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ω-Conotoxin GVIA Binding to a High-Affinity Receptor in Brain: Characterization, Calcium Sensitivity, and Solubilization

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We describe unique, high-affinity binding sites for ω[^{125}I]conotoxin GVIA in membranes from rat brain and rabbit sympathetic ganglia which appear to be primarily associated with N-type voltage-dependent calcium channels. The dissociation constant (K_D) for the toxin in rat brain membranes is 60 pM. Physiologic extracellular concentrations of calcium inhibit toxin binding noncompetitively (IC_{50} = 0.2 mM). The regional distribution of the binding sites in rat brain differs markedly from that of dihydropyridine calcium antagonist receptors associated with L-type calcium channels. In detergent-solubilized brain membranes, toxin binding retains the same affinity, specificity, and ionic sensitivity as in particulate preparations.

Several subtypes of voltage-sensitive calcium channels are responsible for major functions of excitable tissues (Nilius et al., 1985; Nowycky et al., 1985). Calcium antagonist drugs of the dihydropyridine, phenylalkylamine, and benzothiazepine classes interact with the L subtype (Nowycky et al., 1985). The N subtype plays a role in neurotransmitter release in the brain (Miller, 1987). The role of T channels is not clear. The snail toxin ω-conotoxin GVIA (ω-CTX) interacts potently and persistently with N and L channels but not T channels (McCleskey et al., 1987). The toxin also blocks PNS (Kobayashi et al., 1982; Keer and Yoshikami, 1984) and CNS (Reynolds et al., 1986) neurotransmission, presumably through an effect on N channels.

In earlier studies, saturable binding sites for ω[^{125}I]CTX have been identified in chick brain synaptosomes (Cruz and Olivera, 1986; Cruz et al., 1987). However, the affinity of the binding sites was substantially less than the toxin's potency in blocking transmitter release, and calcium only had weak effects on binding. In another study, rat brain synaptic membranes were used to demonstrate 2 ω[^{125}I]CTX binding sites (Abe et al., 1986). This study also used a toxin of relatively low specific activity. In the present study employing ω[^{125}I]CTX of high specific activity, we have demonstrated a single binding site in rat brain membranes with low picomolar affinity, characterized its regional localization, demonstrated sensitivity to physiological extracellular calcium concentrations, and solubilized the receptor binding sites.

Materials and Methods

Materials. CTX GVIA was obtained from Peninsula (Belmont, CA) and iodinated as described (Olivera et al., 1984) by Russel Garlick (NEN/DuPont, Boston). Specific activity of ω[^{125}I]CTX was 2200 Ci/mmol. Native ω-CTX MVIIA was purified as described (Olivera et al., 1985) from the fish-hunting sea snail *Conus magus,* and native ω-CTX GVIA was purified as described (Olivera et al., 1984) from *Conus geographus.* The phenylalkylamines, (−)-, (+)-desmethoxyverapamil and verapamil were gifts of Drs. Hollman and Traut (Knoll AG, Ludwigschaten, FRG). Nitrendipine was the gift of Dr. Alexander Scriabine (Miles Laboratories, New Haven, CT), and fostedil (KB 944) was a gift from Dr. Raymond Kauffman (E. Lilly, Indianapolis, IN). [^{3}H]Nitrendipine (78 Ci/mmol) was obtained from NEN/DuPont (Boston). All other chemicals were obtained from commercial sources. Rabbit sympathetic ganglia were obtained frozen from Pel-Freez (Rogers, AR).

Preparation of rat whole forebrain, rat brain regions, and rabbit sympathetic ganglia. Rats (male Wistar rats, 175–250 gm) were sacrificed by cervical dislocation, their brains were rapidly removed, and dissected on ice. Frozen rabbit sympathetic ganglia were thawed. All further steps took place at 4°C. Tissues were homogenized in 50 mM HEPES, pH 7.4, 50 mg wet weight tissue/ml buffer, using a Brinkmann Polytron (setting on half-maximal for 30 sec). Homogenates were centrifuged for 15 min at 40,000 × *g.* The pellet was resuspended in 50 mM HEPES, pH 7.4, 10 mM EDTA. Homogenates were incubated at 4°C for 30 min and recentrifuged at 40,000 × *g* for 10 min. The pellet was resuspended in Chelex-treated 50 mM HEPES, pH 7.4, using a Polytron for rat brain and sonicator for rabbit sympathetic ganglia.

