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μ-Conotoxins Share a Common Binding Site with Tetrodotoxin/Saxitoxin on Eel Electroplax Na Channels

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The binding characteristics of conotoxin GIIIA purified from the venom of a marine snail, *Conus geographus*, with regard to electroplax membranes from *Electrophorus electricus* were studied using a radiolabeled monopropionyl derivative of the toxin (^{3}H-Pr-CGIIIA). ^{3}H-Pr-CGIIIA bound specifically to a single class of saturable binding sites in electroplax membranes with a dissociation constant of 1.1 ± 0.2 nM and a maximal binding capacity of 11 ± 2 pmol/mg of protein. The latter value was similar to the number of specific binding sites (10 ± 2 pmol/mg of protein) for ^{3}H-lysine-tetrodotoxin (^{3}H-Lys-TTX). Monopropionyl CGIIIA and CGIIIA had similar inhibitory effects on the binding of ^{3}H-Lys-TTX (1 nM) to electroplax membranes with IC_{50} values of 3.5 and 0.9 nM, respectively.

The association and dissociation of ^{3}H-Pr-CGIIIA and electroplax membranes were much slower than those of ^{3}H-Lys-TTX and the membranes. μ-Conotoxins (CGIIIA and CGIIIB) and guanidinium toxins (TTX and saxitoxin) inhibited ^{3}H-Pr-CGIIIA (1 nM) binding to electroplax membranes with IC_{50} values of 0.6, 1.1, 7.1, and 2.2 nM, respectively. However, several other kinds of neurotoxins and local anesthetics known to interact with Na channels did not affect ^{3}H-Pr-CGIIIA binding.

These findings indicate that μ-conotoxins must be classified in the same group of Na channel inhibitors as guanidinium toxins, since they competed with guanidinium toxins for binding sites on the Na channel. The peptide μ-conotoxins should be useful in studies on the functional and structural domains of Na channel proteins.

The voltage-dependent Na channel plays an important role in the generation of the action potential responsible for the electrical signaling of excitable cells. The use of radiolabeled neurotoxins as specific ligands made it possible to isolate the Na channels from the eel electroplax (Agnew et al., 1978), rat brain (Hartshorne and Catterall, 1981) and rat skeletal muscle (Barchi, 1983). A number of neurotoxins that bind with high affinity and specificity to Na channels have been very useful in studying the functional and structural properties of these channels (Narahashi, 1974; Catterall, 1980), and it is tempting to use the toxins to identify the physiologically functional domains on the Na channel proteins whose primary structures have been determined by recombinant DNA techniques (Noda et al., 1984, 1986).

The μ-conotoxins from the venom of the marine snail *Conus geographus* consist of 7 homologous peptides, each composed of 22 amino acids (Cruz et al., 1985). In the μ-conotoxin family, conotoxins GIIIA and GIIIB were shown to be identical with Geographutoxins I and II (Sato et al., 1983), respectively. μ-Conotoxins preferentially abolish the action potential of the skeletal muscle in a fashion similar to that of guanidinium toxins such as TTX and saxitoxin (STX). But μ-conotoxins scarcely affect the Na channels of nerves. Experimental findings on batrachotoxin-activated Na channels incorporated into planar lipid bilayers were consistent with the above findings—namely, CGIIIA blocked Na channels of muscle, but had no detectable effect on those of brain. The kinetics of this blocking effect was similar to that of TTX (Cruz et al., 1985). In our previous study (Yanagawa et al., 1986), low concentrations of CGIIIA blocked the binding of radiolabeled TTX (^{3}H-Lys-TTX) to eel electroplax Na channels, but much higher concentrations of the toxin were needed to block the binding of the ligand to rat brain. Recently Ohizumi et al. (1986b) reported results that were very consistent with ours, namely, they observed that CGIIIB effectively blocked ^{3}H-STX binding to rat or rabbit skeletal muscle, but that CGIIIA did not block ^{3}H-STX binding to rat brain or cervical ganglia.

