THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 259, No. 13, Issue of July 10, pp. 8353-8357, 1984
Printed in U.S.A.

Ciguatoxin Is a Novel Type of Na^+ Channel Toxin*

(Received for publication, November 9, 1983)

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Purified ciguatoxin at 0.1 to 10 ng/ml inhibits the net accumulation of neurotransmitters (γ-aminobutyric acid and dopamine) by brain synaptosomes. This action is due to a stimulation of neurotransmitter release. The half-maximum effect of the toxin is observed at 0.62 ng/ml. The effect of ciguatoxin is completely inhibited by tetrodotoxin ($K_{0.5}$ = 4 nM). Electrophysiological studies on neuroblastoma cells indicate that ciguatoxin induces a membrane depolarization which is prevented by tetrodotoxin and which is due to an action that increases Na^+ permeability. Under appropriate conditions ciguatoxin creates spontaneous oscillations in the membrane polarization level and repeated action potentials. Ciguatoxin stimulates $^{22}Na^+$ entry through the voltage-dependent Na^+ channels of neuroblastoma cells and rat skeletal myoblasts when it is used in synergy with veratridine, batrachotoxin, pyrethroids, sea anemone, or scorpion toxins. The half-maximum effect of ciguatoxin on $^{22}Na^+$ flux in the presence of veratridine occurs at a concentration of 0.5 ng/ml. Stimulation of $^{22}Na^+$ flux by ciguatoxin is abolished by tetrodotoxin. These results taken together indicate that ciguatoxin belongs to a new class of toxins acting on Na^+ channels.

Ciguatera, a specific endemic affliction of many tropical islands, is due to alimentary consumption of fresh reef fish (1). The origin of ciguatera poisoning is a toxin named CTX[1] (2) which is synthesized by a dinoflagellate (3). The structure of this toxin which is among the most potent marine toxins (1) is still unknown; however, it has been reported that it is an oxygenated polyether compound (4).

This paper analyzes the effects of CTX on neurotransmitter accumulation and release by rat brain synaptosomes, on $^{22}Na^+$ flux in nerve and muscle cells, and on the electrical properties of the neuroblastoma membrane.

* This work was supported by the Centre National de la Recherche Scientifique, by Grant 83.C.0918 from the Ministère de l'Industrie et de la Recherche, and by the Fondation pour la Recherche Médicale. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ Recipient of a NATO Science Fellowship (Z.W.O) and also supported by the Centre National de la Recherche Scientifique.

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[1] The abbreviations used are: CTX, ciguatoxin; TTX, tetrodotoxin; GABA, γ-aminobutyric acid; AS_{II} and AS_V, toxins II and V from *Anemonia sulcata*; Aa_{II}, toxin II from *Androctonus australis*; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

EXPERIMENTAL PROCEDURES

Materials—[^{3}H]GABA, [^{3}H]dopamine, and Biofluor were obtained from New England Nuclear. ^{22}NaCl was from the Commissariat à l'Energie Atomique (Saclay, France). Na valproate (Depakine) was obtained from Labaz Laboratories (France). Pargyline, ouabain, veratridine, and other chemicals were from Sigma. AS_{II} and AS_V were obtained according to Schweitz *et al.* (5). Aa_{II} was obtained according to Miranda *et al.* (6). TTX was from Sankyo Chemical Company (Tokyo, Japan). Deltamethrine was from Procida (Marseille, France). Batrachotoxin was a generous gift of Dr. J. Daly (National Institutes of Health, Bethesda, MD).

Preparation of CTX—CTX was extracted from muscle of *Gymnothorax javanicus* and then purified partially using the procedure described by Chanteau *et al.* (7). After the last step, the toxic fraction has a LD_{50} of 30 μg/kg (intraperitoneally) in mice. This fraction was further purified using high performance liquid chromatography (Waters Associates, Inc.) on a Lichrosorb RP-18 column (Merck) using CH_3CN/H_2O (65:35, by volume) as eluting solvent. Fig. 1 shows this last step of the purification of the toxin. The position of the active fraction in the eluate was determined by its lethality to mice (8) and by its effects on synaptosomal neurotransmitter transport (see later). The active fraction of CTX from the high performance liquid chromatography step was stored in lyophilized form or in methanol under nitrogen at −30 °C. Under these conditions activity is stable for at least 1 month. The quality of the purification procedure is shown by the following facts: (i) the peak of activity emerges in parallel with a peak of absorption at 208 nm (Fig. 1) and (ii) after rerunning the active fraction obtained in Fig. 1 on the same high performance liquid chromatography column with a linear 40–80% methanol gradient for 40 min at a flow rate of 1 ml min^{-1}, only one peak of activity emerges. Intraperitoneal (100 μl) and intracisternal (5 μl) injections into the fourth ventricle (9) were made into 20-g female Swiss mice. Intraperitoneal injection of purified CTX was lethal in 50% of the mice at a dose of 0.5–1 μg/kg. This is the most active preparation of CTX so far obtained. CTX is much more active when administrated by intracisternal injection since as little as 20 ng/kg induced complete paralysis of hind limbs, convulsions, and death of mice in times ranging from 10 to 60 min. The symptoms (8) induced in mice were the same at all stages of purification.

