Proc. Natl. Acad. Sci. USA
Vol. 77, No. 11, pp. 6884–6888, November 1980
Neurobiology

Molecular characterization, reconstitution, and "transport-specific fractionation" of the saxitoxin binding protein/Na^+ gate of mammalian brain

(excitable membranes/artificial lipid vesicles/action potential/purification)

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Communicated by David H. Hubel, July 21, 1980

ABSTRACT **The saxitoxin (STX) binding protein has been solubilized by sodium cholate, both from axolemma and from synaptosomal membranes of mammalian brain. On the basis of agarose gel filtration and sedimentation properties in H_2O and ^2H_2O, the solubilized particle has the following molecular properties: Stokes radius, 120 Å; partial specific volume, 0.85 cm^3/g; mass, 1,020,000 daltons; frictional ratio f/f_0, 1.6. The solubilized STX binding protein was incorporated into unilamellar (≈550-Å) artificial phosphatidylcholine vesicles. Based on the expectation that the STX binding protein contains functional monovalent cation gating activity ("action potential Na^+ gate") that can be activated by veratridine and inhibited by tetrodotoxin, a strategy was devised for partial purification of the reconstituted sodium gate/STX binding protein by "transport-specific fractionation." When the entire vesicle population was preloaded with 0.4 M cesium ion, addition of veratridine allowed Cs^+ efflux from specifically those vesicles containing the ion gate; the concomitant reduction in intravesicular density permitted the ion gate/STX binding protein to be fractionated on density gradients. These observations demonstrate functional reconstitution and partial (30- to 50-fold) purification of the STX binding protein/Na^+ gate of mammalian brain.**

The primary axonal action potential consists of an inward movement of sodium ion followed by an outward movement of potassium ion (1). The Na^+ gating component of the action potential is specifically blocked by the potent poisons tetrodotoxin (TTX) and saxitoxin (STX) at low concentrations (K_i = 1–10 nM) (2). The dissociation constants and binding kinetics derived from studies with these radiolabeled toxins are similar to those derived from electrophysiological and $^{22}Na^+$ flux studies on TTX and STX inactivation of Na^+ gating. This suggests that the binding sites for the radioligands are the sites for inactivation of the Na^+ gate (2, 3). Binding of STX to axonal membrane and to synaptosomes (pinched-off nerve endings) has been characterized (4, 5). Although much progress has been made towards purification of detergent-solubilized STX/TTX binding proteins from electric eel (6) and muscle (7), the precise molecular weight of a detergent-solubilized STX/TTX binding protein is unknown. More significantly, ion gating activity by the solubilized binding protein reconstituted into artificial vesicles has yet to be demonstrated: the relationship of the binding protein to the ion gate is as yet undefined.

We report here the quantitative molecular properties of the cholate-solubilized STX binding protein of mammalian brain. We have reconstituted the solubilized STX binding protein into artificial vesicles and have partially purified the protein by "transport-specific fractionation." This approach (8, 9) involves reconstitution of the transport system of interest into artificial vesicles before purification so as to insert only one or, at most, a few membrane proteins into each artificial vesicle. The transport properties of the protein of interest are then used as a physical tool (e.g., transport-specific changes in vesicle density are created) to separate vesicles containing the transport system from the rest of the crude preparation and thus result in their purification. Because reconstitution of transport activity is *required* for the viability of this approach, we have concomitantly demonstrated preservation of veratridine-activated TTX-inhibited ion gating by the solubilized STX binding protein after it is incorporated into lipid vesicles.

