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The Interaction of Ervatamine and Epiervatamine with the Action Potential Na^+ Ionophore

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SUMMARY

FRELIN, C., P. VIGNE, G. PONZIO, G. ROMEY, Y. TOURNEUR, H. P. HUSSON, AND M. LAZDUNSKI. The interaction of ervatamine and epiervatamine with the action potential Na^+ ionophore. *Mol. Pharmacol.* **20**:107-112 (1981).

The alkaloid ervatamine blocks the voltage-sensitive Na^+ conductance. EC_{50} for different cell lines and for synaptosomes range between 1-3 and 5-12 μM. The toxic compound also alters the K^+ conductance, although less efficiently than the Na^+ conductance. Epiervatamine, a structural analogue of ervatamine, is 4-8 times more potent than ervatamine. Dose-response curves for ervatamine and epiervatamine show that the compounds bind to a single class of binding sites. Competition experiments with a radiolabeled tetrodotoxin (TTX) derivative indicate that these binding sites are distinct from the TTX receptor site. Ervatamine and epiervatamine seem to inhibit the Na^+ current (or Na^+ flux) by acting on the channel-gating mechanism. $^{22}Na^+$ uptake measurements with neuroblastoma cells show that epiervatamine is a competitive inhibitor of the action of batrachotoxin, a well known specific activator of the Na^+ channel. The dissociation constant of epiervatamine from its receptor site is 1.7 μM, very similar to that of batrachotoxin K_d = 1.3 μM from a receptor site which could then be the same as that for epiervatamine. Although ervatamine and epiervatamine, like TTX, can inhibit the action that sea anemone and scorpion toxins have on Na^+ channels, none of them antagonize the binding of these radiolabeled polypeptide toxins to their own receptor sites on the gating system of the channel. A comparison has been made of the effects of ervatamine and epiervatamine on one hand, and yohimbine and tetracaine on the other hand. The mechanisms of action of yohimbine, ervatamine, and epiervatamine appear to be very similar. However, epiervatamine is 10 times more potent than yohimbine.

INTRODUCTION

Ervatamine is an acyl-indolic alkaloid first isolated from an Australian tree, *Ervatamia orientalis*, which belongs to the Apocynaceous family (1). Other alkaloids like reserpine and yohimbine have been isolated from plants of the same family. Ervatamine has been synthesized by Husson *et al.* (2). Very little was known about the pharmacological properties of the alkaloids before reports by Pichon and Sauviat (3) and Lees and Pichon (4) showed that ervatamine blocks the fast Na^+ conductance in isolated giant axons of the cockroach and of the squid. The block occurs in a frequency-dependent manner.

In this paper, we analyze the mechanism of action of ervatamine and one of its derivatives, epiervatamine, on various neuronal preparations: N1E 115 neuroblastoma cells, C_9 cells, and brain synaptosomes. We used three different approaches: voltage-clamp experiments, ion flux methods, and binding experiments with a radiolabeled TTX[2] derivative and radiolabeled sea anemone and scorpion toxins. A comparison is made with the action of yohimbine and tetracaine. The structural formulas for ervatamine, epiervatamine, and yohimbine are shown in Fig. 1.

MATERIALS AND METHODS

Veratridine and yohimbine were obtained from Aldrich Chemical Company, Inc. (Milwaukee, Wisc.), ouabain

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[1] Institute de Chimie des Substances Naturelles.

[2] The abbreviations used are: TTX, tetrodotoxin; Hepes, 4-(2-hydroxyethyl-1-piperazineethanesulfonic-acid; ATX_{II}, *Anemonia sulcata* $toxin_{II}$; $ScTX_{II}$, *Androctonus australis* $toxin_{II}$.

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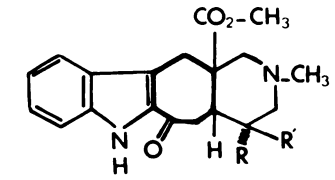
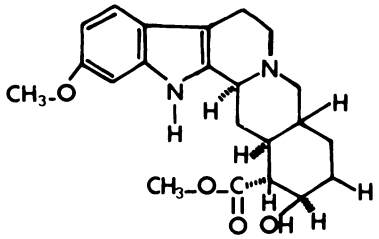


ERVATAMINE and EPIERVATAMINE



YOHIMBINE

FIG. 1. *The structure of ervatamine, epiervatamine, and yohimbine* Ervatamine, R = H, R′ = C_2H_5; epiervatamine, R = C_2H_5, R′ = H.