Solubilization of ω[^{125}I]CTX binding sites. CHAPS and Triton X-100 detergents, 0.1–2%, were added to fresh crude membranes and homogenized with 8 strokes of a Teflon–glass homogenizer. After 30–60 min incubation at 4°C, the homogenate was centrifuged at 100,000 × *g* for 60 min, and the supernatant was aspirated. In some experiments, the detergent-solubilized extracts were concentrated using an ultrafiltration apparatus (Amicon) at 50 psi. In other experiments, the pellet and supernatant were used immediately.

ω[^{125}I]CTX binding assay. ω[^{125}I]CTX was diluted from a lyophilized powder into double-distilled H_2O and dispensed in 50 μl aliquots into glass test tubes. Appropriate concentrations of crude or detergent-solubilized membranes, diluted into Chelex-treated 50 mM HEPES, pH 7.4, were added to a total assay volume of 1 ml. Assays also contained 0.4% BSA and appropriate concentrations of drugs or ions dispensed in 1:10 or 1:20 dilutions. The final ω[^{125}I]CTX concentration was 15–30 pM. At ligand concentrations above 60 pM, nonspecific binding increased more than specific binding so that routine assays also employed lower ligand concentrations. Mixtures were filtered simultaneously with a Brandel Cell Harvester (Gaithersburg, MD) using glass fiber filters (Scheicher and Schuell, No. 32) presoaked with the filtering buffer for crude membranes or 0.5% polyethylenimine for detergent-solubilized membranes. The filters then were washed at 4°C with three 4 ml aliquots of 160 mM choline chloride, 10 mM Tris, pH 7.7, and 0.2% BSA. Radioactivity was assessed by liquid scintillation spectrophotometry or by a gamma counter. [^{3}H]Nitrendipine binding was assayed as described (Murphy et al., 1983).

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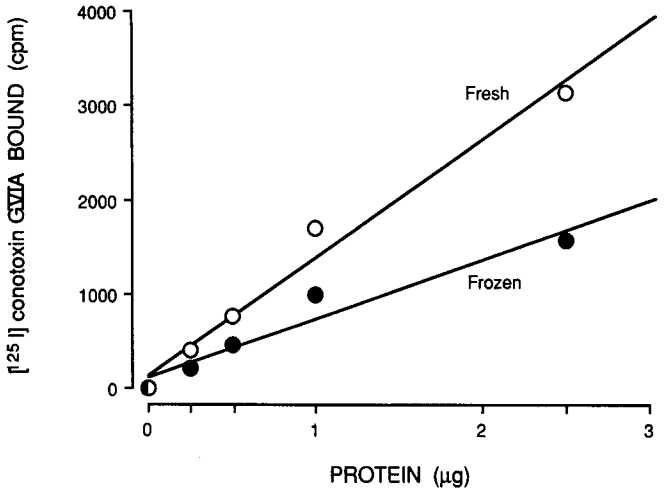

Figure 1. Membrane protein linearity of ω[^{125}I]CTX binding to rat forebrain membranes, both freshly prepared (*open circles*) and previously frozen at −20°C (*filled circles*). Ligand concentration was 28 pM in this experiment. Individual points are means of duplicate determinations. This experiment was replicated 3 times with similar results.

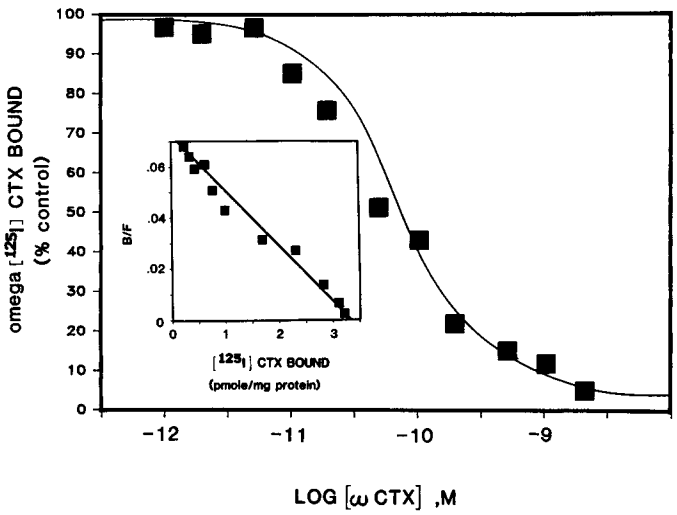

Figure 2. Saturation analysis of ω[^{125}I]CTX binding to rat forebrain membranes. ω[^{125}I]CTX, 24 pM, was incubated with the indicated concentrations of unlabeled toxin in triplicate. *Inset,* Scatchard replot of the same data. In the 1-site fit depicted, the background values, determined by the LIGAND computer program, are subtracted. Data are from a representative experiment performed in duplicate. Essentially identical results were obtained in 14 independent experiments. No analysis of saturation data using the LIGAND program yielded a statistically significant 2-site fit of the Scatchard plot.