METHODS

Preparation of Membranes. All procedures were at 0–4°C unless otherwise specified. Axolemma was prepared from bovine brain as described (5). Synaptosomal membranes were prepared by a procedure derived from the observations of Catterall (4). Gray matter from freshly killed calves (10–20 g) was rinsed in 0.32 M sucrose/0.5 mM Tris·HCl/0.1 mM phenylmethanesulfonyl fluoride, pH 7.4, and homogenized in 9 ml of the same solution per g of tissue with 10 strokes of a size B Teflon pestle homogenizer. The homogenate was centrifuged at 7500 × *g* for 10 min in a Sorvall SS-34 rotor; the supernatant was then centrifuged for 60 min at 14,000 × *g* as above. The resulting pellets were homogenized (four strokes, as above) in 18 ml of 5 mM Tris·HCl, pH 8.1, per g of initial gray matter. Solid sucrose was then dissolved in the medium to a sucrose concentration of 15% (wt/vol). The resulting lysed crude synaptosomal mixture was placed (22 ml per tube) in sealable tubes for the Beckman 50.2 Ti rotor; a 3-ml 32.5% sucrose cushion was added. After centrifugation at 150,000 × *g* for 50 min the band at the 32.5%/15% sucrose interface was collected and diluted to ≈0.5 mg of protein per ml with 10% (vol/vol) glycerol, 10 mM Tris·HCl, pH 7.0. The yield was 3–5 mg/g of tissue, with STX binding activity of 5–10 pmol/mg. Protein assays were by a modified Lowry method (10). [^{3}H]STX was prepared and binding assays were performed by rapid ion exchange as described (5), with Hydrofluor (National Diagnostics, Somerville, NJ) substituted for Aquasol in scintillation counting.

Solubilization of STX Binding Protein. A suspension of cholic acid (Sigma) at 31 mg/ml, 125 mM NaCl, 25 mM Tris, 37.5 mM $MgCl_2$, 0.5 mM EDTA, 5 mM 2-mercaptoethanol was prepared; the cholic acid suspension was dissolved by titration to pH 7.4 with 6 M NaOH. Egg phosphatidylcholine (prepared as in ref. 8) from a 125 mg/ml aqueous suspension in 5 mM 2-mercaptoethanol was added to the 31 mg/ml cholate solution to a final concentration of 25 mg/ml. The suspension was clarified by incubation for 1 hr at room temperature, then

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Abbreviations: TTX, tetrodotoxin; STX, saxitoxin.

chilled to 0°C. Total lipid phosphate was determined by the method of Ames (11). Axolemma or synaptosomal membranes were pelleted at 70,000 × *g* for 30 min in a Beckman 50 Ti rotor. The pooled pellets were rapidly resuspended by mechanical homogenization in an A Teflon pestle homogenizer (four strokes) to a final protein concentration of 1 mg/ml in the above solution. The homogenizer was kept immersed in ice water to minimize frictional heating. The resulting suspension was incubated for 5 min on ice, then spun at 25,000 rpm for 8 min at 2°C in the 50 Ti rotor. EDTA from a 1.0 M stock solution, pH 8.0 (titrated with NaOH), was added to the optically clear supernatant to a final concentration of 60 mM. This supernatant solution contained 20–30% of the initial STX binding activity and 70–85% of the initial membrane protein.

Reconstitution of the STX Binding Protein/Ion Gate into Artificial Vesicles. The above supernatant was immediately subjected to overnight (14–15 hr) hollow fiber dialysis (9, 10). The dialysate (400 ml/ml of supernatant) was 100 mM NaCl/5 mM 2-mercaptoethanol/4 mM EDTA/10 mM Tris·HCl, pH 7.0. The resulting vesicle preparation was used for further characterization and purification as described. Typically, 9 ml of vesicles was produced per batch, although the procedure could be substantially scaled up (50 ml per batch) by the use of C. DAK 0.6 D artificial kidney units (12) (Cordis Dow, Miami, FL) for dialysis. After dialysis, 30–50% of the binding activity in the supernatant was recovered.

Sucrose Density Gradient Ultracentrifugation. Linear ^1H_2O gradients (7–17% sucrose), and ^2H_2O gradients (0–10% sucrose) were poured in 5-ml cellulose nitrate ultracentrifugation tubes. Gradients contained 50 mM KCl, 50 mM imidazole, 5 mM 2-mercaptoethanol, 2.2 mM EDTA, cholic acid at 2 mg/ml, and phosphatidylcholine at 0.5 mg/ml. Synaptosomal membranes were solubilized with cholate/lipid as above. Supernatant, 0.7 ml, was layered onto the gradients. The calibrating proteins were layered on parallel gradients as a 1 mg/ml solution as above. Centrifugation was in a Beckman SW 65 rotor, 50,000 rpm at 4°C, 7.5 hr for ^1H_2O gradients and 2.7 hrs for ^2H_2O gradients; 0.33-ml fractions were collected and assayed for [^{3}H]STX binding. Water-soluble proteins for calibration included bovine IgG (Sigma), $s_{20,w}$ = 7.0 S (13); bovine liver L-glutamate dehydrogenase (Sigma), 11.45 S (14); and human IgM (Cappel Labs, Cochranville, PA), 19 S (13).