In this study we prepared a radiolabeled derivative of CGIIIA and examined its binding characteristics to eel electroplax membranes. The results of two kinds of competition experiments, inhibition of ^{3}H-Lys-TTX binding to eel electroplax membranes by CGIIIA and inhibition of ^{3}H-Pr-CGIIIA binding to these membranes by TTX and STX, indicated that CGIIIA and the guanidinium toxins TTX and STX bind to a common site on the Na channel.

Materials and Methods

Purification of μ-conotoxins. Specimens of *C. geographus* were transported in dry ice from Okinawa Island and stored at −80°C. The following procedures were carried out at 0–4°C. The venom ducts of these specimens were homogenized in 10 volumes of 0.1 *N* HCl in a polytron. The homogenate was centrifuged at 100,000 × *g* for 1 hr, and the supernatant was saved. The pellet was rehomogenized with 5 volumes of 0.1 *N* HCl and centrifuged at 100,000 × *g* for 1 hr. The supernatants were combined and lyophilized. The lyophilized material was dissolved in 5 ml of 1% (vol/vol) acetic acid, and insoluble material was removed by centrifugation at 100,000 × *g* for 1 hr. The supernatant was applied to a column (2.1 × 195 cm) of Sephadex G-25 (fine). Fractions (I–V) were obtained by monitoring absorbance at 254 nm. Of these fractions, fraction II, containing the Na channel inhibitors, was lyophilized and

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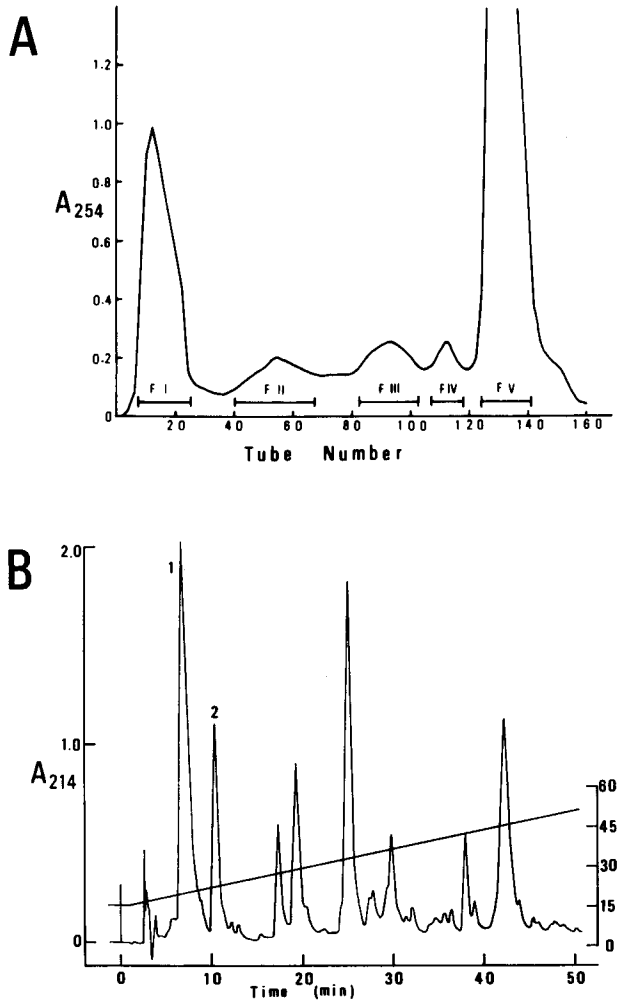

Figure 1. Purification of μ-conotoxins. *A,* Gel filtration of the lyophilized extract (170 mg) on a column (2.1 × 195 cm) of Sephadex G-25. The flow rate was 21 ml/hr and 2.5-ml fractions were collected. Fraction II, containing μ-conotoxins, was lyophilized. *B,* Reversed-phase HPLC of fraction II. Fraction II was dissolved in 300 μl of 0.1% trifluoroacetic acid (solvent *A*) and applied to a column (1.9 × 15 cm) of μBondapak C18 (Waters); toxins were eluted with a linear gradient of 0–60% acetonitrile in 0.1% trifluoroacetic acid (solvent *B*), using an automated gradient controller (Waters). The flow rate was 10 ml/min. CGIIIA (2 mg; peak 1) and CGIIIB (1.2 mg; peak 2) were obtained.