and tetracaine from Sigma Chemical Company (St. Louis, Mo.), TTX from Sankyo Company, Ltd. (Tokyo, Japan), ^{22}NaCl, ^{86}RbCl and L-[^{3}H]leucine from the CEA (Saclay, France). Batrachotoxin was kindly given to us by Drs. J. Daly (National Institutes of Health, Bethesda, Md.) and T. Tokuyama, Osaka City University (Osaka, Japan). The sea anemone toxin$_{II}$ was purified from *Anemonia sulcata* according to Beress *et al.* (5) and the toxin$_{II}$ from *Androctonus australis* venom according to Miranda *et al.* (6). Ervatamine and epiervatamine were synthesized as described elsewhere (2). [^{3}H]en-TTX (27 Ci/mmol) was prepared as described by Chicheportiche *et al.* (7). Iodination of sea anemone toxin$_{II}$ and scorpion toxin$_{II}$ was performed as described previously (8). Specific activities were 1400 Ci/mmol for scorpion toxin and 2000 Ci/mmol for sea anemone toxin$_{II}$.

Cells from the N1E 115 neuroblastoma clone were grown as previously described (9) by using 24 wells of Costar tissue culture clusters and were allowed to differentiate in a medium containing 1% fetal calf serum and 1.5% dimethyl sulfoxide. The C_9 cell line is derived from a bladder metastasis of an *N*-ethyl-*N*-nitrosourea-induced rat brain tumor (10, 11). C_9 cells were grown as previously described (12). Neuroblastoma and C_9 cells were labeled with L-[^{3}H]leucine (0.2 μCi/ml) for 48 hr before the uptake experiments to measure protein recovery from the ^{3}H counts.

Initial rates of $^{22}Na^+$ uptake by N1E 115 neuroblastoma and C_9 cells were determined as follows. Cells were incubated for 20 min at 37° in a Na^+-free medium consisting of 140 mM choline chloride, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 20 mM Hepes-Tris (pH 7.4), and neurotoxins. It has been verified that this incubation time was long enough to ensure the complete association to equilibrium of all the toxins used, including ervatamine and epiervatamine. Cells were then shifted to a medium consisting of 130 mM choline chloride, 5.4 mM KCl, 10 mM NaCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 20 mM Hepes-Tris (pH 7.4) and supplemented with neurotoxins, 0.5 mM ouabain, and 0.1 μCi/ml of ^{22}NaCl. The initial rates of $^{22}Na^+$ uptake were determined after 1 min of incubation under conditions previously used in this laboratory (9).

Rat brain synaptosomes were prepared according to Abita *et al.* (13). Rates of sea anemone toxin$_{II}$-induced $^{86}Rb^+$ release from preloaded synaptosomes were measured as described elsewhere (14).

Rat brain synaptosomal membranes were prepared according to Krueger *et al.* (15). Binding experiments with [^{3}H]en-TTX were carried out as described elsewhere (7, 16). Competition experiments between [^{3}H]en-TTX and either unlabeled TTX, ervatamine, epiervatamine, tetracaine, or yohimbine, were performed as follows: synaptic membranes (90 μg of proteins) were incubated at 4° for 5 min in 300 μl of 25 mM Tris-HCl buffer at pH 7.6 containing 50 mM choline chloride and varying amounts of competitor. [^{3}H]en-TTX (5 nM) was then added and equilibration was allowed to proceed for 15 min. [^{3}H]en-TTX bound to membranes was determined by filtering 90-μl aliquots through wetted GF/B glass fiber filters (Whatmann, Inc.; Clifton, N. J.). Filters were washed twice in less than 5 sec with 2.5 ml of an ice-cold 20 mM Tris-HCl buffer at pH 7.6 containing 100 mM choline chloride and counted in 8 ml of Picofluor 30 (Packard Instrument Company; Downers, Grove, Ill.).

Binding experiments involving synaptosomes and either [^{125}I]ATX$_{II}$ or [^{125}I]ScTX$_{II}$ were performed as previously described (8). As for [^{3}H]en-TTX binding experiments, competitors were added to synaptosomes before the labeled neurotoxins.

In voltage-clamp measurements, culture dishes containing N1E 115 neuroblastoma cells were directly used after replacing the culture medium by an Earle solution (NaCl, 115 mM; KCl, 5.4 mM; $MgSO_4$, 0.4 mM; $CaCl_2$, 1.8 mM; Hepes-NaOH, 25 mM; and glucose, 5 mM) buffered at pH 7.4. The culture dish was placed on the warm stage of an inverted microscope (Leitz-Diavert, Wetzlar, West Germany) and the temperature was maintained at 34 ± 1°. Voltage-clamp experiments were performed by using a new suction pipette method (17–19). The technique described by Lee *et al.* (19) for snail neurons was adapted to neuroblastoma cells. The internal solution was 10 mM NaH_2PO_4, 1 mM $MgCl_2$, and 115 mM glutamic acid adjusted to pH 7.1 with KOH and to an osmotic pressure of 305 mOsm with sucrose. The ionic currents were simultaneously digitized and stored by a digital computer (Intertechnique-Plurimat S) and displayed on storage oscilloscope (Tektronix 5103N).