RESULTS

Properties of the Cholate-Solubilized STX Binding Protein. As shown in Fig. 1, the STX binding activity, cholate-solubilized in the presence of phosphatidylcholine, elutes from a Sepharose 4B gel filtration column at a position corresponding to a mean Stokes radius (16) of ≈120 Å. In order to determine the molecular weight and the degree of asymmetry (frictional ratio, f/f_0) of the solubilized particle, the partial specific volume ($\bar{v}$) must be determined; the lipid/cholate-solubilized STX binding protein may have a $\bar{v}$ substantially higher than that (≈0.74 cm^3/g) typical for most soluble proteins (13), due to the binding of substantial quantities of the less dense phosphatidylcholine ($\bar{v}$ = 0.98 cm^3/g; cf. ref. 14). Determination of $\bar{v}$ entailed the comparison of the sedimentation behavior of the solubilized STX binding protein in ^1H_2O vs. ^2H_2O sucrose gradients, a method employed by others studying solubilized membrane proteins (16, 17). The solubilized STX binding protein behaved as would a water-soluble protein of 11.8 S in H_2O/sucrose gradients, but—presumably due to bound lipid—behaved as would a water-soluble protein of 7.1 S on the denser ^2H_2O/sucrose gradients. The $\bar{v}$ thus calculated was 0.85 cm^3/g, yielding a molecular weight of 1,020,000 (±8%) for the solubilized particle, and an f/f_0 of 1.6.

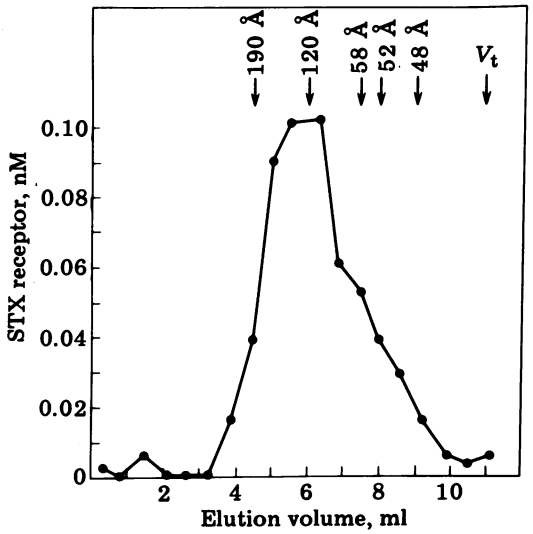

FIG. 1. Sepharose 4B elution profile of the solubilized STX binding activity. Synaptosomal membranes were solubilized with cholate/lipid. Supernatant, 0.7 ml, was loaded onto a 23 × 0.94 cm column of Sepharose 4B (Pharmacia) that had been preequilibrated at 4°C with 50 mM KCl/50 mM imidazole/5 mM 2-mercaptoethanol/2.2 mM EDTA/2 mg of cholic acid per ml/0.5 mg of phosphatidylcholine per ml. Column flow rate was 2.4 ml/hr. The [^{3}H]STX binding assay was performed on 250-μl aliquots of fractions in the presence and absence of 1 μM unlabeled STX. Recovery of binding activity was 98%. The column was calibrated as described (15) with uniform latex particles, Stokes radius = 190 Å, and "Huangosomes," 120 Å. Additional protein calibration standards were ferritin, 58 Å (Boehringer Mannheim); bovine IgG, 52 Å (Sigma); human plasminogen, 48 Å (Worthington). Each protein was loaded as a 1 mg/ml solution in 0.7 ml of elution buffer. V_t, total volume.

Assuming that the solubilized particle is composed primarily of protein, cholate, and phospholipid, it can be estimated from the experimentally determined $\bar{v}$ that about 46% of the mass of the particle is phospholipid. The amount of cholate in the particle cannot be readily estimated because the $\bar{v}$ of cholate, 0.75 cm^3/g (18), is very similar to that of a water-soluble protein. Thus, a firm *upper* limit for the molecular weight of the protein component of the solubilized particle is 560,000.