Table 1. Amino acid analysis of materials in peaks 1 and 2 of Figure 1B

Amino acid	Peak 1 material (residues/22)	CGIIIA	Peak 2 material (residues/22)	CGIIIB
Asp	2.04 (2)	2	1.92 (2)	2
Thr	0.93 (1)	1	1.00 (1)	1
Glu	2.03 (2)	2	–	–
Ala	1.12 (1)	1	1.15 (1)	1
½Cys[a]	5.91 (6)	6	5.73 (6)	6
Met[b]	–	–	0.92 (1)	1
Lys	3.74 (4)	4	4.12 (4)	4
Arg	2.97 (3)	3	4.06 (4)	4
Hyp	3.17 (3)	3	3.09 (3)	3

[a] Measured as cysteic acid.

[b] Measured as methionine sulfone.

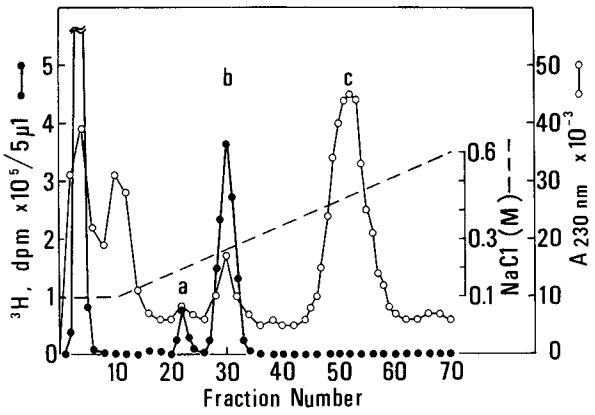

Figure 2. Purification of ^{3}H-Pr-CGIIIA. CGIIIA (60 nmol) was ^{3}H-propionylated, as described in Materials and Methods. The reaction mixture was applied to a CM–Sephadex C-25 column (0.75 × 4.5 cm) equilibrated with 0.1 M NaCl in 20 mM sodium phosphate buffer (pH 7.4), and eluted with a linear gradient of 0.1–0.6 M NaCl in the same buffer. Absorbance at 230 nm (○) and the radioactivity (●) of 5-μl aliquots of each fraction were determined. The NaCl concentration was estimated theoretically (– – –). The flow rate was 2 ml/hr and 1.0-ml fractions were collected. The yields of di^{3}H-propionyl CGIIIA (peak *a*) and mono^{3}H-propionyl CGIIIA (peak *b*) were 0.2 and 3.8 nmol, respectively.

further fractionated by reversed-phase high-performance liquid chromatography (HPLC) as follows: Fraction II was dissolved in 0.3 ml of 0.1% trifluoroacetic acid (solvent A) and applied to a column (1.9 × 15 cm) of μBondapak C18 (Waters) that was operated in a HPLC apparatus (Waters). The peptides were eluted with a linear gradient of 60% acetonitrile in 0.1% trifluoroacetic acid (solvent B) at a flow rate of 10 ml/min, using an automated gradient controller (Waters). Fractions were collected manually and lyophilized.

Amino acid analysis. The amino acid compositions of purified peptides were determined in a Hitachi amino acid analyzer after the hydrolysis of samples in 6 *N* HCl *in vacuo* at 110°C for 20 hr. Values for threonine were corrected for destruction. Half-cysteine and methionine residues were determined as cysteic acid and methionine sulfone, respectively, after oxidation with performic acid (Hirs, 1967).