Results

General properties of ω[^{125}I]CTX binding

ω[^{125}I]CTX binding to rat brain membranes is linear with tissue protein over the range 0.01–2.5 μg (Fig. 1). Linearity of ligand binding with tissue is extremely dependent on the use of very low concentrations of tissue protein and low concentrations of ligand. Linearity is apparent only with tissue concentrations <2 μg protein/ml. Earlier studies in chick brain membranes employed higher tissue concentrations (Abe et al., 1986; Cruz and Olivera, 1986; Cruz et al., 1987).

ω[^{125}I]CTX binding is temperature sensitive. At 25°C toxin binding reaches plateau levels by 7 min, while about 20 min is required for equilibrium binding at 4°C. Routine binding assays are conducted for 30 min at 25°C. In typical experiments employing 1 μg protein/ml and 20 pM ω[^{125}I]CTX, total binding is 3000 cpm and nonspecific binding, measured in the presence of 0.1 μM unlabeled toxin, is 1000 cpm.

Ligand binding is not markedly dependent upon pH, with similar levels of equilibrium binding observed over the range of pH 7–8.9.

ω[^{125}I]CTX binding deteriorates with storage of rat brain membranes. If tissue is frozen at −20°C immediately upon preparation of the membranes and thawed and assayed 24 hr later, binding is reduced by 50% (Fig. 1). Accordingly, fresh brain tissue is employed for routine experiments.

Saturation and kinetics of ω[^{125}I]CTX binding

ω[^{125}I]CTX is saturable (Fig. 2). At 25°C binding is half-maximal at 50 pM unlabeled toxin. Scatchard analysis reveals a single population of binding sites with a dissociation constant (K_D) of 60 pM and a maximal number of binding sites (B_{max}) of about 3 pmol/mg protein.

ω[^{125}I]CTX associates fairly rapidly and dissociates quite slowly (Fig. 3). At 25°C binding reaches plateau levels at 3–4 min and half-maximal levels at 0.5 min (Fig. 3*A*). The plot of time versus ln ($B_e/B_e - B$) is linear, consistent with a simple bimolecular reaction. The k_{ob} is 0.784 min^{-1}, and the calculated rate constant for association (k_1) is 2.6 × 10^{10} min^{-1} M^{-1}. The K_{ob} for ω[^{125}I]CTX at 4°C is 0.2 min^{-1}.

Dissociation of ω[^{125}I]CTX was examined by incubating the toxin with rat brain membranes to equilibrium, whereupon dissociation was initiated either by the addition of 100 nM unlabeled ω-CTX (Fig. 3*B*) or 50-fold dilution of the buffer (data not shown), and residual binding was measured at several time intervals. The rate of dissociation of ω[^{125}I]CTX is essentially the same by both of these techniques, suggesting the absence of cooperative interactions. When plotted on semilogarithmic paper, the dissociation is linear with 50% dissociation apparent at about 12 hr. The calculated rate constant for dissociation (k_{-1}) is 0.001 min^{-1} both by measurements utilizing excess ligand (Fig. 3*B*) or infinite dilution (data not shown). In some experiments we attempted to monitor dissociation at 4°C. Less than 20% dissociation was apparent at 24 hr. The kinetically derived dissociation constant, obtained from the ratio k_{-1}/k_1 is 40 fM, a value substantially lower than the K_D from equilibrium experiments. The discrepancy between K_D values determined in equilibrium and kinetic experiments might reflect irreversible or pseudoirreversible ligand binding with some of the dissociation deriving from decomposition of the ligand.

Influences of peptides and drugs on ω[^{125}I]CTX binding

ω-CTX itself is the most potent competitor for ω[^{125}I]CTX binding, with 50% inhibition apparent at 0.061 nM (Table 1). The MVIA toxin, derived from a different species of snail than ω-CTX, but similar in chemical structure and toxicity, has an IC_{50} of 0.5 nM.