RESULTS

Voltage-clamp experiments describing the effects of ervatamine on ionic channels in neuroblastoma cells are represented in Figs. 2 and 3. The effect of ervatamine on the Na^+ conductance was measured in the presence of 10 mM tetraethylammonium, which is known to block specifically the outward K^+ current (20, 21). The effect of the drug on the K^+ conductance was analyzed in the presence of 0.1 μM TTX, which is known to block specifically the Na^+ conductance (21). Fig. 2A shows the blockade of the Na^+ current by ervatamine at a concentration of 50 μM. With one stimulation every 40 sec, the Na^+ current was maximally inhibited to 80% of its control value after a 20-min application of the toxic compound. If after 4 min of toxic application, when 50% of the Na^+

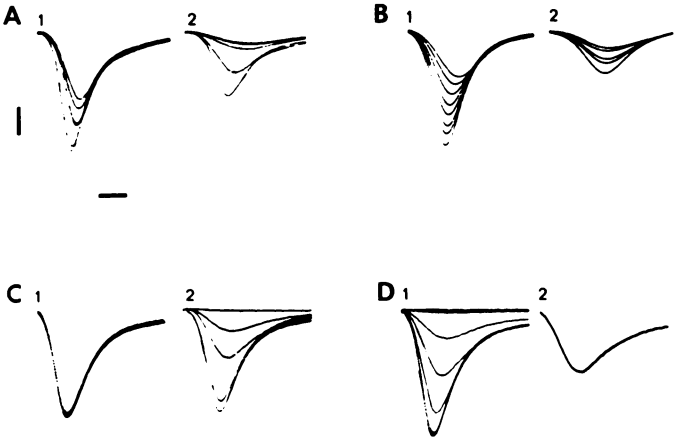


FIG. 2. *Voltage-clamp analysis of the effects of ervatamine (A), epiervatamine (B), yohimbine (C), and TTX (D) on the Na⁺ current of N1E 115 neuroblastoma cells*

In each record, the K^+ current was blocked with 10 mM tetraethylammonium. Depolarizing voltage pulses of 70-mV amplitude and 10-msec duration were applied from a holding potential of −90 mV. Because of the complete Na^+ inactivation obtained at the end of each voltage pulse, it was possible to suppress the initial capacitive current simply by adding the ON-response of the current to the OFF-response.

In (1), successive recordings were taken at 40-sec (A–C) or at 10-sec (D) intervals after toxin application. In (2), the use-dependent action of the toxins have been tested by stimulating at a frequency of 10 stimulations/sec; recordings presented in the figures have been collected at intervals of 0.5 sec.

A. The Na^+ current was inhibited to 80% of its control value after a 20-min application of 50 μM ervatamine (not shown): 50% of the Na^+ channels were blocked after a 4-min application of the toxin (A1). A fast repetitive stimulation applied at this level of Na^+ current inhibition then blocked nearly completely the Na^+ channels in less than 2 sec (A2).

B. A 20-min application of 50 μM epiervatamine blocked completely the Na^+ current (not shown): 70% of the Na^+ channels were blocked after a 5-min application of the toxin (B1). The fast repetitive stimulation then blocked 60% of the remaining active channels in 2 sec.

C. The Na^+ current remained unchanged after a 15-min application of 10 μM yohimbine (C1). After this period of incubation with the toxin, the fast repetitive stimulation completely blocked the Na^+ channels in a few seconds (C2).

D. The blocking action of 0.1 μM TTX on the Na^+ current is fast and complete (D1). No use-dependent effect was seen by following the result of a fast repetitive stimulation (30 stimulations/sec) applied for 1 sec just after 50% of the Na^+ channels were blocked by 0.1 μM TTX (D2). Current scale: 20 nA; time scale: 1 msec.

channels are blocked, the neuroblastoma cell is stimulated at a frequency of 10 stimulations/sec, then nearly a complete block of functional channels occurs in less than 2 sec. The same type of observation was made in Fig. 2B with epiervatamine. Here again, repetitive depolarizing pulses increase the rate of inhibition of the Na^+ current.