Accumulation and Release of Neurotransmitter by Synaptosomes—Synaptosomes were prepared from decerebellate brains of Sprague-Dawley rats (200 g) using standard techniques (10). [^{3}H]GABA and [^{3}H]dopamine accumulation measurements were carried out as previously described (10, 11). Rates of [^{3}H]GABA release from preloaded synaptosomes were measured as described by Abita *et al.* (10).

(Na^+,K^+)-ATPase Activity Measurements—(Na^+,K^+)-ATPase activity was measured as previously described (12).

Cell Culture—N1E 115 neuroblastoma cells were grown as previously described (13) using 24-well Costar tissue culture clusters. Cells were differentiated in the presence of medium containing 1% fetal calf serum and 1.5% dimethyl sulfoxide. Primary cultures of rat skeletal muscle cells were used at the myoblast stage after 4 days in culture (14).

Electrophysiological Measurements—Culture dishes containing differentiated neuroblastoma cells (N1E 115) (13) were mounted on a warmed stage (30 °C) of an inverted phase microscope. Immediately prior to the measurements, the culture medium was replaced by an external solution containing 115 mM NaCl, 5.4 mM KCl, 0.4 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM glucose, buffered by 25 mM HEPES/

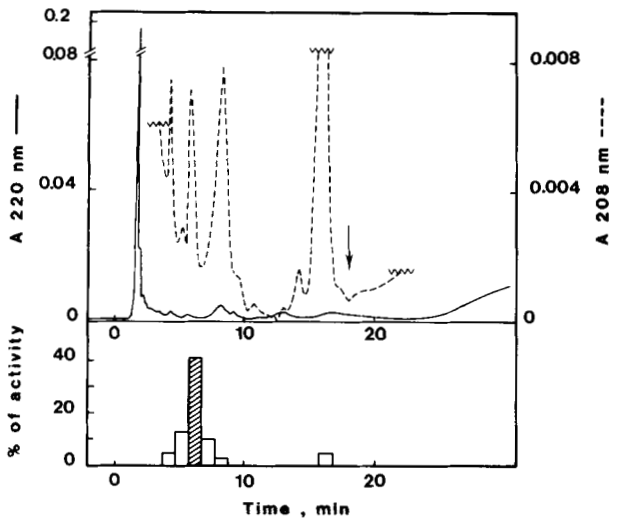

FIG. 1. **Final purification step of CTX by HPLC.** Partially purified CTX (3.4 μg) was solubilized in 70 μl of CH_3CN/H_2O (65:35, by volume) mixture and loaded on a Lichrosorb RP-18 column (7 μm, RT 20-4) with a Lichrosorb RP-18 precolumn (45 μm, 0.4 × 1 cm) equilibrated with the solvent described above. The flow rate was 1 $ml \cdot min^{-1}$. The column was first eluted with the equilibration solvent, then at 18 min (*arrow*) with a linear gradient from 65 to 95% CH_3CN for 10 min, and then the elution was continued with 95% of CH_3CN. The chromatography was monitored by absorbance at 220 and at 208 nm. One-ml fractions were collected and the activity of each fraction after solvent evaporation under nitrogen was measured as the capacity to inhibit [^{3}H]dopamine accumulation by synaptosomes (*lower part* of the figure). The percentage of activity indicated in the figure corresponds to the activity recovered as compared to the total activity loaded (100%). The activity profile has also been followed using the lethality of CTX to mice. Only the active fractions on [^{3}H]dopamine uptake are toxic to mice. The active fraction, used in this present work, is represented in a *shaded form*; it clearly corresponds to a single peak observed at 208 nm which emerges at 6–7 min.

NaOH at pH 7.4. Toxins were added directly to the external medium. Membrane potential measurements were performed using the suction pipette method (15). Pipette resistances were 300–500 kilo-ohms, and the pipette solution contained 10 mM NaCl, 115 mM K^+ glutamate, and 10 mM HEPES buffered at pH 7.1 with glutamic acid. Osmolarity was adjusted to 285 mosm/liter with sucrose.

$^{22}Na^+$ Uptake Measurements—Initial rates of $^{22}Na^+$ uptake were determined as previously described (13, 14).

Binding of Neurotoxins—Binding of ^{125}I-labeled sea anemone toxin V ($-AS_V$) (5), of the ^{125}I-labeled toxin II from *Androctonus australis Hector* ($-Aa_{II}$) (16) and ^{125}I-*Tityus* toxinγ (17), and of [^{3}H]ethylenediamine TTX (18) to synaptosomes was determined as previously described.