Incorporation of the STX Binding Protein into Artificial Phospholipid Vesicles. Brain axolemma or synaptosomal membrane, solubilized with a mixture of cholate and phosphatidylcholine, was subjected to hollow-fiber dialysis (9, 12); the resulting detergent removal caused formation of small unilammellar lipid vesicles with incorporated proteins, as shown in studies on reconstitution of other transport systems (8–10). In this case, 20–35% (n = 22, mean 28%) of the STX binding activity of either axolemma or synaptic membranes reproducibly remains after cholate removal. The agarose gel filtration column profile of STX binding activity (Fig. 2) precisely parallels that of vesicle phospholipid (mean vesicle diameter, 550 Å). In contrast to the rapid decay of STX binding activity observed in the initial solubilized mixture (half-life ≈ 2 hr), the binding activity after cholate dialysis is quantitatively stabilized for at least several days (Fig. 3). After cholate/dialysis, the saturable STX binding component is comparable in STX dissociation constant, as measured in the 100 mM Na^+ dialysis buffer, to that of the starting material (0.95 and 0.8 nM, respectively).

Fig. 4 shows an isopycnic density gradient profile of vesicle phospholipid and STX binding activity. The STX binding activity on this shallow gradient is closely associated with the lipid vesicles. But unlike the gel filtration column profile, there is some skewing of the density of STX binding activity with re-

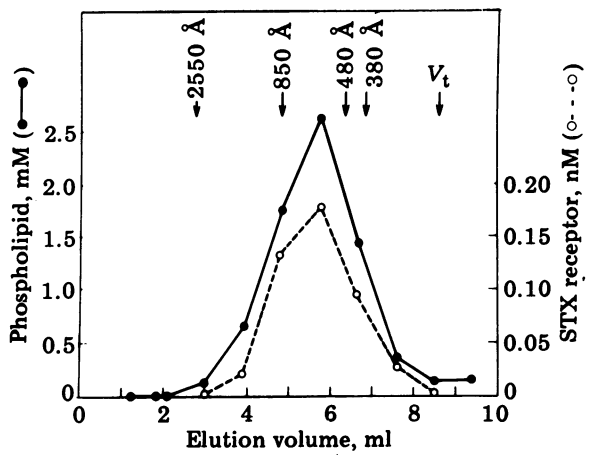

FIG. 2. Bio-Gel A-150m (Bio-Rad) agarose gel filtration profile of artificial vesicle phospholipid and axolemma STX receptor binding activity after reconstitution by cholate/dialysis. The column was equilibrated at 4°C with 50 mM KCl/50 mM imidazole/5 mM 2-mercaptoethanol/700 mM glycerol/1 mM EDTA, pH 6.5. Vesicles, 0.7 ml, were loaded on the column. Operation and calibration of the column are described in ref. 15. Recovery of phospholipid, 93%; recovery of [^{3}H]STX binding activity (300-μl aliquots assayed in the presence or absence of M unlabeled STX), 109%.

spect to that of vesicle phospholipid: the mean isopycnic density of STX binding activity is reproducably (n = 5) about 0.008 g/cm^3 higher than the mean density of the artificial vesicles. A likely reason for this relates to the rather high observed molecular weight of the solubilized STX binding component: a 550-Å unilamellar vesicle has $\approx 10^7$ daltons of phospholipid (10) of density $\approx$1.02 g/cm^3 (20); a vesicle containing $\approx$400,000 daltons more protein (density 1.25 g/cm^3) than the average vesicle protein content would have a calculated density that is higher by approximately the value observed. The column and gradient profiles of the STX binding activity after reconstitution were similar whether axolemma or synaptic membrane was the source of starting material.