A comparison has been made in Fig. 2C with the indolealkylamine alkaloid yohimbine, which has been shown to produce a use-dependent inhibition of the sodium current in the squid giant axon (22). Figure 2C shows the very important use-dependent action of yohimbine on neuroblastoma cells. Figure 2D confirms the well known fact that the action of TTX on Na^+ channels is not use-dependent. Figures 3A1 and A2 show the action of ervatamine and epiervatamine on the K^+ channel. Concentration of 0.1 mM ervatamine or 50 μM epiervatamine, which completely blocked the Na^+ current, only blocked partially the K^+ conductance.

Comparative current-voltage relationships for steady-state K^+ currents before and after application of 50 μM epiervatamine are presented in Fig. 3B.

Veratridine and sea anemone toxins are specific of the Na^+ channel (23, 24). These two toxins act in synergy to produce an important stimulation of $^{22}Na^+$ uptake through the Na^+ channel of neuroblastoma and other cells, which is completely blocked by TTX (9, 12). Both cell lines used in the present work differ in their sensitivity to TTX. The Na^+ channel of neuroblastoma cells is highly sensitive to TTX (EC_{50} = 5 nM); it is much less sensitive to this toxin in C_9 cells (EC_{50} = 0.3 μM) (9, 12). Figure 4 shows that like TTX, ervatamine and epiervatamine are able to inhibit completely the increase of $^{22}Na^+$ uptake produced by the mixture of veratridine and ATX_{II}. Epiervatamine (EC_{50} = 3 μM) is more potent than ervatamine (EC_{50} = 12 μM). The sensitivity of Na^+ channels to ervatamine and epiervatamine is the same in neuroblastoma cells and in C_9 cells.

Figure 4 presents a comparison of the effects of ervatamine (or epiervatamine) on one hand and of yohimbine and the local anaesthetic molecule tetracaine on the other hand. Blockade of Na^+ channels by yohimbine and by tetracaine was observed with EC_{50} of 120 and 10 μM respectively. Dose-response curves for TTX, ervatamine, epiervatamine, and yohimbine are characterized by Hill coefficients close to 1.

Since there is obviously an analogy of action between yohimbine and ervatamine (or epiervatamine), and since yohimbine has been reported to be a competitive inhibitor of the action of batrachotoxin on the Na^+ channel (25), the analysis of interactions between the epiervatamine and the batrachotoxin receptor has been made in Fig. 5. To determine whether epiervatamine blocks batrachotoxin activation of sodium channels in a competi-

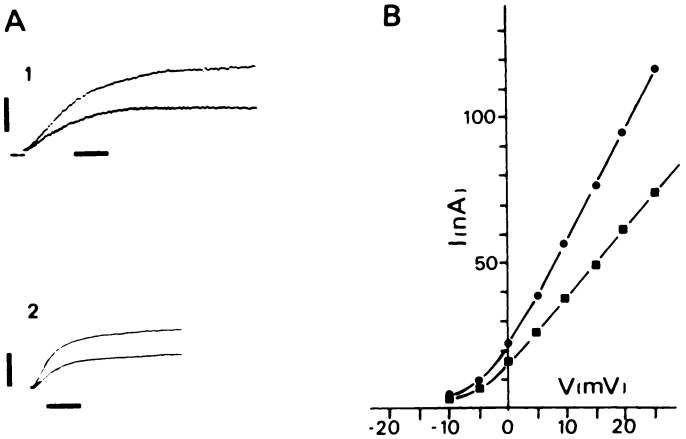


FIG. 3. *Voltage-clamp analysis of ervatamine and epiervatamine actions on K⁺ currents of N1E 115 neuroblastoma cells*

A. The Na^+ current was blocked with 0.1 μM TTX, and the membrane potential was clamped at 5 mV after a step change from a holding potential of −70 mV. Partial inhibition of the K^+ current after a 20-min application of 0.1 mM ervatamine (A1) or 50 μM epiervatamine (A2). Current scale: 20 mA; time scale: 2.5 msec (A1) and 5 msec (A2).

B. Current voltage relations for steady-state K^+ currents before (●) and after (■) application of 50 μM epiervatamine. Similar results were obtained with 0.1 mM ervatamine.