Rat myelin basic protein and polylysine (22 kDa) have IC_{50} values of 2 and 5 nM, respectively. Whereas ω-CTX reduces binding in a competitive fashion, Scatchard analysis indicates noncompetitive inhibition by both myelin basic protein and polylysine (Fig. 4). Myelin basic protein and polylysine exert similarly potent inhibition of ligand binding to a cannabinoid receptor (Nye et al., 1988), presumably reflecting complementary charged sites on the receptors.

Table 1. Effects of drugs, peptides, and proteins on ω[^{125}I]CTX binding to rabbit sympathetic ganglia membranes and intact and solubilized rat forebrain membrane

	IC_{50} (nM)		
	Rat forebrain		Rabbit sympathetic ganglia membranes
	Membrane	Solubilized	
Conus peptides			
ω-CTX GVIA, native	0.061	0.069	0.092
ω-CTX GVIA, synthesized	0.090	0.105	0.120
ω-CTX MVIIA, native	0.5	N.D.	N.D.
Polycationic proteins			
Rat myelin basic protein	2	21	N.D.
Polysine (22K)	5	15	15
Aminoglycoside antibiotics			
Amikacin	15,000	N.D.	14,000
Gentamycin	10,000	N.D.	18,000
Kanamycin	200,000	370,000	240,000
Neomycin	3,000	6,000	7,000
Streptomycin	51,000	63,000	65,000
Tobramycin	18,000	N.D.	27,000
L-channel Ca^{2+} antagonists			
Verapamil	>10,000	>10,000	>10,000
(−)Desmethoxy-verapamil	>10,000	>10,000	>10,000
(+)Desmethoxy-verapamil	>10,000	N.D.	>10,000
Nitrendipine	>10,000	>10,000	>10,000
(+)PN 200-110	>10,000	N.D.	>10,000
Diltiazem	>10,000	>10,000	>10,000
Fostedil	>10,000	N.D.	>10,000

The effects of toxin peptides, polycationic proteins, aminoglycoside antibiotics, and L-channel Ca^{2+} antagonists on ω[^{125}I]CTX were assessed in rat brain, membrane and solubilized, and rabbit sympathetic ganglion membranes. Data are IC_{50} values from a single representative experiment replicated on 6 occasions for toxin peptides, 4 occasions for polycationic proteins, 4 occasions for aminoglycosides, and 3 occasions for Ca^{2+} antagonists.