In contrast to the above, STX binding activity associated with axolemma or synaptic membrane eluted in the void of the agarose column and sedimented to a much higher isopycnic density (>1.10 g/cm^3) when the membranes were not subjected to the reconstitution procedure. These observations—analogous to those made in our laboratory on a reconstituted synaptosomal Ca^{2+} transport system (9)—demonstrate that the STX binding

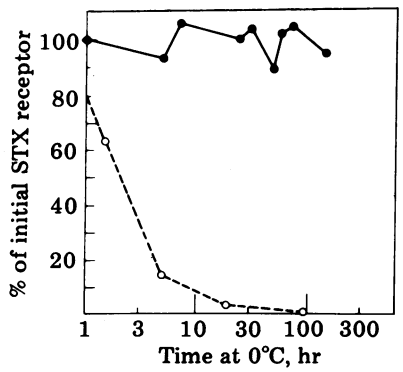

FIG. 3. Retention of [^{3}H]STX receptor binding as a function of time. Synaptosomal membranes were solubilized with cholate/lipid. The fraction of the initial [^{3}H]STX binding activity (○) was determined on 100-μl aliquots after incubation for the indicated time intervals. In parallel, a portion of these solubilized synaptosomal membranes was subjected to the reconstitution procedure. The fraction of [^{3}H]STX binding as a function of time (●) was determined, using 100-μl aliquots of the resulting vesicle preparation.

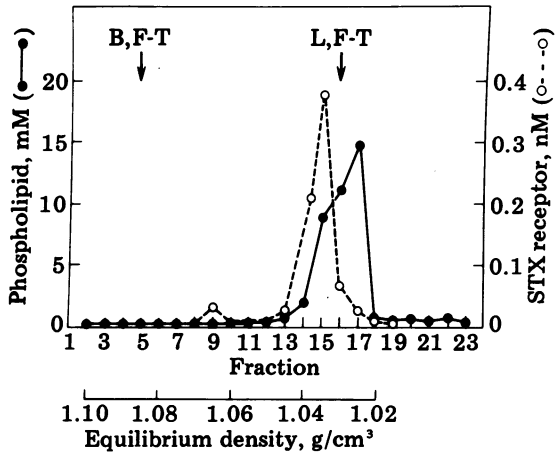

FIG. 4. Isopycnic density gradient profiles of [^{3}H]STX binding (○) and vesicle phospholipid (●) of reconstituted material. Axolemma was solubilized and subjected to cholate/dialysis. Eight milliliters of the resulting vesicle preparation was layered onto chilled linear gradients formed in tubes for the Beckman VTI50 rotor from 14.5 ml of 700 mM glycerol (light phase) and 15.5 ml of 700 mM sucrose (heavy phase), over a 2.5-ml 50% sucrose cushion. All gradient solutions contained 50 mM potassium acetate, 50 mM imidazole, and 5 mM 2-mercaptoethanol at pH 6.5. After centrifugation at 50,000 rpm for 90 min, 1.5-ml fractions were collected and assayed for [^{3}H]STX binding (200 μl, ± μM STX), and lipid phosphate. B,F-T and L,F-T denote the positions of the main peaks of STX binding activity and vesicle phospholipid, respectively, of lobster axolemma (prepared as for ref. 19) subjected to the freeze/thaw-sonication procedure (19); see text for details.

component has indeed been translocated into the unilammellar artificial vesicles that result from the cholate dialysis method. We explored in parallel the use of the freeze/thaw-sonication technique (21) for reconstitution of the STX binding activity. We were able to confirm the findings of Villegas *et al.* (19) that this manipulation, when applied to lobster nerve membranes, resulted in enhancement of observed veratridine-stimulated TTX-inhibitable Na^+ uptake (data not shown). However, the isopycnic gradient profile of the STX binding activity after this freeze/thaw-sonication showed that it migrated as a distinct peak to a substantially higher density ($\approx$1.09 g/cm^3) than the bulk of the artificial vesicle phospholipid, as denoted by the arrows in Fig. 4.

Transport-Specific Fractionation of the Reconstituted STX Binding Protein/Ion Gate. The artificial vesicles containing reconstituted synaptic membrane protein were rendered temporarily permeable to Cs^+ by adding a small amount of the detergent Lubrol. The Lubrol was subsequently removed by treatment with Bio-Beads (21), enabling preloading of the reconstituted vesicles with 350 mM cesium acetate (CsOAc). Most of the intravesicularly trapped Cs^+ remains inside, even after extended incubation at 4°C (Fig. 5). Intravesicular Cs^+ is immediately discharged by addition of valinomycin, an ionophore that—due to its high selectivity for *both* Cs^+ and K^+ (22)—should produce rapid Cs^+/K^+ exchange.