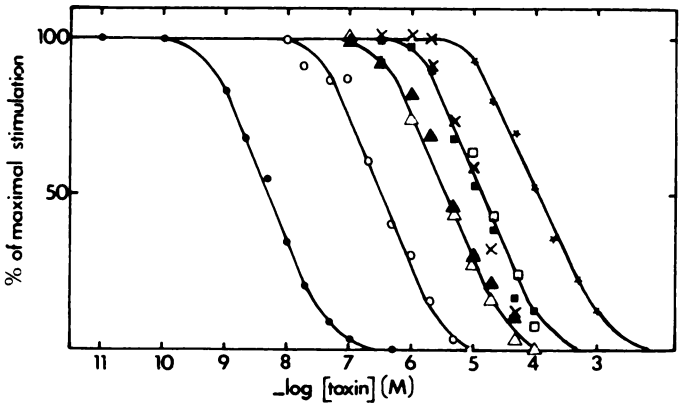


FIG. 4. *Inhibition of toxin-stimulated $^{22}Na^+$ uptake in N1E 115 neuroblastoma cells and C_9 cells*

The initial rates of $^{22}Na^+$ uptake stimulated by 10 μM ATX_{II} and 100 μM veratridine were inhibited by increasing concentrations of TTX (● or ○), ervatamine (■ or □), epiervatamine (▲ or △), tetracaine (×), and yohimbine (★); neuroblastoma cells (filled symbols, ×, ★) and TTX-insensitive C_9 cells (open symbols). *Curves* have been fitted according to Jacques *et al.* (9) and are characterized by the following EC_{50} and Hill coefficients: TTX (N1E 115: EC_{50} = 5 nM, n_H = 1.0 C_9: EC_{50} = 0.3 μM, n_H = 1.0), ervatamine (N1E 115: EC_{50} = 12 μM, n_H = 1.0; C_9: EC_{50} = 17 μM, n_H = 1.1), epiervatamine (N1E 115: EC_{50} = 3.3 μM, n_H = 0.9; C_9: EC_{50} 3.1 μM, n_H = 0.9), yohimbine (N1E 115: EC_{50} = 120 μM, n_H = 0.9), tetracaine (N1E 115: EC_{50} = 11 μM, n_H = 1.4; C_9: EC_{50} = 5.1 μM, n_H = 1.4).

tive manner, $^{22}Na^+$ uptake measurements have been made at different concentrations of batrachotoxin with various fixed amounts of epiervatamine. Lineweaver-Burk plots in Fig. 5 indicate that experimental data fall on straight lines with slopes varying with epiervatamine concentration. These Lineweaver-Burk plots correspond to the equation $1/V = (1/V_{max}) + (K_{0.5}/V_{max}B)$, where V is the velocity of $^{22}Na^+$ influx measured at a given concentration of batrachotoxin and of epiervatamine, V_{max} is the maximal rate at "infinite" concentration of batrachotoxin, $K_{0.5}$ is the apparent dissociation constant of batrachotoxin for a given concentration of epiervatamine, and B is the batrachotoxin concentration. $K_{0.5}$ values are 1.3, 2.6, and 6.5 μM at 0, 2, and 7 μM of epiervatamine. They change markedly at different epiervatamine concentrations, whereas V_{max} remains essentially unchanged. This property is that of a competitive inhibition. Since batrachotoxin and epiervatamine interact competitively, the concentration of epiervatamine required for 50% inhibition of $^{22}Na^+$ uptake stimulated by batrachotoxin is of course larger than the true dissociation constant for epiervatamine. The data in Fig. 5 were used to calculate the dissociation constant (K_d) of the epiervatamine-receptor complex in neuroblastoma cells: K_d = 1.7 μM.

The properties of the Na^+ channel can be studied *in vitro* by using brain synaptosomes (14). ATX_{II} has been shown to stimulate $^{86}Rb^+$ efflux through synaptosomal Na^+ channels. This efflux is inhibited by TTX (EC_{50} = 6.2 nM), but not by K^+ channel blockers such as tetraethylammonium and 2,4-diaminopyridine (14). Figure 6 shows that ervatamine and epiervatamine also inhibit ATX_{II}-stimulated $^{86}Rb^+$ efflux from preloaded synaptosomes with EC_{50} values which are 5 and 1.2 μM, respectively. Yohimbine and tetracaine have a similar effect with EC_{50} values of 8 and 1 μM, respectively.

The EC_{50} value found for ervatamine is close to the dissociation constant found through competitive inhibition with batrachotoxin on neuroblastoma cells (1.7 μM).