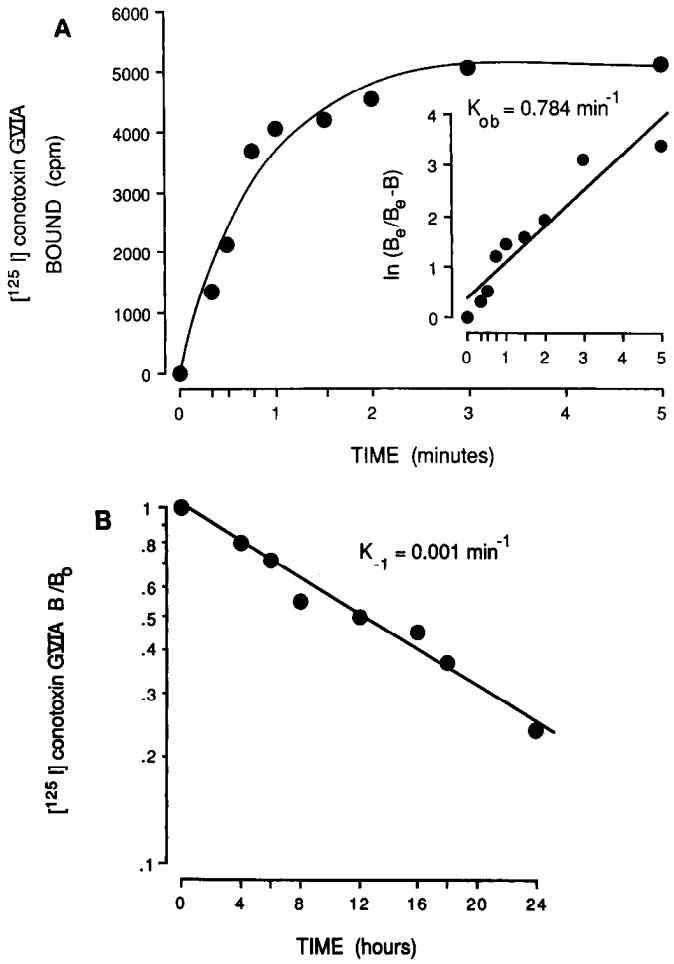

Figure 3. Association (*A*) and dissociation (*B*) of ω[^{125}I]CTX with rat forebrain membranes. Total and nonspecific ω[^{125}I]CTX binding to brain membranes was determined as in Materials and Methods. *A*, Specific ω[^{125}I]CTX binding in cpm plotted against duration of incubation. The concentration of ω[^{125}I]CTX was 28 pM. *Inset*, Replot of the same data: $\ln(B_e/B_e - B)$ versus time, where B_e = bound ω[^{125}I]CTX at equilibrium and B = bound toxin at a particular time point. The replot is linear and is used to calculate K_{ob}. *B*, The dissociation of ω[^{125}I]CTX from brain membranes equilibrated with 25 pM ω[^{125}I]CTX for 30 min. Dissociations were initiated with 100 nM unlabeled toxin. Each point of the dissociation has a set of controls (total and blank) equilibrated for the entire duration of the dissociation. Blanks (nonspecific binding) were determined in the presence of 100 nM unlabeled ω-CTX. Results are the means of duplicate determinations. The experiment was replicated on 3 occasions with similar results.

The aminoglycoside antibiotics exert potent neurotoxicity, which is reversed by calcium (Lietman, 1985; Sande and Mandell, 1985). In preliminary experiments we have found that the aminoglycoside antibiotics inhibit $^{45}Ca^{2+}$ uptake into synaptosomes through voltage-dependent calcium channels in proportion to their relative neurotoxicity. A series of 6 aminoglycoside antibiotics all inhibit ω[^{125}I]CTX binding. Scatchard analysis indicates noncompetitive inhibitory patterns (Fig. 4). Neomycin, which is the most neurotoxic of these drugs, is the most potent inhibitor of ligand binding.

Calcium antagonist drugs which are known to act through the L subtype of calcium channels all fail to inhibit ω[^{125}I]CTX binding at 10 μM. These findings confirm previous observations with lower specific activity ω[^{125}I]CTX (Abe et al., 1986; Cruz and Olivera, 1986).

By contrast to the potent influences upon toxin binding of myelin basic protein and polylysine, these substances do not inhibit [^{3}H]nitrendipine binding to brain membranes at 10 μM concentration. Similarly, the aminoglycoside antibiotics at 1 mM fail to affect [^{3}H]nitrendipine binding, except for streptomycin whose IC_{50} is 330 μM.

Brain regional and tissue distribution of ω[^{125}I]CTX GVIA binding

Voltage-dependent calcium channels are not uniformly distributed throughout the brain. The L-type channels labeled with [^{3}H]nitrendipine demonstrate heterogeneous distributions (Table 2) with maximal binding in the hippocampus, lowest binding in the brain stem, and intermediate levels in the cerebral cortex, confirming previous results obtained by both biochemical (Gould et al., 1982) and autoradiographic techniques. The distribution of ω[^{125}I]CTX binding differs substantially from that of [^{3}H]nitrendipine binding (Gould et al., 1985). More than 10-fold variations are apparent between the highest levels in the

Table 2. Distribution of ω[^{125}I]CTX and [^{3}H]nitrendipine binding in different neuronal regions

Tissue	ω[^{125}I]CTX		[^{3}H]Nitrendipine	
	K_D (pM)	B_{max} (pmol/mg protein)	K_D (pM)	B_{max} (pmol/mg protein)
Cerebral cortex	67	8.3	133	0.149
Cerebellum	54	3.7	124	0.046
Thalamus/hypothalamus	82	3.6	115	0.103
Corpus striatum	71	2.0	129	0.149
Hippocampus	46	6.4	110	0.172
Midbrain	113	3.5	—	<0.01
Brain stem	40	0.55	—	<0.01
Pituitary	313	2.2	N.D.	N.D.
Rabbit sympathetic ganglion	150	1.1	N.D.	N.D.

Other tissue examined without detectable ω[^{125}I]CTX binding include kidney, heart, trachea, bladder, vas deferens, spleen, lung, hindleg skeletal muscle, aorta, testes, and liver.

cerebral cortex and the lowest values in the brain stem. The hippocampus and cerebral cortex have fairly similar levels, whereas next highest values in the cerebellum and hypothalamus-thalamus and midbrain are less than half those of the cortex.

The K_D values for ω[^{125}I]CTX binding are similar in most brain regions, though the K_D level is significantly higher in the midbrain than in other areas (Table 2). The K_D value in the pituitary is higher than in the principal brain areas.

