( \Gamma_2 \) by means of equation 3. The slope of this part of the curve is 5.8 dynes/cm. \( \Gamma_2 \), the STX surface concentration thus calculated, is 2.3 \( \times \) 10\(^{-10}\) mole/cm\(^2\) or 1.4 \( \times \) 10\(^{14}\) STX molecules/cm\(^2\). It follows that the ratio of STX to cholesterol molecules in the monolayer, that can be obtained from values of \( \pi \) measured at 39 A\(^2\)/cholesterol molecule (2.6 \( \times \) 10\(^{14}\) molecules/cm\(^2\)), is 0.54. It should be noted that the
largest STX effect on the surface pressure of the cholesterol films is observed at 39 A²/molecule in the present experiments. The same calculations for TTX (R. Villegas, and F. V. Barnola, unpublished results) indicate that, under similar experimental conditions, the ratio of TTX to cholesterol molecules is about the same.

Experiments with Cholesterol Derivatives

The effect of STX on monolayers of cholesteryl acetate, cholesterol methyl ether, and cholestanol was studied. These experiments were carried out to

![Surface pressure-area diagrams of monolayers of (a) cholesteryl acetate and (b) cholesterol methyl ether, spread on 10⁻⁴ M STX (solid circles) and STX-free subphases (open circles). Each value is the mean ± standard error of eight experimental measurements for (a), and eight for (b). Subphase, 0.15 M NaCl solution; pH 7.4; temperature 25°C.](image)

![Surface pressure-area diagrams of monolayers of cholestanol spread on 10⁻⁴ M STX (solid circles) and STX-free subphases (open circles). Each value is the mean ± standard error of seven experimental measurements. Subphase, 0.15 M NaCl solution; pH 7.4; temperature 25°C.](image)
investigate whether the structural features of cholesterol required for its interaction with STX are similar to those previously determined for interaction with TTX (1).

Fig. 4 shows the $\pi-A$ diagrams of cholesteryl acetate and cholesterol methyl ether monolayers spread on $10^{-6}$ M STX and STX-free subphases at 25°C. The diagrams indicate that STX produces an expansion of the monolayers of both of these cholesterol derivatives.

Fig. 5 shows the $\pi-A$ diagrams of cholestanol monolayers also spread on $10^{-6}$ M STX and STX-free subphases at 25°C. The diagrams show that STX does not expand the cholestanol monolayer.

Experiments with Membrane Lipid Monolayers

The effect of STX on monolayers of total nerve plasma membrane lipids and their polar and nonpolar fractions was studied. The total lipids extracted from whole nerve plasma membranes were utilized. Total lipids of nerve membrane fraction I were used in some experiments.

Fig. 6 shows the $\pi-A$ diagrams of monolayers of total lipids and the polar and nonpolar fractions spread on $10^{-6}$ M STX and STX-free subphases at 25°C. The diagrams indicate that STX produces expansion of the nonpolar lipid monolayer but, in contrast, does not appear to affect the total and polar lipid monolayers. The same results were obtained with total lipids of nerve membrane fraction I as with total lipids of whole nerve plasma membranes.

![Figure 6](https://example.com/figure6.png)

**Figure 6.** Surface pressure–area diagrams of monolayers of (a) the total lipids extracted from whole nerve fiber plasma membranes, and those corresponding to their (b) polar, and (c) nonpolar fractions, spread on $10^{-6}$ M STX (solid circles) and STX-free subphases (open circles). Each value represents the mean ± standard error of eight experimental measurements for (a), eight for (b), and six for (c). Subphase, 0.15 M NaCl solution; pH 7.4; temperature 25°C.
Experiments with Mixed Lecithin-Cholesterol Monolayers

The results obtained with total lipid monolayers suggest that the cholesterol-STX interaction is affected by the presence of other types of lipid molecules in the monolayer.

Experiments with films of purified egg lecithin and three different egg lecithin-cholesterol mixtures were performed. The fractional molar concentrations of lecithin in the lecithin-cholesterol mixtures were 0.20, 0.33, and 0.60.

Fig. 7 shows the \( \pi - A \) diagrams of the lecithin-cholesterol mixtures and
pure lecithin films spread on $10^{-6}$ M STX and STX-free subphases at 25°C. The diagrams reveal that the increase in the fractional molar concentration of lecithin diminishes the expansion of the cholesterol monolayers caused by STX. Fig. 7 shows that STX expands only the monolayer prepared with a mixture of 0.8:0.2 cholesterol/lecithin molecules.

**Spectrophotofluorometric Experiments with STX and TTX**

The fluorescence spectra of sonicated nerve membrane fraction I was obtained as described under Methods. It was found that the addition of 2 µg of STX to 33 µg of nerve membrane fraction I (10 µg of membrane protein) suspended in 1 ml of 0.15 M NaCl solution at pH 7.4, does not change the emission of the aromatic amino acid residues. Similar experiments carried out with TTX produced similar results.

**DISCUSSION**

The experiments described indicate a TTX-like interaction of STX with cholesterol monolayers. As shown in Fig. 1, the interaction is revealed by an expansion of the cholesterol monolayer.