Rat brain synaptosomes have TTX receptors (7). The Scatchard plot for [^{3}H]en-TTX binding to synaptosomal membranes is presented in Fig. 7A. It is exactly the same in the absence and in the presence of ervatamine at a concentration of 0.1 mM. The maximal binding capacity is 3.2 pmoles/mg of protein and the dissociation constant for the [^{3}H]en-TTX receptor is 3.8 nM. [^{3}H]en-TTX binding to Na^+ channels is completely inhibited by unlabeled TTX and the competition curve (Fig. 7B) indicates a dissociation constant of 2 nM for the complex formed between native TTX and its receptor. Ervatamine and epiervatamine are unable to compete with [^{3}H]-

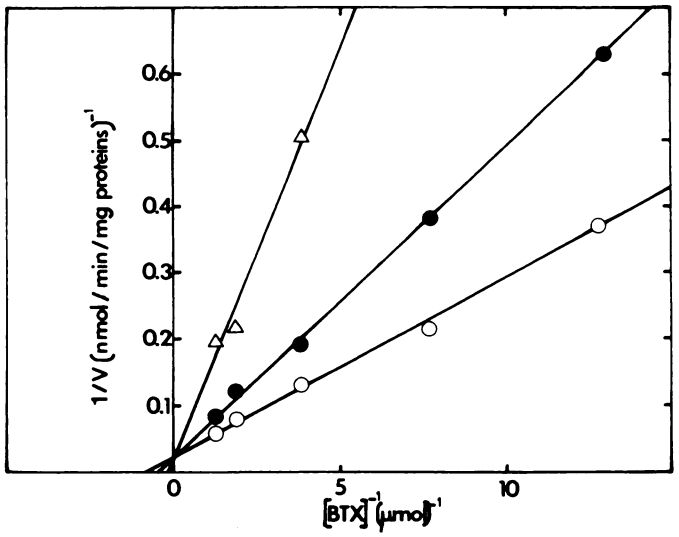


FIG. 5. *Lineweaver-Burk plots for the competitive inhibition of batrachotoxin (BTX)-activated $^{22}Na^+$ uptake by epiervatamine*

Cells were incubated with increasing concentrations of BTX and 0 μM (○), 2 μM (●), and 7 μM (△) epiervatamine. Half-maximum effects for BTX action are 1.3, 2.6, and 6.5 μM at 0, 2, and 7 μM epiervatamine.

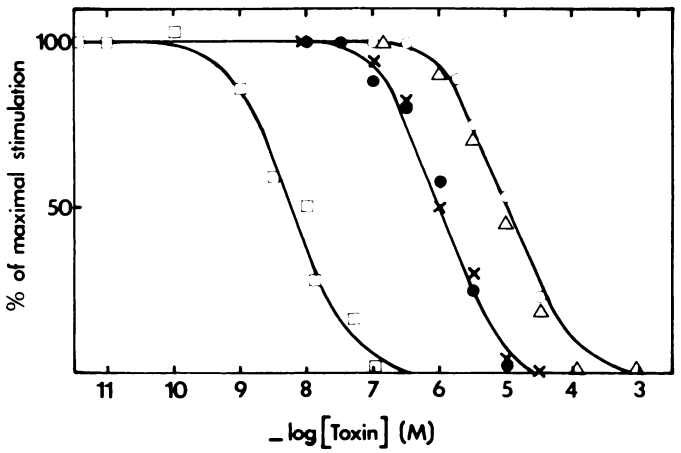


FIG. 6. *Inhibition of 10 μM ATX_{II}-induced $^{86}Rb^+$ release by rat brain synaptosomes by TTX (□), epiervatamine (●), ervatamine (○), yohimbine (△), and tetracaine (×)*

Curves were fitted according to Jacques *et al.* (9) and are characterized by the following EC_{50} and Hill coefficients: TTX (EC_{50} = 6.2 μM, n_H = 0.9), ervatamine (EC_{50} = 5.1 μM, n_H = 1.0), epiervatamine (EC_{50} = 1.2 μM, n_H = 1.2), yohimbine (EC_{50} = 8.2 μM, n_H = 1.1), tetracaine (EC_{50} = 1.0 μM, n_H = 1.0).