The above observations provided the impetus for designing a gradient system for separating these artificial vesicles on the basis of their density, which can be varied by controlling intravesicular Cs^+ content. Fig. 6*A* shows the behavior of the reconstituted vesicle preparation on such a gradient. CsOAc-preloaded vesicles migrate to a mean density of 1.04–1.05 g/cm^3. When valinomycin is added to the CsOAc-preloaded vesicles, the concomitant discharge of CsOAc causes the vesicles to remain near the top of the gradient (mean density 1.025 g/cm^3). In contrast, addition of veratridine (0.25 mM) does not significantly change the distribution of vesicle phospholipid

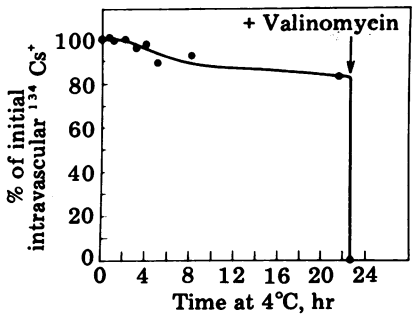

FIG. 5. Retention of Cs^+ by artificial vesicles. Synaptosomal membranes were solubilized and subjected to cholate/dialysis. Imidazole was added to a final concentration of 27 mM from a 0.7 M, pH 6.0, stock solution. Cesium acetate (Alfa) containing $^{134}Cs^+$ (New England Nuclear), 5×10^7 cpm/ml, was then added from a 50% wt/vol stock solution to a final concentration of 400 mM. Lubrol PX (Sigma) was added to 0.02% from a 1% stock solution, and the vesicle preparation was incubated at 0°C for 1 hr. This permeabilized vesicle preparation was then stirred overnight at 0°C with Bio-Beads (Bio-Rad) at 0.4 g/ml, prepared as in ref. 21. The resulting Cs^+-preloaded vesicles were separated from the Bio-Beads by filtration through glass wool in the tip of a pasteur pipette. Two milliliters of the vesicles was exchanged into 350 mM potassium acetate/50 mM imidazole/5 mM 2-mercaptoethanol, pH 6.5, on a 10-ml column of Sephadex G-50 fine (see ref. 22). Intravesicular $^{134}Cs^+$ content was then measured as a function of time (●) in 100-μl aliquots of the exchanged vesicles, using the Sephadex column assay (10). The arrow indicates the intravesicular $^{134}Cs^+$ content assayed 2 min after addition of valinomycin (1:100 dilution from a 0.2 mg/ml stock solution in ethanol).

with respect to the untreated control, indicating that veratridine—which is known to open the action potential Na^+ gate—does *not* have a significant nonspecific ionophoric effect on the vast majority of the reconstituted vesicles.

After veratridine treatment, the behavior of vesicles containing the reconstituted STX binding component differs in an important respect from that of the bulk of the vesicle preparation. Fig. 6*B* shows the distribution of the STX binding component measured on the same set of three gradients. Despite the fact that addition of veratridine does *not* affect the density of the *majority* of the vesicles, those vesicles that contain the STX binding component *are* shifted toward the top of the gradient by veratridine addition; this is presumably because veratridine *selectively* opens a pore associated with the STX binding protein, allowing sufficient Cs^+/K^+ exchange to cause the density shift. As shown, in the untreated vesicle preparation most of the STX binding activity is in the denser region of the gradient; addition of valinomycin causes the vesicles containing the STX binding protein to remain near the top of the gradient with the rest of the vesicle preparation, presumably by generating a Cs^+/K^+ exchange pathway in parallel that is independent of the activation of any neuronal ion gate. Vesicles preincubated with veratridine as above, but in the presence of 30 μM TTX, exhibited similar profiles of phospholipid and STX binding activity—on gradients containing 30 μM TTX (not shown)—to those exhibited by vesicles treated with neither drug (Fig. 6). This demonstrates TTX blockage of the effect of veratridine. The recovery of STX binding activity after the repeated pelleting and washing of the gradient fractions necessary to remove the TTX (see legend, Fig. 6) was about 1/4 of that in the three sets of gradients shown in Fig. 6.