RESULTS AND DISCUSSION

Fig. 2 (*inset*) shows that CTX drastically decreases the net uptake of [^{3}H]GABA and of [^{3}H]dopamine by brain synaptosomes. The dose-response relation (Fig. 2) for the CTX inhibition of neurotransmitter accumulation indicates a Hill coefficient of 1.0 and a half-maximum effect of CTX at $K_{0.5}$ = 0.87 ng/ml.

TTX which by itself has only small effects on GABA or dopamine accumulation prevents CTX effects on neurotransmitter uptake (Fig. 3, *inset*). TTX inhibition of CTX action (Fig. 3) reveals a $K_{0.5}$ of 4 nM. This value corresponds closely with the dissociation constant of TTX (K_D = 5 nM) for the Na^+ channel in synaptosomes (10, 18).

Net uptake of neurotransmitters by synaptosomes is controlled both by the rates of influx and release. The decrease of the net uptake of neurotransmitters in the presence of CTX is mainly due to an increased rate of neurotransmitter release

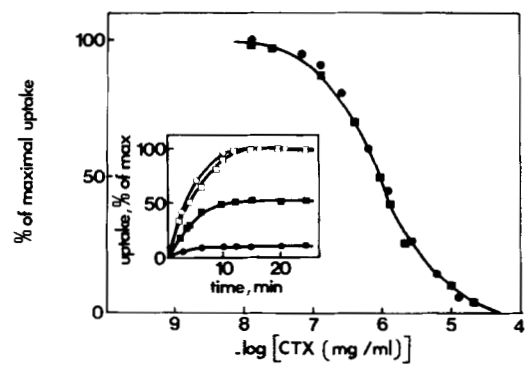

FIG. 2. **The influence of CTX on net accumulation levels of [^{3}H]GABA and [^{3}H]dopamine by rat brain synaptosomes.** Dose-response curve for CTX inhibition of the net uptake of [^{3}H]GABA (■) and of [^{3}H]dopamine (●) by synaptosomes. Time of uptake was 15 min. CTX-insensitive rates of [^{3}H]GABA and [^{3}H]dopamine uptakes were 3.7 nmol/mg of protein and 0.9 pmol/mg of protein, respectively. These values were subtracted from the total rate of uptake. *Inset*, the time course of [^{3}H]GABA (□, ■) and [^{3}H]dopamine (○, ●) uptake by synaptosomes. Synaptosomes (140–300 μg/ml) were equilibrated in the presence (■, ●) or in the absence (□, ○) of 10 ng/ml of CTX at 22 °C for 30 min (GABA uptake experiments) or at 30 °C for 15 min (dopamine uptake experiments). Uptake experiments were then initiated by the addition of labeled neurotransmitter and carried out as previously described (10, 11). Maximum uptake levels of [^{3}H]GABA and [^{3}H]dopamine were 7.0 nmol/mg of protein and 8.4 pmol/mg of protein, respectively.

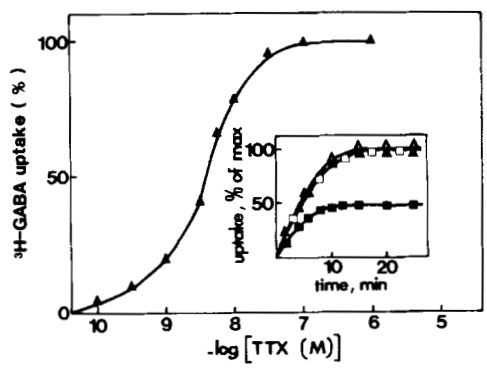

FIG. 3. **Antagonism by TTX of CTX inhibition of [^{3}H]GABA accumulation by rat brain synaptosomes.** Dose-response curve for TTX reversal of the CTX (10 ng/ml)-induced inhibition of the net [^{3}H]GABA uptake, measured after 15 min of incubation. 0 and 100% [^{3}H]GABA uptakes were 3.8 and 7.0 nmol/mg of protein, respectively. *Inset*, time course of [^{3}H]GABA uptake in the absence of toxin (□), in the presence of 10 ng/ml of CTX (■), in the presence of 1 μM TTX (△), and in the presence of 10 ng/ml of CTX and 1 μM TTX (▲).

(Fig. 4, *inset*). The concentration dependence of CTX-stimulated release of neurotransmitters (Fig. 4) yields a value of $K_{0.5}$ = 0.62 ng/ml, a value very similar to that found in Fig. 2. Accelerated neurotransmitter release due to CTX is also inhibited by 1 μM TTX (Fig. 4, *inset*).