^{3}H-labeling of CGIIIA. *N*-Succinimidyl 2,3-^{3}H-propionate (105 Ci/mmol) was obtained from Amersham. The following procedures were carried out at 0–4°C. The solvent was evaporated from the reagent (10 nmol) with a stream of N_2 gas, and CGIIIA (60 nmol) in 400 μl of 0.1 M borate buffer, pH 8.5, was added. The mixture was incubated for 15 min and diluted with 2 ml of 0.1 M NaCl in 20 mM sodium phosphate buffer, pH 7.4 (buffer A). The mixture was then applied to a CM–Sephadex C-25 column (0.75 × 4.5 cm) equilibrated with buffer A and the column was washed with 4–6 volumes of buffer A. ^{3}H-Propionyl derivatives of CGIIIA were separated from unlabeled CGIIIA by elution with a linear NaCl gradient (0.1–0.6 M) in 20 mM sodium phosphate buffer, pH 7.4. The absorbance at 230 nm and the radioactivity of each fraction of eluate were measured.

Standard binding assays. Electroplax membranes from *Electrophorus electricus* were prepared as described previously (Yanagawa et al., 1986). Protein was measured by the method of Lowry et al. (1951), with bovine serum albumin as standard.

Eel electroplax membranes (22–62 μg protein/ml) were incubated at 4°C in 2 ml of incubation medium consisting of 40 mM choline chloride, 0.1% (wt/vol) bovine serum albumin, 0.01% (wt/vol) NaN_3, 20 mM Tris-HCl, pH 7.4, and an appropriate concentration of mono^{3}H-propionyl CGIIIA (^{3}H-Pr-CGIIIA) or ^{3}H-Lys-TTX. After incubation, samples were centrifuged at 28,000 × *g* for 15 min at 0°C. The supernatant fluids were decanted and the pellets solubilized in 1 ml of 0.4% SDS in 0.4 *N* NaOH and neutralized with 5 *N* HCl. After addition of 9 ml of Scintisol EX-H (Dojindo Laboratories, Kumamoto, Japan), radioactivity was measured in a Beckman liquid scintillation counter. Nonspecific binding was determined in the presence of unlabeled CGIIIA (1 μM) or unlabeled TTX (1 μM), and specific binding was calculated by subtracting nonspecific binding from total binding. Variations from these conditions for individual experiments are noted in the figure and table legends.

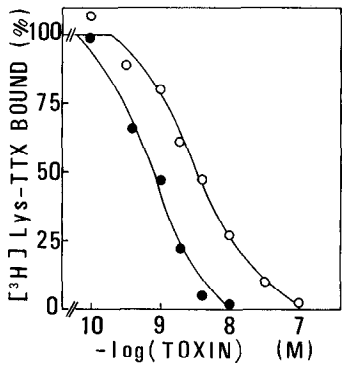

Figure 3. Inhibition of ^{3}H-Lys-TTX binding by native CGIIIA (●) and Pr-CGIIIA (○). Eel electroplax membranes were incubated for 30 min at 4°C with 1 nM ^{3}H-Lys-TTX in the presence of the indicated concentrations of CGIIIA or Pr-CGIIIA. Bound ^{3}H-Lys-TTX was measured by the centrifugation procedure. Nonspecific binding, measured in the presence of 1 μM TTX, was subtracted from observed values.

Toxins and chemicals. Citrate-free TTX was obtained from Sankyo, Tokyo. ^{3}H-Lys-TTX was synthesized as described by Chicheportiche et al. (1980); its specific radioactivity was 30 Ci/mmol. STX was the generous gift of Dr. Y. Kishi. Aconitine and veratridine were from Sigma. Toxin γ from *Tityus serrulatus* venom (TiTxγ) was prepared by Dr. J. R. Giglio. *Parasicyonis* toxin (PaTX) was prepared from the sea anemone *Parasicyonis actinostoloides* by a slight modification of the method of Ishikawa et al. (1979). N-Succinimidyl propionate was synthesized by a method similar to that used in the synthesis of *N*-hydroxysuccinimidyl acetate (Rappoport and Lapidot, 1974).