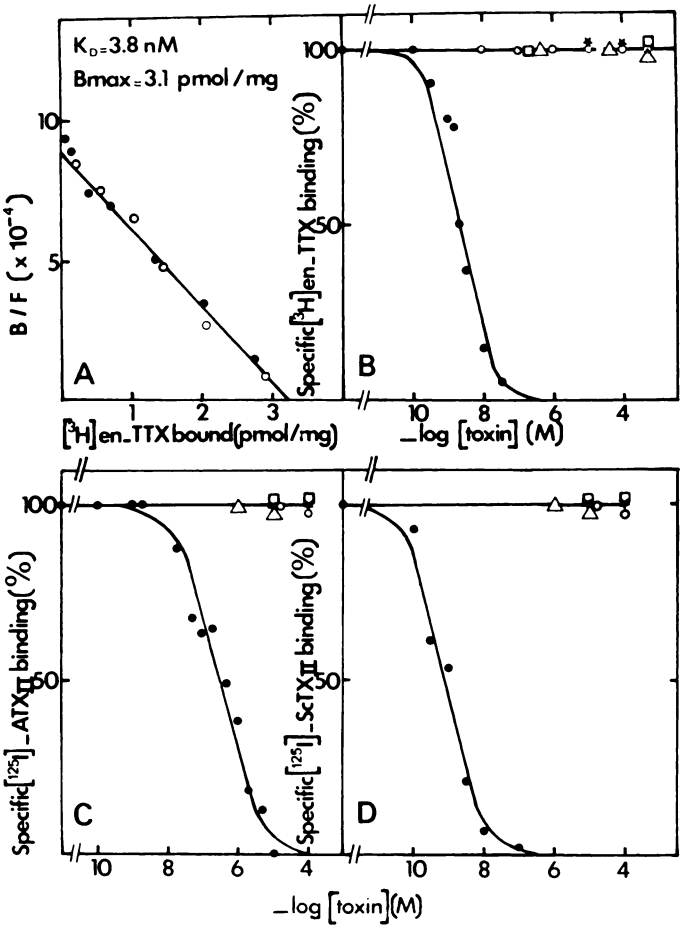


FIG. 7. *The effects of various compounds on [^{3}H]en-TTX, [^{125}I]-ATX$_{II}$, and [^{125}I]ScTX$_{II}$ binding*

A. Scatchard plot for the binding of [^{3}H]en-TTX to rat brain membranes in the absence (○) and in the presence (●) of 0.1 mM ervatamine. Membranes (90 μg) were incubated in 300 μl of 200 mM chlorine chloride, 25 mM Tris HCl buffer at pH 7.6 with increasing concentrations of [^{3}H]en-TTX. After 5 min of incubation at 4°, the radioactivity specifically bound to membranes was determined as described (7, 16). Nonspecific binding was determined as usual in the presence of a large excess (5 μM) of unlabeled TTX (7, 16). Specific binding is the difference between total binding and nonspecific binding and average 83% at 3.8 nM [^{3}H]en-TTX.

B. The effect of native unlabeled TTX (●), ervatamine (○), epiervatamine (□), yohimbine (★), and tetracaine (△) on [^{3}H]en-TTX binding to brain membranes.

C. The effect of native ATX$_{II}$ (●), ervatamine (○), epiervatamine (□), yohimbine (★), and tetracaine (△) on [^{125}I]ATX$_{II}$ binding to rat brain synaptosomes. Experiments were performed at 10 nM [^{125}I]ATX$_{II}$ and the specific binding was 70% of the total binding.

D. The effect of native ScTX$_{II}$ (●), ervatamine (○), epiervatamine (□), yohimbine (★), and tetracaine (△) on [^{125}I]ScTX$_{II}$ binding to rat brain synaptosomes. Experiments were performed at 0.5 nM [^{125}I]-ScTX$_{II}$ and the specific binding was 75% of the total binding.

en-TTX for binding to its specific receptor even at concentrations as high as 0.5 mM (Fig. 7B). This lack of competition to [^{3}H]en-TTX binding is also observed for yohimbine and for tetracaine.

Polypeptide toxins like scorpion and sea anemone toxins act on the gating system of the Na^+ channel; they specifically slow down its inactivation (24, 26). Their receptor sites are distinct from those of other toxic compounds which alter the normal mechanism of the gating system such as veratridine, batrachotoxin, or pyrethroids (8, 27). Both radiolabeled ScTX$_{II}$ and radiolabeled ATX$_{II}$ bind to a single class of sites on rat brain synaptosomes (8). [^{125}I]ATX$_{II}$ or [^{125}I]ScTX$_{II}$ binding to the Na^+ channels in synaptosomal membranes are competitively inhibited by unlabeled ATX$_{II}$ or by unlabeled ScTX$_{II}$ (Fig. 7). The competition curve indicates dissociation constants of 0.5 μM and 1 nM for the ATX$_{II}$-receptor complex and the ScTX$_{II}$-receptor complex, respectively. Ervatamine and epiervatamine, like TTX, are unable to compete with [^{125}I]ATX$_{II}$ or [^{125}I]ScTX$_{II}$ even at concentrations as high as 0.1 mM (Fig. 7), *i.e.*, at concentrations much higher than those necessary to completely inhibit ATX$_{II}$-stimulated $^{86}Rb^+$ efflux from synaptosomes. Yohimbine and tetracaine are also without effect on [^{125}I]ATX$_{II}$ and [^{125}I]ScTX$_{II}$ binding.