The inhibition of net neurotransmitter accumulation and the stimulation of neurotransmitter release by CTX, as well as the inhibition of CTX effects by TTX, are similar to those previously observed with veratridine or with scorpion and sea anemone toxins (10). These toxins are known either to produce permanent activation of the Na^+ channel (veratridine) or to prolong drastically the lifetime of the open form of the channel (scorpion and sea anemone toxins) (19–21). The simplest explanation for the effects of CTX on net neurotransmitter accumulation and release is that the toxin activates voltage-dependent Na^+ channels producing a depolari-

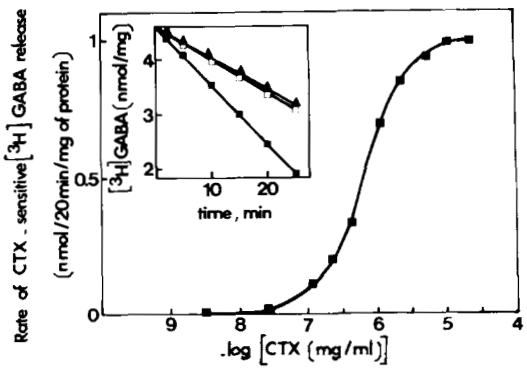

FIG. 4. **The influence of CTX on the release of [^{3}H]GABA by synaptosomes.** Dose-response curve for CTX activation of [^{3}H] GABA release from synaptosomes. Time of release was 20 min. The rate of CTX-sensitive [^{3}H]GABA release was the difference between the rate of release measured in the presence of a given concentration of CTX and the rate measured in the absence of toxin. *Inset,* time course of [^{3}H]GABA release by synaptosomes that have been incubated with [^{3}H]GABA for 20 min in the absence of toxins under conditions previously described (Fig. 2). Synaptosomes were separated from external GABA by centrifugation, and efflux was initiated as previously described (10), in the absence of toxin (□), in the presence of 10 ng/ml of CTX (■), and in the presence of 10 ng/ml of CTX and 1 μM TTX (▲).

zation of the synaptosomal membrane. This depolarization would in turn open Ca^{2+} channels provoking Ca^{2+} entry and neurotransmitter release. Membrane depolarization and transmitter release appear not to be due to an inhibition by CTX of the (Na^+,K^+)-ATPase since CTX is without effect on the activity of this enzyme (data not shown). Transmitter release is not due to an effect of CTX on Ca^{2+} channels since classical Ca^{2+} channel antagonists, D_{600} (5 μM) and nitrendipine (1 μM), had no effect (not shown). Conclusions obtained from this study of the CTX effects on neurotransmitter accumulation and release by synaptosomes are also consistent with independent observations obtained recently with partially purified CTX (22, 23) which have shown that the toxin induces a marked release of norepinephrine from presynaptic sites in the neuromuscular junction of the guinea pig vas deferens which was abolished in the presence of TTX.

The action of CTX on the electrical properties of neuroblastoma cells is presented in Fig. 5. Fig. 5*A* shows a control action potential evoked by a short depolarizing stimulus from a steady polarized level of −75 mV. Immediately after addition of CTX the membrane starts to depolarize. A steady membrane potential level between −50 mV and −20 mV is attained within 10 min (−48 mV in the particular experiment of Fig. 5*A*). After a hyperpolarizing stimulus of sufficient strength the membrane exposed to CTX does not immediately return to its depolarized level; instead the membrane potential remains close to the membrane potential obtained before addition of CTX for 1–10 s. Then the membrane slowly depolarizes again, and during this spontaneous depolarization, membrane potential oscillations and repeated action potentials are observed. This evoked passage between two membrane potential levels could be repeated a great number of times in the same cell for periods of several hours. Spontaneous transitions of membrane potential between a CTX-induced depolarized level and a more negatively polarized level are also occasionally observed and are very similar to the evoked transitions. The effects of CTX are quasi-irreversible (or very slowly reversible) since superfusion of neuroblastoma cells with a CTX-free solution fails to reverse the effects of CTX even after 2 h.

The effects of CTX on the electrical activity of neuroblastoma cells are the same when the toxin is applied to cells exposed to an external medium containing 25 mM tetraethylammonium with CsF in the pipette (Fig. 5*B*). Under these conditions K^+ channels are blocked (24). Moreover, addition of TTX (1 μM), a specific Na^+ channel blocker, in the external medium not only blocks electrical activity but also reverses CTX-induced membrane depolarization (Fig. 5*B*). Removal of TTX by perfusion with a CTX- and TTX-free medium induces the reappearance of the effects of CTX. This is because TTX action is easily reversible whereas CTX action is not. These results taken together strongly support the idea that the action of CTX is specific for voltage-dependent Na^+ channels. This conclusion is also consistent with the observation that unpurified CTX induces a TTX-sensitive depolarization of the skeletal muscle membranes (25).