Results

Purification of μ-conotoxins

An outline of the purification procedure has been reported (Yanagawa et al., 1986). The soluble venom was fractionated into 5 distinct fractions on a Sephadex G-25 column (Fig. 1*A*). Fraction II, containing μ-conotoxins, was fractionated further by reversed-phase HPLC chromatography (Fig. 1*B*). Several fractions, including peak 1 and peak 2, were separated and lyophilized. Amino acid analyses indicated that the materials in peak 1 and peak 2 were CGIIIA and CGIIIB, respectively (Table 1) (Sato et al., 1983; Cruz et al., 1985).

Preparation and characterization of ^{3}H-Pr-CGIIIA

CGIIIA is a basic peptide composed of 22 amino acids. This toxin is unsuitable for direct radioiodination (e.g., the chloramine-T procedure), since it contains no tyrosine residue. Therefore, *N*-succinimidyl 2,3-^{3}H-propionate was used in the synthesis of a radiolabeled derivative of CGIIIA. Mainly to obtain the mono^{3}H-propionyl derivative of CGIIIA, we added excess toxin over ^{3}H reagent in the propionylation procedure. Figure 2 shows a chromatogram of the reaction products on a CM-Sephadex C-25 column. Two well-separated peaks of ^{3}H-propionyl derivatives of CGIIIA (peaks a and b) were obtained with unbound ^{3}H reagent, which was not adsorbed on the column, and unlabeled toxin (peak c), which was eluted at a higher concentration of NaCl than were the ^{3}H-propionyl derivatives.

For the determination of the molar ratios of ^{3}H-propionyl residues to toxin in the materials in peaks a and b, 300 nmol of toxin was condensed with 100 nmol of *N*-succinimidyl propionate with low specific radioactivity (10.5 Ci/mol) under the conditions described in Materials and Methods. A chromatogram similar to that in Figure 2 was obtained, and the materials

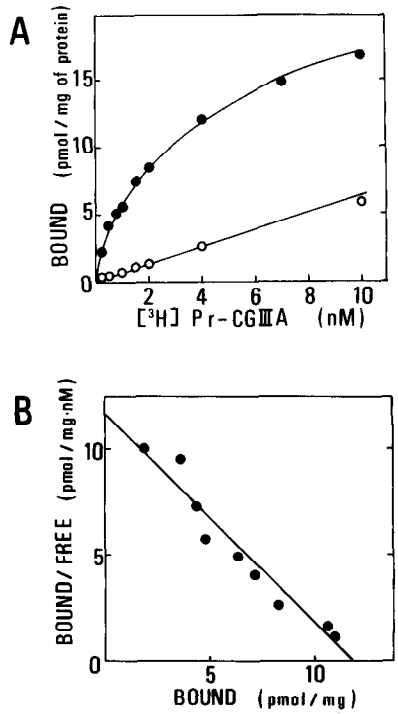

Figure 4. Binding of ^{3}H-Pr-CGIIIA to eel electroplax membranes. Eel electroplax membranes were incubated for 60 min at 4°C with various concentrations of ^{3}H-Pr-CGIIIA and the bound radioactivity was measured by the centrifugation procedure. *A*, Total binding (●) and nonspecific binding measured in the presence of 1 μM CGIIIA (○) are plotted versus the free ^{3}H-Pr-CGIIIA concentration. *B*, Specific binding was calculated as the difference between total and nonspecific bindings and is presented as a Scatchard plot.

in peaks a and b were subjected to amino acid analysis to determine their toxin contents. Radioactivity was measured in a scintillation counter, and the specific radioactivities of peaks a and b were calculated to be 21.0 and 10.5 Ci/mol, respectively. Thus, peak a was dipropionyl CGIIIA and peak b was monopropionyl CGIIIA.