Dose-response experiments, similar to those carried out with TTX (1), show that the increment in area per cholesterol molecule is a function of the STX concentration in the subphase. As shown in Fig. 2, the STX dose-response data are approximated by a rectangular hyperbola calculated by assuming an apparent dissociation constant of $4.0 \times 10^{-7}$ M for the STX-cholesterol monolayer complex. The value of the apparent dissociation constant for the STX-cholesterol monolayer complex is similar to that determined for the TTX-cholesterol monolayer complex ($2.6 \times 10^{-7}$ M) in previous work (1). However, they are both about $10^2$ times higher than those calculated by Hille (8) for STX and TTX with frog nerve membrane, and by Cuervo and Adelman (15) for TTX with the squid axon membrane. The different dissociation constants for the toxin-cholesterol monolayer and the toxin-nerve membrane receptor complexes may be due to differences between the monolayer and toxin binding site of the nerve membrane systems. It should be noted that the values calculated for the cholesterol-toxin (STX and TTX) complexes are extremely low, indicating a very specific interaction.

As shown in Fig. 2 of the present work for STX and Fig. 7 of reference 1 for TTX, the largest expansion caused by the toxins is 2–2.5 A²/cholesterol molecule. The small increment in the steroid area caused by STX and TTX, which have areas, measured from molecular models, larger than that for cholesterol, indicates that there is no penetration of the toxins into the monolayer. It appears more likely that the increase in area is due to a change in the arrangement of the cholesterol molecules caused by association of the toxin with the monolayer.
The simplest interpretation of the dose-response curve is that one STX molecule interacts with a single type of site in the cholesterol monolayer, as has been proposed for the cholesterol-TTX interaction (1). The site might be formed by two cholesterol molecules, because the largest surface concentration ratios of cholesterol to STX and to TTX are approximately 2. At an area of 39 Å²/molecule the largest effect of the toxins on the monolayer is observed, suggesting that, at this area, the cholesterol monolayer presents the most favorable distribution of sites for the interaction. Thus, the interaction is a feature of the cholesterol monolayer rather than of the individual cholesterol molecule itself.

The apparent dissociation constants of the STX-cholesterol and TTX-cholesterol monolayer complexes indicate that the standard free energies of association of the toxins with the monolayer sites are about -9.0 kcal/mole. These values, while large, are well within the range of those determined by noncovalent interactions (16). The driving force for the association may be, in part, a charge-dipole interaction between the guanidinium group of the toxins and the hydroxyl groups at the monolayer. However, short-range nonpolar forces may also make a major contribution to the free energy of association. This interpretation is consistent with the specificity of the toxin-monolayer association, since short-range interactions require a high degree of steric compatibility among the molecules involved.

The interaction between cholesterol and the toxins is rather specific. Experiments carried out with STX suggest that the structural features of the steroid required for the interaction may be similar but not necessarily identical to those determined for its interaction with TTX. The requirements are a partial negative charge at C₅ and a double bond between C₅ and C₆. These were established by studying the association of several cholesterol derivatives with TTX (1).

The interaction between the steroid monolayer and STX is not sensitive to changes in the polarity of the substituent at C₄, at least those occurring among the alcohol, acetate ester, and methyl ether groups. On the other hand, the TTX experiments indicate that this toxin interacts with cholesteryl acetate but not with cholesterol methyl ether. It follows, therefore, that the association between steroids and TTX is more sensitive to changes in the polarity at C₄ than is the interaction between steroids and STX.

The importance of the double bond between C₅ and C₆ for the interaction with STX is indicated by the lack of effect of STX on the cholestanol monolayer. A similar result was observed in cholestanol experiments with TTX. It is possible that by establishing certain stereochemical relationships within the steroid nucleus the double bond between C₅ and C₆ provides a correct conformation of the molecules of the monolayers required for the interaction with STX. It is also possible that the unsaturation would have an inductive effect that would favor the localization of a partial negative charge at C₅.
The lack of knowledge about the nature of the STX-cholesterol monolayer association precludes any further interpretation of this interaction. It should be noted that STX and TTX share a guanidinium group (2–4). Since the membrane is permeable to the guanidinium ion, this is the part of the molecule most likely to produce a direct block of the Na current by reducing the number of Na\(^+\) channels.

Differences have been observed between the results of the STX and TTX experiments with the total nerve plasma membrane lipids and the polar and nonpolar fractions. STX produces only a small expansion of the nonpolar lipid monolayer, while TTX expands the total and nonpolar lipid films as well.

![Figure 8. Egg lecithin–cholesterol mixed monolayers. Area per molecule versus lecithin mole fraction. The values (solid circles) were obtained from the diagrams of the monolayers in STX-free subphases shown in Figs. 1 and 7 by extending the linear portion of the \(\pi-A\) diagrams to intersect the x-axis. Experimental (---), and theoretical (-----) lines.](image)

The lack of interaction of STX with the total lipid monolayer, which contains about 25% by weight of cholesterol, is considered to be due to the presence of polar lipids. Experiments with egg lecithin–cholesterol mixtures support this interpretation.