Electrophysiological experiments with neuroblastoma cells were also carried out in an external medium containing 10 mM instead of 1.8 mM Ca^{2+}. Under these conditions, the Ca^{2+} current due to the slow Ca^{2+} channels of neuroblastoma cells is sufficiently high to produce a Ca^{2+} channel component in the action potential and to be seen by voltage clamp techniques (26, 27). CTX is without effect on the electrical properties of neuroblastoma in a 10 mM Ca^{2+} medium (data not shown). This result shows: (i) that CTX has no action on slow Ca^{2+} channels confirming the specificity of the toxin for Na^+ channels, (ii) that CTX action on Na^+ channels is antagonized by Ca^{2+}. The same lack of effect of TTX has been found in 10 mM Ca^{2+} medium. Antagonism between Ca^{2+} and Na^+ channel toxins has been observed with veratridine (28).

Measurements of $^{22}Na^+$ flux have been used successfully in recent years to analyze the pharmacological and mechanistic properties of the Na^+ channel (13, 14, 29). Two cells in culture have been used in our work, neuroblastoma cells (N1E 115 clone) which have TTX-sensitive Na^+ channels ($EC_{50(TTX)}$ = 1 nM) (13) and rat skeletal muscle myoblasts which have TTX-resistant Na^+ channels ($EC_{50(TTX)}$ = 1 μM) (14). CTX has no significant action by itself on the rate of $^{22}Na^+$ uptake by these cells (Fig. 6). This observation may seem surprising in view of the fact that CTX has a depolarizing effect which seems to be due to an action on the Na^+ channel (Fig. 5). However, in order to obtain a CTX-induced stimulation of $^{22}Na^+$ uptake one would need to have a Na^+ entry through Na^+ channels opened by the toxin which is significantly higher than the Na^+ entry into the excitable cells via other Na^+ entry systems. For example, the Na^+/H^+ exchange system represents a large part of the Na^+ entry in excitable cells when Na^+ channels are closed. It appears that $^{22}Na^+$ uptake elicited by CTX alone is not significantly higher than uptake through other pathways. The lack of increase of the TTX-sensitive Na^+ uptake by CTX suggests that a small population of Na^+ channels is affected by the toxin and/or that CTX-modified Na^+ channels do not remain permanently open. This view is consistent with the observations that CTX only produces a partial depolarization of the membrane (which remains negatively polarized even after application of the toxin). However, CTX acts in synergy with alkaloid toxins veratridine and batrachotoxin, with polypeptide toxins from sea anemone and scorpion venom, and with the pyrethroid molecule deltamethrine to increase the rate of $^{22}Na^+$ uptake (Figs. 6 and 7). This acceleration of $^{22}Na^+$ uptake is due to an activation of Na^+ channels since the effect of CTX is antagonized by TTX (Fig. 7). The dose-response curve for CTX action on $^{22}Na^+$ flux in neuroblastoma cells in the presence of veratridine (Fig. 7) shows half-maximal response at 0.5 ng/ml, a value similar to that found in synaptosomes (Figs. 2 and

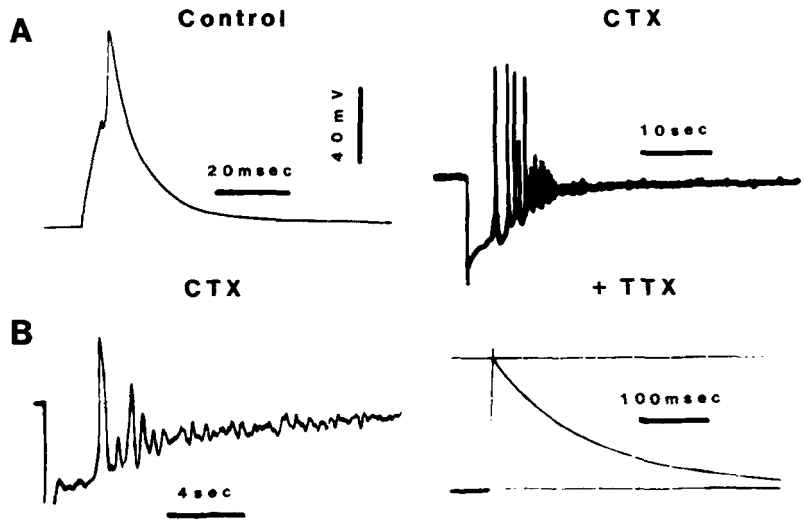

FIG. 5. **Electrophysiological action of CTX on electrically evoked action potentials in neuroblastoma cells.** *A, left,* control action potential before adding CTX, evoked by a depolarizing stimulus from a steady hyperpolarized membrane potential level of −75 mV. *Right,* electrical activity recorded 2 min after the addition of 10 ng/ml of CTX to the external solution. The membrane was depolarized by the action of the toxin to −48 mV, and a hyperpolarizing stimulus (−1.5 nA for 100 ms) evoked slow transient membrane action potentials. *B, left,* electrical activity recorded 4 min after the addition of 1 ng/ml of CTX in the presence of 25 mM tetraethylammonium. Membrane was depolarized to −20 mV 16 min after application of the toxin, and the transient membrane hyperpolarization was evoked by a hyperpolarizing stimulus of −0.5 nA for 1 s. Action potential duration is prolonged because K^+ current was blocked by tetraethylammonium. *Right,* effect of the addition of 1 μM TTX on the membrane potential of the same cell. The cell was repolarized by the action of TTX to the control level of −70 mV, and even a large depolarizing stimulus no longer evoked an active electrical response. Zero and resting membrane potentials are indicated.