We examined a wide range of peripheral tissues for ω[^{125}I]CTX binding. Substantial levels of saturable high-affinity binding sites are detected only in rabbit sympathetic ganglia membranes (Table 2). The K_D value for ω[^{125}I]CTX in this tissue is similar to values in brain membranes, suggesting that the same or closely similar sites are labeled. However, binding site density is lower in sympathetic ganglia than in any of the brain regions examined except for brain stem. Since frozen ganglia were employed and ω[^{125}I]CTX binding is lower in frozen than fresh brain, values for ganglia binding may be underestimates. The drug specificity of the ω[^{125}I]CTX site in ganglia membranes is similar to the site in rat forebrain (Table 1). None of the non-neuronal tissues examined have detectable levels of ω[^{125}I]CTX binding (Table 2).

Effect of cations on ω[^{125}I]CTX GVIA binding

If ω-CTX labels voltage-dependent calcium channels, one might anticipate it to be influenced by physiological concentrations of calcium. However, in an earlier study, 30 mM calcium was required to reduce binding substantially (Cruz and Olivera, 1986). In the present study, employing much lower ligand and tissue concentrations, we detect more potent effects of calcium (Table 3). Fifty percent inhibition of binding is apparent with 0.2 mM calcium. Similar effects are observed with the wide range of divalent cations. The most potent inhibitors of binding are La^{3+} and Hg^{2+}, with respective IC_{50} values of 0.09 and 0.03 mM. Scatchard analysis indicates that the inhibitory effect of calcium is noncompetitive (Fig. 4). Similar cation effects are observed in ω[^{125}I]CTX binding to sympathetic ganglia.

Solubilization of ω[^{125}I]CTX GVIA binding sites

To ascertain whether ligand binding is retained in solubilized membranes, we treated rat brain membranes with 1% CHAPS or Triton X-100. Both solubilized preparations display substantial levels of specific ω[^{125}I]CTX binding at 25°C.

We have conducted routine assays with CHAPS. Similar binding levels occur in preparations solubilized with 0.5, 0.75, and 1.0% CHAPS, and 0.75% CHAPS is employed routinely. ω[^{125}I]CTX binding is linear with tissue protein over the range 1–100 μg (data not shown). Essentially all ω[^{125}I]CTX binding in the membranes is recovered in the solubilized preparations.

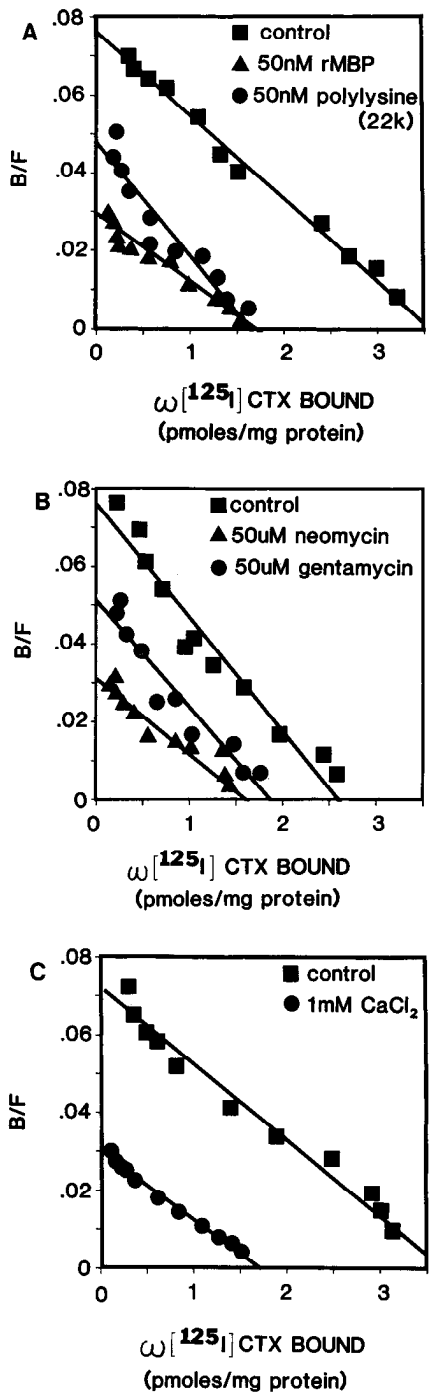

Figure 4. The effects of various agents on saturation of ω[^{125}I]CTX binding to brain membranes. *A*, Effects of no additive (*filled squares*), 50 nM rat myelin basic protein (*filled triangles*), or 50 nM 22K polylysine (*filled circles*). *B*, Effects of no additive (*filled squares*), 50 μM neomycin (*filled triangles*), or 50 μM gentamycin (*filled circles*). *C*, Effects of no additive (*filled squares*) or 1 mM $CaCl_2$ (*filled circles*).