DISCUSSION

The alkaloids ervatamine and epiervatamine block the voltage-sensitive Na^+ conductance. The mechanism of this blockade is distinct from that found for TTX, the specific blocker of the Na^+ channel. (a) Unlike TTX, ervatamine and epiervatamine are not specific blockers of the Na^+ channel; they also partially block the K^+ conductance. (b) Unlike the effect of TTX on the Na^+ channel, the inhibiting effect of ervatamine and epiervatamine is accelerated by repetitive depolarizing pulses. (c) Ervatamine and epiervatamine are unable to compete with [^{3}H]en-TTX for binding to the TTX receptor. (d) Ervatamine and epiervatamine have the same apparent affinities for their receptor sites in two different types of cell lines, N1E 115 neuroblastoma cells and C_9 cells. In contrast, the TTX sensitivity of the N1E 115 neuroblastoma cells is 16 times higher than that of C_9 cells. (e) TTX is a noncompetitive inhibitor of the action of veratridine and batrachotoxin (28). In contrast, we find that epiervatamine is a competitive inhibitor of batrachotoxin action.

The first way by which ervatamine (or epiervatamine) could reduce the sodium current would be by occlusion of the Na^+ channel. The presence of this large molecule inside of the mouth of the pore would certainly impede Na^+ transport. This has been the classical mechanism proposed for the action of TTX and saxitoxin (29). However, very recent results indicate that these two toxins do not bind to the selectivity filter of the Na^+ channel (30), as was previously believed. On the other hand, ervatamine (or epiervatamine) might bind to components of the Na^+ channel which control the gating system. The competition between epiervatamine and batrachotoxin would be consistent with such a mechanism. Batrachotoxin binding to its receptor site would chemically activate the Na^+ channel, thereby stabilizing a permeable form of the Na^+ channel, whereas ervatamine binding to the batrachotoxin site would have the inverse effect of stabilizing a nonpermeable form of the channel. In such a mechanism in which the two toxic molecules bind to the same site, ervatamine binding to the batrachotoxin site would of course prevent binding of batrachotoxin. A comparison of the apparent dissociation constant of complexes formed with epiervatamine (K_d = 1.2–1.7 μM) and batrachotoxin (K_d = 1.3 μM) with the Na^+ channel indicates that the two molecules have very similar affinities. The existence of a common receptor site for ervatamine

(or epiervatamine) and batrachotoxin is not definitely demonstrated. One cannot eliminate a mechanism in which ervatamine binds to a site distinct from the batrachotoxin receptor and by an allosteric effect reduces the binding of batrachotoxin to its own site.

There are other identified sites on the Na^+ channel which are involved in the control of the gating system. These sites bind polypeptide toxins extracted from sea anemone and from scorpion venom. Ervatamine and epiervatamine counteract the action of polypeptide toxins as shown in Fig. 6, but they do not bind to the polypeptide toxin receptors as shown in Fig. 7.

The competitive behavior observed between batrachotoxin and epiervatamine is similar to the competitive behavior observed between the indolealkylamine alkaloid yohimbine which is also extracted from plants of the *Apocynaceous* family (22, 25) and batrachotoxin. Moreover, the use-dependent inhibition of the sodium current of squid axon and neuroblastoma cells observed with yohimbine (22, 25) is also similar to the use-dependent inhibition observed with ervatamine and epiervatamine (31, 32). The three molecules seem to bind preferentially to the open form of the Na^+ channel as previously discussed for yohimbine (22, 25). There are therefore good reasons to believe that ervatamine, epiervatamine, and yohimbine have similar mechanisms of actions. This view is supported by results obtained in this paper, which shows that all the effects seen with ervatamine or epiervatamine are also seen with yohimbine (Figs. 2, 4, and 6). Moreover, yohimbine, like ervatamine or epiervatamine, binds neither to the TTX receptor nor to the receptor for polypeptide neurotoxins. As for ervatamine and epiervatamine, a single class of binding sites has been found for yohimbine. The apparent dissociation constant of the yohimbine-receptor interaction has been found to be 120 μM by using neuroblastoma cells as the source of the Na^+ channel as compared to values of 12 and 3 μM for ervatamine and epiervatamine, which have a much higher affinity for the Na^+ channel. Values found from Fig. 5 for the interaction of these three molecules with the Na^+ channel in synaptosomes are 10, 5, and 1.2 μM for yohimbine, ervatamine, and epiervatamine, respectively.

Tetracaine data have been presented for the sake of comparing the potency of several different molecules (ervatamine, epiervatamine, yohimbine, tetrodotoxin, and tetracaine) which all block the sodium channel. Ervatamine has a potency which is similar to that of tetracaine.

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Note added in proof. In a recent study, Sauviat (33) described the effects of ervatamine on ionic currents in frog atrial fibers. The observed effects are very similar to the ones reported in this paper.

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