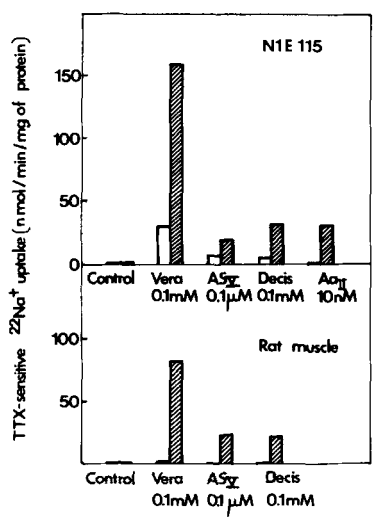

FIG. 6. **Synergistic effects of CTX and other neurotoxins specific for the Na^+ channel on the initial rate of TTX-sensitive $^{22}Na^+$ uptake by neuroblastoma cells and rat muscle cells.** Initial rates of $^{22}Na^+$ uptake were determined after 1 min of uptake in the presence of various neurotoxins used alone (*open bars*) or in combination with 2.9 ng/ml of CTX (*hatched bars*). The rate of TTX-insensitive $^{22}Na^+$ uptake was subtracted from all data. *Vera,* veratridine; *Decis,* deltamethrine.

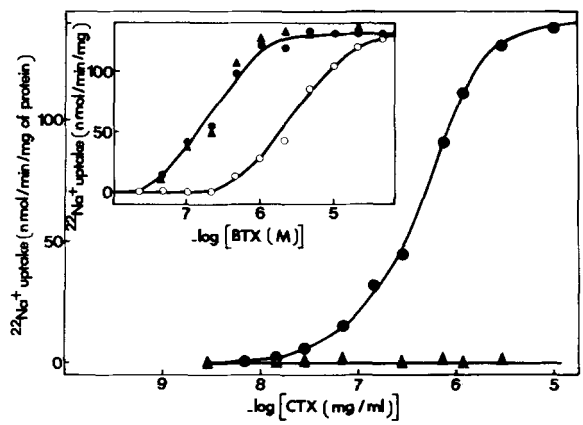

FIG. 7. **Dose-response curve for CTX action on $^{22}Na^+$ uptake neuroblastoma cells.** Initial rates of $^{22}Na^+$ uptake were determined as described elsewhere (13, 14) in the presence of 0.1 mM veratridine and increasing CTX concentrations (●) and of 0.1 mM veratridine plus 0.1 μM TTX and increasing CTX concentrations (▲). The contribution due to the presence of veratridine was subtracted in both curves. The Na^+ uptake component which is shown in the dose-response curve corresponds to the following situation: Na^+ uptake measured in the presence of 0.1 mM veratridine plus CTX minus Na^+ uptake measured in the presence of 0.1 mM veratridine alone (50 nmol/min/mg). The same conditions were used in the experiments carried out in the presence of TTX. *Inset,* dose-response curve for batrachotoxin (*BTX*) action on the rate of TTX-sensitive $^{22}Na^+$ uptake in the absence of CTX (○), in the presence of 2.9 ng/ml of CTX (●), and, for comparison, in the presence of 1 μM AS_{II} (▲).

4). The *inset* of Fig. 7 shows how CTX increases batrachotoxin action on $^{22}Na^+$ flux. CTX at a saturating concentration shifts the dose-response curve for batrachotoxin to a lower concentration range. The EC_{50} for batrachotoxin action is reduced from 3.0 to 0.23 μM in the presence of 2.9 ng/ml of CTX. This shift is identical to that found with a saturating concentration of the sea anemone toxin AS_{II} (Fig. 7, *inset*) which is also known to be synergistic in its action with batrachotoxin (13).