The first gradient (Fig. 4) generated a 6- to 8-fold purification of the STX binding protein over the initial specific activity (0.6–0.9 pmol/mg) of the reconstituted vesicle preparation. Measurement of the specific activity of STX binding of the top 2/3 of the STX binding peak corresponding to +VTN (veratridine) of Fig. 6*B* showed a 30- to 50-fold final purification, with yield averaging 26% ($n = 4$).

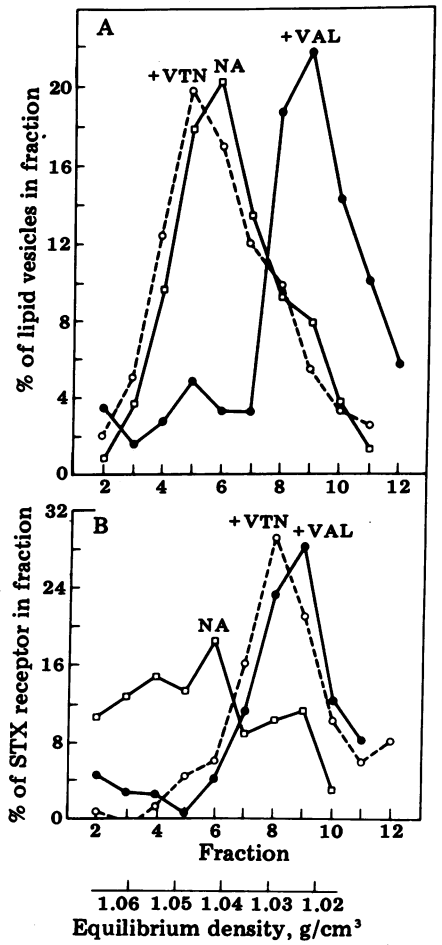

FIG. 6. Density gradient profiles of vesicle phospholipid (*A*) and [^{3}H]STX binding activity (*B*) for reconstituted vesicles formed from synaptosomal membranes. Vesicles containing the STX binding protein were first partially purified by isopycnic density gradient centrifugation as described in Fig. 4. The peaks of STX binding from three of these gradients (corresponding to fractions 14 and 15, Fig. 4) were pooled. The pooled peaks were preloaded with Cs^+ by Lubrol/Bio-Bead treatment as in Fig. 5 and subsequently dialyzed at 4°C, using hollow fibers for 15 min against 500 ml of 350 mM potassium acetate/50 mM imidazole/5 mM 2-mercaptoethanol/1 mM EDTA, pH 6.5. Vesicles were removed and divided into three equal (4-ml) aliquots. One aliquot was used as a control, to which a 1:100 dilution of ethanol was added (NA). To the second, valinomycin was added by 1:100 dilution from a 0.2 mg/ml stock in ethanol (VAL). To the third, veratridine (K & K Labs) was added by 1:100 dilution from a 25 mM stock in ethanol (VTN). After 15-min incubation on ice, each of the three aliquots was added to separate chilled linear gradient formed in VTI50 rotor tubes from 14 ml of 350 mM potassium acetate, etc. (see above) as "light phase," and 15 ml of the same plus 400 mM sucrose as the "heavy phase," over a 2-ml cushion of 50% sucrose in light phase. Centrifugation was at 2°C, 50,000 rpm for 75 min. Fractions (3 ml) were collected at 4°C. Binding activity was determined after diluting 2.5 ml of each fraction with 21 ml of chilled distilled water in sealable tubes for the Beckman 50.2 Ti rotor, placing a 1-ml cushion of 500 mM sucrose/10 mM imidazole/5 mM 2-mercaptoethanol/25 mM potassium acetate/1 mM EDTA, pH 7.0, in each tube and centrifuging for 2 hr at 40,000 rpm. The vesicles from each fraction formed a sharp band at the interface between the cushion and the upper phase. Each band was collected and diluted to 1.0 ml; 400-μl aliquots of each fraction were assayed for [^{3}H]STX binding, ± 1 μM unlabeled STX. Control preparations (not illustrated) were run as for VTN, but vesicles were preincubated and gradients were formed in the presence of 30 μM TTX. Before measurement of binding activity, fractions were twice diluted to 25 ml with TTX-free gradient light phase and pelleted at 40,000 rpm for 2 hr, as above in a Beckman 50.2 Ti rotor. Each final pellet was resuspended in 1 ml of 500 mM sucrose cushion solution (see above) and assayed for [^{3}H]STX binding.