To identify the residue of modified amino acid in monopropionyl CGIIIA, a 5-nmol sample with low specific radioactivity was dinitrophenylated as described by Sanger and Thompson (1953), hydrolyzed, and then analyzed by an amino acid analyzer. CGIIIA was treated similarly, and the results are shown in Table 2. After dinitrophenylation, 2 arginine and no lysine residues were recovered from CGIIIA, which contains 3 arginine and 4 lysine residues. On the other hand, 2 arginine and 1 lysine residues were recovered from dinitrophenyl monopropionyl

Table 2. Amino acid analysis of dinitrophenyl CGIIIA and dinitrophenyl monopropionyl CGIIIA (residues/mol)

Amino acid	CGIIIA	Dinitrophenyl CGIIIA	Dinitrophenyl monopropionyl CGIIIA
Asp	2	2	2
Lys	3.89 (4)	0.07 (0)	0.95 (1)
Arg	2.92 (3)	2.04 (2)	2.11 (2)

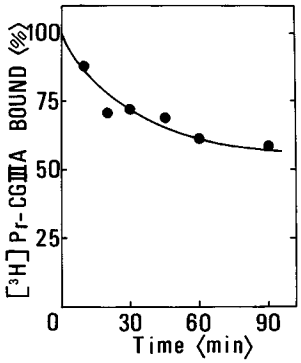

Figure 5. Time course of dissociation of ^{3}H-Pr-CGIIIA from eel electroplax membranes. ^{3}H-Pr-CGIIIA (1 nM) was preincubated with eel electroplax membranes for 60 min at 4°C. At time *0*, unlabeled CGIIIA (final concentration, 1 μM) was added and incubation was continued for the indicated times at 4°C. Then the reaction mixture was centrifuged at 28,000 × *g* for 15 min at 0°C. The supernatant was removed and the radioactivity of the pellet measured. Values are expressed as percentages of the maximal value for specific binding of ^{3}H-Pr-CGIIIA.

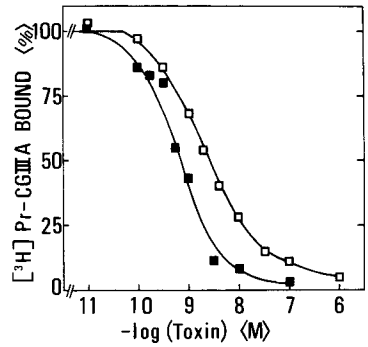

Figure 6. Inhibition of ^{3}H-Pr-CGIIIA binding to eel electroplax membranes by CGIIIA and saxitoxin. Eel electroplax membranes were incubated for 60 min at 4°C with 1 nM ^{3}H-Pr-CGIIIA in the presence of the indicated concentrations of CGIIIA (■) or saxitoxin (□). Bound ^{3}H-Pr-CGIIIA was measured by the centrifugation procedure. Nonspecific binding, measured in the presence of 1 μM CGIIIA, was subtracted from the observed values.

CGIIIA. Thus it is evident that 1 lysine residue found in the hydrolysate of monopropionyl CGIIIA was derived from ε-*N*-propionyl lysine in the modified peptide. The position in CGIIIA of this preferentially propionylated lysine was not identified by the present study.

For the binding study, mono^{3}H-propionyl CGIIIA (sp. radioact., 105 Ci/mmol), referred to as ^{3}H-Pr-CGIIIA, was used throughout this study. Possible contamination by di^{3}H-propionyl CGIIIA and unlabeled CGIIIA was avoided by using only fractions 29–31 (Fig. 2) for binding experiments. Monopropionyl CGIIIA of low specific radioactivity (10.5 Ci/mol), prepared as described above and referred to as Pr-CGIIIA, was used in place of cold CGIIIA derivative in the following experiments.