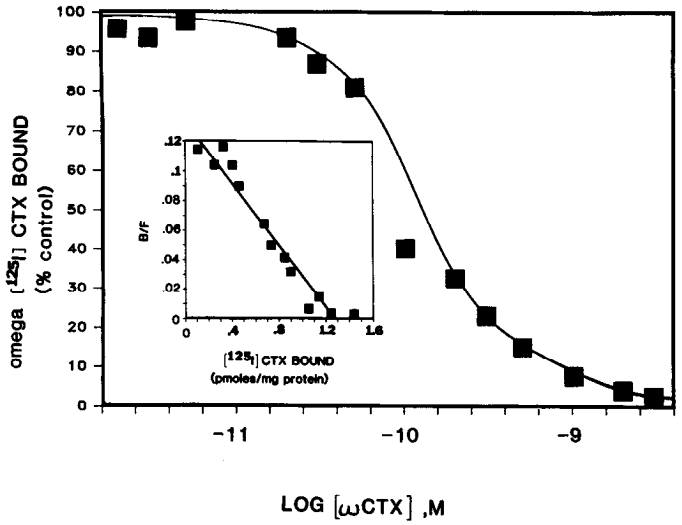

Figure 5. Saturation analysis of ω[^{125}I]CTX binding to solubilized rat forebrain membranes. Concentration of ω[^{125}I]CTX was 34 pM. *Inset,* Scatchard replot of the same data. Data are from a representative experiment performed in duplicate. Essentially identical results were obtained in 6 independent experiments performed on different days with different solubilized preparations.

Scatchard analysis reveals a single population of binding sites with a K_D of 60 pM, essentially the same as observed in intact membranes (Fig. 5).

Additional evidence that solubilized binding sites are the same as those that are in the membrane includes the closely similar effects of peptides, drugs, and cations on binding in solubilized and particulate fractions (Tables 1 and 3). The association and dissociation rates of ω[^{125}I]CTX in solubilized preparation are similar to those in crude membranes (Fig. 6).

Table 3. Effects of cations on ω[^{125}I]CTX binding to rabbit sympathetic ganglia membranes and intact and solubilized rat forebrain membranes

	IC_{50} (mM)		
	Rat forebrain		Rabbit sympathetic ganglia membranes
	Membrane	Solubilized	
Group IA			
NaCl	31	35	33
KCl	42	30	38
LiCl	35	50	33
CsCl	47	40	42
Group IIA			
$CaCl_2$	0.22	0.20	0.25
$BaCl_2$	0.20	0.14	0.17
$SrCl_2$	0.37	0.19	0.21
$MgCl_2$	0.60	0.85	0.76
Transition and lanthanide series			
$CoCl_2$	0.15	0.16	0.11
$CdCl_2$	0.21	0.32	0.25
$LaCl_3$	0.09	0.05	0.07
$Pb(NO_3)_2$	0.34	0.52	0.47
$HgCl_2$	0.03	N.D.	0.06
$CuCl_2$	0.02	N.D.	N.D.
$NiCl_2$	0.35	0.57	0.53

The effects of 8–16 concentrations of various cations were examined in triplicate on ω[^{125}I]CTX binding. Data are from a representative experiment replicated 3 times.