Pyrethroids are also knwon to have the same type of action on batrachotoxin action (30). Neurotoxins active on the Na^+ channel have been distinguished in 5 separate classes (20, 21, 31, 32) including (i) the Na^+ channels antagonists TTX and saxitoxin, (ii) the lipid-soluble toxins (veratridine, batrachotoxin, grayanotoxins, etc.) that cause persistent activation of

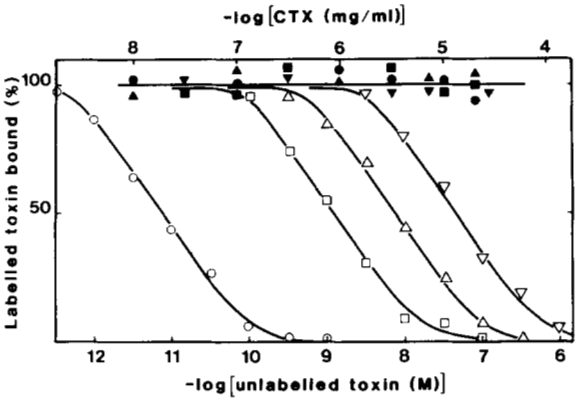

FIG. 8. **The influence of CTX on the specific binding of several radiolabeled toxins to their respective receptor sites on Na⁺ channels in synaptosomes.** Competition between ^{125}I-*Tityus* toxin γ and unlabeled *Tityus* toxin γ (○) or CTX (●). Competition between ^{125}I-Aa_{II} and unlabeled Aa_{II} (□) or CTX (■). Competition between ^{125}I-AS_V and unlabeled As_V (▽) or CTX (▼). Competition between the tritiated derivative of TTX, [^{3}H]ethylenediamine TTX (18), and unlabeled TTX (△) or CTX (▲). Experiments have been carried out as previously described (5, 16–18). In these experiments, synaptosomes have been incubated for 30 min at 20 °C with the indicated concentrations of the unlabeled toxins (*Tityus* toxin γ, Aa_{II}, AS_V, TTX, and CTX) before adding the labeled toxin, the addition being followed by a second incubation of 30 min to 120 min depending on the labeled toxins (5, 16–18). The following concentrations of labeled toxins have been used: ^{125}I-*Tityus* toxin γ, 10^{-11} M; ^{125}I-Aa_{II}, 0.4 nM; ^{125}I-AS_V, 10 nM; and [^{3}H]ethylenediamine TTX, 1 nM. Protein concentrations in synaptosomes were 4 μg/ml, 1 mg/ml, 1 mg/ml, and 0.5 mg/ml for binding experiments with ^{125}I-*Tityus* toxin γ, ^{125}I-Aa_{II}, ^{125}I-AS_V, and [^{3}H]ethylenediamine TTX, respectively. The term unlabeled toxin in the abscissa refers to *Tityus* toxin γ, Aa_{II}, AS_V, and TTX. Ordinate values show the specific binding components of the different toxins which are defined as the difference between the total and the nonspecific binding (5, 16–18).

Na^+ channels, (iii) the polypeptide toxins including sea anemone toxins and some of the scorpion toxins (*Leiurus*, *Androctonus*) that slow Na^+ current inactivation, (iv) the scorpion toxins (*Centruroides*, *Tityus*) that are primarily active on Na^+ channel activation. They shift the voltage dependence of the activation of Na^+ channels and induce a Na^+ channel activity at negative potentials at which Na^+ channels are normally closed (in the resting state) (32). These toxins do not produce a persistent membrane depolarization. (v) Another class is the pyrethroids that transform fast Na^+ channels into slower ones.

CTX is synergistic in its action with toxins of class (ii), (iii), and (v). Therefore, CTX cannot obviously associate with the respective receptors of these 3 toxin classes. This conclusion is confirmed for toxins of class (iii) by the fact that CTX has no effect on the binding of ^{125}I-labeled AS_V and Aa_{II} to their receptor sites in synaptosomes (Fig. 8).

TTX is a noncompetitive inhibitor of CTX action since the concentration dependence of TTX inhibition of CTX action on [^{3}H]GABA uptake (Fig. 3) is independent of CTX concentration (data not shown). Therefore, TTX and CTX obviously bind to different receptor sites. This conclusion is confirmed by the observation that CTX does not prevent [^{3}H]ethylenediamine TTX binding to the TTX receptor site in synaptosomes (Fig. 8).

Finally CTX has no effect on ^{125}I-*Tityus* toxin γ binding to receptor sites of class (iv) in synaptosomes (Fig. 8).

In conclusion, CTX appears to be a toxin acting selectively on voltage-dependent Na^+ channels in nerve and muscle cells as well as on synaptic terminals. The electrophysiological behavior of CTX is unlike any other Na^+ channel toxins. $^{22}Na^+$ flux measurements and experiments involving competition of CTX with a variety of other labeled Na^+ channel toxins indicate that CTX belongs to a new class of Na^+ channel toxins. There are now six different classes of such toxins.

Acknowledgments—We are very grateful to Dr. A Lombet, D. Pauron, and J. Barhanin for carrying out binding experiments, to Dr. G. Romey for very useful discussions, to Drs. J. Taylor and M. Starzak for their careful reading of the manuscript, and to M. Valetti for secretarial assistance.