Effect of Pr-CGIIIA on the binding of ^{3}H-Lys-TTX to electroplax membranes

To assess the interaction of Pr-CGIIIA with voltage-dependent Na channels, we examined the ability of Pr-CGIIIA to displace ^{3}H-Lys-TTX (1 nM) specifically bound to Na channels in electroplax membranes. In this case the specific radioactivity of Pr-CGIIIA (10.5 Ci/mol) was about 1/3000th that of ^{3}H-Lys-TTX (30 Ci/mmol).

The results (Fig. 3) showed that CGIIIA and Pr-CGIIIA displaced ^{3}H-Lys-TTX (1 nM) dose-dependently, with IC_{50} values of 0.9 nM and 3.5 nM, respectively. Thus, it is evident that the mono^{3}H-propionyl derivative of CGIIIA has a similar affinity to native CGIIIA for electroplax Na channels.

Table 3. Inhibition of specific ^{3}H-Pr-CGIIIA binding to eel electroplax membranes by neurotoxins

Neurotoxin	IC_{50} (nM)
Conotoxin GIIIA	0.6 ± 0.1
Conotoxin GIIIB	1.1 ± 0.1
Saxitoxin	2.2 ± 0.4
Tetrodotoxin	7.1 ± 0.9

In competition experiments, various concentrations of the indicated neurotoxins were incubated with eel electroplax membranes and 1 nM ^{3}H-Pr-CGIIIA. The IC_{50} values, which are the concentrations for half-maximal inhibition of binding, are shown as means ± SD from 3 separate experiments.

Specific binding of ^{3}H-Pr-CGIIIA to electroplax membranes

Figure 4 shows the results of binding experiments. The specific binding, calculated as the difference between total and nonspecific bindings, was a saturable function of the ^{3}H-Pr-CGIIIA concentration. Scatchard analysis of the specific binding gave a straight line, indicating that ^{3}H-Pr-CGIIIA bound to a single class of binding sites. The dissociation constant of the complex formed between ^{3}H-Pr-CGIIIA and electroplax membranes (K_d) was calculated to be 1.1 ± 0.2 nM (n = 3), and the maximal binding sites (B_{max}) to be 11 ± 2 pmol/mg of protein (n = 3).

Time course of dissociation of ^{3}H-Pr-CGIIIA bound to electroplax membranes

Specific ^{3}H-Pr-CGIIIA (1 nM) binding approached a plateau value after incubation for 45 min at 4°C. The addition of excess unlabeled CGIIIA (1 μM) after incubation for 1 hr at 4°C caused slow dissociation of the bound ^{3}H-Pr-CGIIIA (Fig. 5). More

Table 4. Binding of ^{3}H-Pr-CGIIIA to eel electroplax membranes in the presence of other kinds of neurotoxins and local anesthetics

Addition	Concentration	^{3}H-Pr-CGIIIA bound (% of control)
Veratridine	100 μM	107 ± 1
Aconitine	100 μM	95 ± 1
PaTX[a]	1 μM	99 ± 5
TiTxγ[b]	100 nM	95 ± 1
Lidocaine	1 mM	89 ± 8

Eel electroplax membranes were incubated for 60 min at 4°C with 1 nM ^{3}H-Pr-CGIIIA in the presence of the indicated neurotoxins or local anesthetics. Bound ^{3}H-Pr-CGIIIA was meaured by the centrifugation procedure. Nonspecific binding, measured in the presence of 1 μM CGIIIA, was subtracted from observed values. Some compounds were dissolved in ethanol and necessary corrections were made to the apparent binding values. Values are means ± SD from three different experiments.

[a] *Parasicyonis* toxin from a sea anemone, *Parasicyonis actinostoloides*.

[b] Toxin γ from *Tityus serrulatus*.

than 50% of the maximal binding was retained after incubation for 90 min. On the other hand, specific ^{3}H-Lys-TTX binding (1 nM) reached a maximum within 5 min upon incubation at 4°C, and the displacement of ^{3}H-Lys-TTX from its receptor by unlabeled TTX (1 μM) approached more than 95% of the maximal value within 5 min.