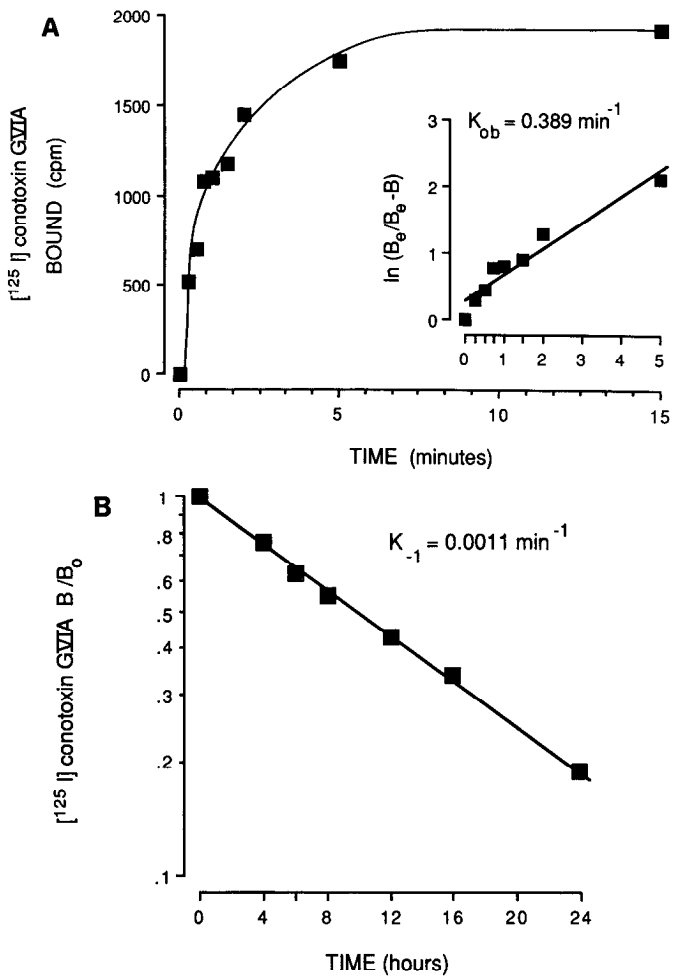

Figure 6. Association and dissociation of ω[^{125}I]CTX to solubilized rat forebrain membranes. Total and nonspecific ω[^{125}I]CTX binding to brain membranes was assayed as described in Materials and Methods. *A,* Specific ω[^{125}I]CTX binding in cpm plotted against the duration of incubation. The concentration of ω[^{125}I]CTX was 33 pM. *Inset,* Same data plotted as described in the legend to Figure 3. *B,* Dissociation of ω[^{125}I]CTX from solubilized brain membranes equilibrated with 21 pM ω[^{125}I]CTX for 30 min. Results are the means of duplicates. These experiments were repeated on 2 occasions with similar results.

Discussion

The main finding of this study is the identification of unique, very high affinity binding sites for ω[^{125}I]CTX in rat brain membranes whose properties fit those of voltage-dependent calcium channels of the N subtype. These sites appear to mediate the marked effects of ω-CTX upon calcium passage through voltage-sensitive channels. Thus, in synaptosomes we observed half-maximal effects of ω-CTX on calcium flux at 50 pM, closely similar to the K_D of ω-CTX for the binding sites (Reynolds et al., 1986). Previous study in which ω-CTX had about 100-fold lesser affinity for binding sites in chick brain synaptosomes may reflect the use of much lower specific activity toxins so that the lowest concentration of radiolabeled ligand employed was 0.1 nM, though there may also be species differences and variations in tissue preparation (Cruz and Olivera, 1986).

Further evidence that ω[125I]CTX binding involves voltage-dependent calcium channels stems from the ability of calcium to inhibit binding at physiological extracellular concentrations. The noncompetitive nature of calcium's inhibition fits with the notion that the ω-CTX binding site is not identical to the calcium pore but is linked to it allosterically.

Three subtypes of voltage-dependent calcium channels have been differentiated by Tsien and associates (Nowycky et al., 1985). The principal calcium antagonist drugs employed clinically act at the L subtype, which is implicated in contractility of cardiac and smooth muscle. Calcium-dependent neurotransmitter release at synapses in the CNS appears to be associated primarily with the N channels and is not markedly affected by the major calcium antagonist drugs (Turner and Goldin, 1985; Reynolds et al., 1986; Miller, 1987). The functions of the T channel are unclear. ω-CTX influences L and N but not T channels (McCleskey et al., 1987). ω-CTX inhibits neurotransmitter release (Kerr and Yoshikami, 1984) and in our studies (Reynolds et al., 1986) does so with a potency comparable to its K_D for the ω-CTX binding sites. The binding of ω[125I]CTX observed here appears to involve primarily or exclusively the N-subtype channels for 2 reasons. First, the binding sites are uninfluenced by the calcium antagonist drugs that act at L channels. Second, rat brain membranes possess about 30 times more ω-CTX binding sites than dihydropyridine binding sites. Thus, even if ω[125I]CTX bound to both channel subtypes, interactions with the L-type channel might not be detectable. The potent, selective interactions of ω-CTX with the N-type calcium channel afford a valuable probe for physiological processes associated with this type of calcium channel.

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