REFERENCES

1. Bagnis, R. (1981) *Oceanol. Acta* **4,** 375–387
2. Scheuer, P. J., Takahashi, W., Tsutsumi, J., and Yoshida, T. (1967) *Science (Wash. D. C.)* **155,** 1267–1268
3. Bagnis, R., Chanteau, S., Chungue, E., Hurtel, J. M., Yasumoto, T., and Inoue, A. (1980) *Toxicon* **18,** 199–208
4. Murakami, Y., Oshima, Y., and Yasumoto, T. (1982) *Bull. Jpn. Soc. Sci. Fish.* **48,** 69–72
5. Schweitz, H., Vincent, J. P., Barhanin, J., Frelin, C., Linden, G., Hugues, M., and Lazdunski, M. (1981) *Biochemistry* **20,** 5245–5257
6. Miranda, F., Kupeyan, C., Rochat, H., Rochat, C., and Lissitzky, S. (1970) *Eur. J. Biochem.* **16,** 514–523
7. Chanteau, S., Bagnis, R., and Yasumoto, T. (1976) *Biochimie* **58,** 1149–1151
8. Hoffman, P. A., Granade, H. R., and McMillan, J. P. (1983) *Toxicon* **21,** 363–369
9. Schanberg, S. M., Schildkraut, J. J., and Kopin, J. J. (1967) *J. Pharmacol. Exp. Ther.* **157,** 311–318
10. Abita, J.-P., Chicheportiche, R., Schweitz, H., and Lazdunski, M. (1977) *Biochemistry* **16,** 1838–1844
11. Vignon, J., and Lazdunski, M. (1984) *Biochem. Pharmacol.* **33,** 700–702
12. Bidard, J. N., Rossi, B., Renaud, J. F., and Lazdunski, M. (1984) *Biochim. Biophys. Acta* **769,** 245–252
13. Jacques, Y., Fosset, M., and Lazdunski, M. (1978) *J. Biol. Chem.* **253,** 7383–7392
14. Frelin, C., Vigne, P., and Lazdunski, M. (1983) *J. Biol. Chem.* **258,** 6272–6276
15. Hugues, M., Romey, G., Duval, D., Vincent, J. P., and Lazdunski, M. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **79,** 1308–1312
16. Vincent, J. P., Balerna, M., Barhanin, J., Fosset, M., and Lazdunski, M. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 1646–1650
17. Barhanin, J., Giglio, J. R., Léopold, P., Schmid, A., Sampaio, S. V., and Lazdunski, M. (1982) *J. Biol. Chem.* **257,** 12553–12558
18. Chicheportiche, R., Balerna, M., Lombet, A., Romey, G., and Lazdunski, M. (1980) *Eur. J. Biochem.* **104,** 617–625
19. Romey, G., Abita, J. P., Chicheportiche, R., Rochat, A., and Lazdunski, M. (1976) *Biochim. Biophys. Acta* **448,** 607–619
20. Lazdunski, M., Fosset, M., Renaud, J. F., Schweitz, H., Vigne, P., and Vincent, J. P. (1983) in *Physical Chemistry of Transmembrane Ion Motions* (Spach, G., ed) pp. 491–504, Elsevier Science Publishers B.V., Amsterdam
21. Lazdunski, M., and Renaud, J. F. (1982) *Annu. Rev. Physiol.* **44,** 463–473
22. Ohizumi, Y., Shibata, S., and Tachibana, K. (1981) *J. Pharmacol. Exp. Ther.* **217,** 475–480
23. Ohizumi, Y., Ishida, Y., and Shibata, S. (1982) *J. Pharmacol. Exp. Ther.* **221,** 748–752
24. Moolenaar, W. H., and Spector, I. (1979) *J. Physiol. (Lond.)* **292,** 297–306
25. Rayner, M. D. (1972) *Fed. Proc.* **31,** 1139–1145
26. Moolenaar, W. H., and Spector, I. (1979) *J. Physiol. (Lond.)* **292,** 307–323
27. Romey, G., and Lazdunski, M. (1982) *Nature (Lond.)* **297,** 79–80
28. Ulbricht, W. (1972) *Pflügers Arch. Eur. J. Physiol.* **336,** 187–199
29. Frelin, C., Vigne, P., and Lazdunski, M. (1981) *Eur. J. Biochem.* **119,** 437–442
30. Jacques, Y., Romey, G., Cavey, M. T., Kartalovski, B., and Lazdunski, M. (1980) *Biochim. Biophys. Acta* **600,** 882–897
31. Jaimovich, E., Ildefonse, M., Barhanin, J., Rougier, O., and Lazdunski, M. (1982) *Proc. Natl. Acad. Sci. U. S. A.* **79,** 3896–3900
32. Barhanin, J., Pauron, D., Lombet, A., Norman, R. I., Vijverberg, H. P. M., Giglio, J. R., and Lazdunski, M. (1983) *EMBO J.* **2,** 915–920