As shown in Fig. 7, a decrease in the expansion of the egg lecithin–cholesterol monolayers occurs when the lecithin molar fraction in the mixture is augmented. Fig. 8 shows the theoretical and experimental areas of the molecules in the lecithin–cholesterol mixed films as a function of the molar fraction of lecithin. It may be observed that the experimental areas for the mixtures are somewhat smaller than the theoretical ones, in all probability due to interaction of the two molecular species composing the film (17–19).

Shah and Schulman (19) have proposed that the apparent condensation of the lecithin–cholesterol films can be explained by the existence of molecular cavities, the insertion of cholesterol into the cavities causing the expected...
proportional increase in the mixed monolayer area to diminish. It is tempting to suggest that this type of lecithin–cholesterol association precludes the cholesterol-STX interaction in the mixed film. This may also be the case for the STX experiments with membrane total lipids. Likewise, the small effect of STX on the monolayers of nonpolar lipid mixtures could also be a consequence of a particular mode of fatty acid–cholesterol association.

The difference between the results obtained with STX and with TTX in the lipid mixtures containing cholesterol may also be due to their different chemical structures, which may either preclude or facilitate the cholesterol-toxin interaction in the mixed lipid films. It is also possible that the other lipids could affect the cholesterol-STX and cholesterol-TTX interactions in the same way, although the magnitude of the effect would be different.

As described under Results, the fluorescence emission of 33 μg of nerve membrane fraction I (10 μg of membrane protein) suspended in 1 ml of 0.15 M NaCl solution at pH 7.4 is not affected by the addition of 2 μg of STX or TTX. Taking the density and thickness of membrane fraction I as 1.09 g/cm² and 100 Å, respectively (11), it can be calculated that the total surface area of the 33 μg of membrane is 30.3 cm². Assuming that there are 13 toxin receptor sites per square micron at one membrane face only (20), the total number of receptor sites in 33 μg of membrane is 4.0 × 10¹⁰. Since the number of molecules in 2 μg of STX (mol wt STX 2 HCl = 370) is 3.3 × 10¹⁰, it is estimated that there are about 8.5 × 10⁴ STX molecules per membrane receptor site. Furthermore, taking an average molecular weight of 120 for the membrane amino acids and assuming that only one out of 20 is aromatic, it is estimated that the ratio of STX molecules to aromatic amino acids in the present experiments is 1.3. These, and similar calculations with TTX, suggest that the number of toxin molecules (STX or TTX) added was larger than those of receptor sites or aromatic amino acids present in the membrane suspension. Thus, the fluorescence measurements may be considered to indicate that there is no general interaction between the toxins and the aromatic residues of the membrane proteins. However, it should be pointed out that these fluorescence experiments are inadequate to detect a specific effect of the toxin on the receptors only, even if the interaction with each receptor would affect 1–100 aromatic amino acids. In such a hypothetical case, following the previous calculations, there would be about 10¹⁴–10¹⁵ unaffected aromatic residues, in addition to those located in the receptors, which would mask the change in emission of the affected ones. It may be relevant to point out that DDT, which delays the turning-off process of the Na current in frog and lobster axons (8, 21), and also reduces the steady-state current in the lobster axons (21), reduces the fluorescence intensity of the aromatic amino acids of the membrane suspensions (22).

The spectrophotofluorometric experiments with STX and TTX do not
permit the exclusion of other types of interactions between the toxins and the membrane proteins.

The fact that STX and TTX interact with cholesterol monolayers may be useful for the understanding of nerve function. If the interaction of the toxins with the nerve membranes is similar to that observed with the monolayers, it may depend to a higher degree on the arrangement than on the number of cholesterol molecules in the axolemma. If cholesterol is a constituent of the STX and TTX receptor in the axolemma, and the Na+ channels are relatively few per unit membrane area (20, 23), only a few molecules of cholesterol are required to take part in Na+ channel function. However, it must be borne in mind that there is a possibility that the actual receptor site in the axolemma is formed for other molecules with a pattern of partial charged groups similar to that present in the steroid monolayers, in which the interaction with the STX and TTX has been observed.

The authors are deeply indebted to Dr. C. Y. Kao who kindly suggested the investigation of the cholesterol-STX interaction; to Dr. E. J. Schantz for his generous gift of STX; to Mr. P. Freund for his collaboration; to Dr. G. Camejo for his suggestions and discussions; to Dr. E. H. Cordes, Dr. Bertil Hille, and Dr. M. Garcia-S. for their critical reading of the manuscript; to Ing. J. Whittembury for his supply of the squid nerve fibers; to Mrs. C. Garcia, G. Cardona, and S. Eljuri; to Messrs. J. Mora and A. Barranco for their assistance in the preparation of the manuscript.

Received for publication 5 April 1971.

REFERENCES

vertical type for biological and chemical work, and a theoretical and experimental comparison with the horizontal type. II. Tight packing of a monolayer by ions. J. Amer. Chem. Soc. 59:2189.