Inhibition of ^{3}H-Pr-CGIIIA binding by various neurotoxins

The inhibitory effects of various concentrations of μ-conotoxins (CGIIIA, CGIIIB) and guanidinium toxins (STX, TTX) on the specific binding of ^{3}H-Pr-CGIIIA to electroplax membranes are shown in Figure 6 and Table 3. Unlabeled CGIIIA and CGIIIB inhibited specific ^{3}H-Pr-CGIIIA binding concentration-dependently with IC_{50} values of 0.6 and 1.1 nM, respectively. STX and TTX, guanidinium toxins that inhibit ion flux through Na channels, had similar effects on ^{3}H-Pr-CGIIIA binding to electroplax membranes, with IC_{50} values of 2.2 and 7.1 nM, respectively.

Table 4 shows that several other neurotoxins and local anesthetics known to affect Na channels did not interfere with ^{3}H-Pr-CGIIIA binding to electroplax membranes. Therefore, these toxins or local anesthetics probably bound to sites other than those of CGIIIA on the Na channel molecule.

Discussion

We have previously reported (Yanagawa et al., 1986) that CGIIIA inhibited the binding of ^{3}H-Lys-TTX to the Na channels of eel electroplax, and suggested that this inhibition was due to competition between CGIIIA and TTX for binding sites. Ohizumi et al. (1986b) obtained results very similar to ours using ^{3}H-STX and rabbit skeletal muscle membranes in place of ^{3}H-Lys-TTX and eel electroplax membranes.

In the present work, we prepared a tritium-labeled monopropionyl CGIIIA and showed that it had a high affinity for electroplax membranes. The binding was saturable and specific, and the K_d and B_{max} of ^{3}H-Pr-CGIIIA binding were comparable with those of ^{3}H-Lys-TTX binding. In these experiments, freshly prepared ^{3}H-Lys-TTX was used, and the parameters for binding of ^{3}H-Pr-CGIIIA and ^{3}H-Lys-TTX to the electroplax membranes were shown to be almost the same—the B_{max} values for ^{3}H-Pr-CGIIIA and ^{3}H-Lys-TTX were 11 ± 2 and 10 ± 2 pmol/mg of protein, and their K_d values were 1.1 ± 0.2 and 1.4 ± 0.5 nM, respectively. As expected, the binding of ^{3}H-Pr-CGIIIA was inhibited by TTX and STX, not by other kinds of neurotoxins and local anesthetics. The IC_{50} value for inhibition of ^{3}H-Lys-TTX binding to electroplax membranes by CGIIIA was almost the same as that for ^{3}H-Pr-CGIIIA binding by TTX or STX. Therefore, these two chemically different types of compounds, peptide and heterocyclic guanidiniums, block Na channels by binding to a common or structurally overlapping site on the Na channel molecule.

These results are consistent with electrophysiological observations on frog and rat skeletal muscle, guinea pig vas deferens, rat and rabbit diaphragms, etc. (Cruz et al., 1985; Ohizumi et al., 1986a, b). In these physiological experiments, μ-conotoxins blocked Na channel activities, as did guanidinium toxins.

In contrast to the similarities described above, the association and dissociation of ^{3}H-Pr-CGIIIA and electroplax membranes were much slower than those of ^{3}H-lysine-TTX and the membranes. Another interesting feature of the peptide toxin CGIIIA is that it interacts more effectively with eel electroplax than with rat brain, whereas the heterocyclic guanidinium toxins interact with both (Yanagawa et al., 1986). Thus, TTX-sensitive Na channels may be classified into CGIIIA-sensitive and CGIIIA-insensitive subtypes. These differences in sensitivity to μ-conotoxins are probably due to differences in the primary structures of Na channel proteins deduced from their cDNA sequence (Noda et al., 1984, 1986).

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