DISCUSSION

These studies demonstrate that the STX binding protein of mammalian brain can be solubilized by cholate in the presence of exogenous lipid and incorporated into small (550-Å) artificial lipid vesicles. The reconstituted STX binding component contains an associated monovalent cation gating structure that can be specifically opened by veratridine at physiological concentrations (3) and blocked by TTX. This is, qualitatively, the pharmacological behavior to be expected if the reconstituted STX binding component contains the action potential Na^+ gate of nerve cells. It is the ability to open this gate with veratridine that allows for its fractionation due to the *specific* reduction of the density of STX-binding gate-containing vesicles by depletion of intravesicular Cs^+.

Measurements of the association with vesicles of the STX binding protein, reconstituted by cholate dialysis at a high lipid-to-protein ratio, are consistent with the expectation that—as we have shown in other reconstituted systems (9, 10)—we are distributing the membrane proteins of the starting material used for reconstitution into small unilamellar artificial vesicles; only one or a few membrane proteins are incorporated per vesicle. In contrast, the density gradient pattern that results after freeze/thaw–sonication of Na^+ gate-containing membranes (19) indicates that what occurs in this case is more likely the fusion of nerve membrane fragments with some exogenous lipid to create a vesicular structure that is more tightly sealed than the original nerve membrane fraction, resulting in an enhanced ability to measure veratridine-stimulated cation uptake.

The results of our gradient sedimentation and gel filtration studies of the cholate-solubilized STX binding protein qualitatively agrees with those of the TTX/STX binding component solubilized from other tissues by using nonionic detergents (6, 7). Our sedimentation studies in ^2H_2O additionally allowed us to determine the molecular weight and frictional ratio of the particle. The picture that emerges is that the cholate-solubilized STX binding protein is large and asymmetric, as predicted (6) for the Lubrol-solubilized electric eel TTX binding protein.

The degree of purification (30- to 50-fold) achieved here is not sufficient for us to clearly identify the component(s) of the STX receptor/ion channel on sodium dodecyl sulfate gels. Purification of the STX receptor/ion gate to homogeneity necessitates refinements of our technique or synergistic use of transport-specific fractionation with more conventional purification strategies. We do not at this point know how quantitatively the properties of the reconstituted STX receptor/ion gate resemble those of the action potential Na^+ gate. This information gap is largely due to inherent methodological limitations involved in ion transport studies of a Na^+ gate that has been reconstituted into small lipid vesicles. Electrophysiological studies have revealed that the axonal Na^+ gate, when opened, conducts ions at an enormous rate: $\approx 10^7$/sec (3). Because a 550-Å vesicle containing 350 mM of a given ion contains only $\approx 10^4$ ions, opening the Na^+ gate for only a millisecond would allow a substantial fraction of intravesicularly trapped Na^+ to leak out. It has been determined in squid axon (23) that the selectivity ratio of the Na^+ gate for Na/K/Cs is 1/0.09/0.016. It is futile to attempt to measure relative cation selectivity in these 500-Å vesicles by using conventional radioisotopic techniques: even if Cs^+ were 1000-fold more slowly gated than Na^+, opening the Na^+ gate with veratridine (4) would theoretically allow nearly complete leakage of Cs^+ out of the vesicle in a few seconds. The above calculations also tend to explain why a relatively high (30 μM) TTX concentration must be used to prevent veratridine-generated Cs^+ efflux: even if the gate were unblocked for a small fraction of the time, significant Cs^+ leakage would occur.

More quantitative comparison of the properties of the reconstituted STX binding protein/ion gate with those of the neuronal action potential Na^+ gate will require fusion of vesicles containing the purified reconstituted protein into a model membrane system (e.g., planar bilayer) that permits direct electrophysiological measurements.

Members of Harvard's Pharmacology and Neurobiology Departments provided useful discussion. This research was supported by National Institutes of Health Grant NS 15236, and by grants from the Sloan Foundation and the Boston Medical Foundation.

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