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EFFECT OF DRUGS ON THE LETHALITY IN MICE OF THE VENOMS AND NEUROTOXINS FROM SUNDRY SNAKES

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R. D. CROSLAND. Effect of drugs on the lethality in mice of the venoms and neurotoxins from sundry snakes. *Toxicon* **29,** 613–631, 1991.—I investigated the efficacy of 10 drugs with respect to reducing the lethality in mice of the following venoms and their respective neurotoxins: *Bungarus caeruleus* venom; *Bungarus multicinctus* venom, α-bungarotoxin, β-bungarotoxin; *Crotalus durissus terrificus* venom, crotoxin; *Notechis scutatus scutatus* venom; *Oxyuranus scutellatus* venom, taipoxin. The drugs diltiazem, nicergoline, primaquine, verapamil and vesamicol protected mice from the lethality of *B. caeruleus* venom, *B. multicinctus* venom, and/or β-bungarotoxin. Dexamethasone provided protection from *B. multicinctus* venom, β-bungarotoxin, crotoxin, *O. scutellatus* venom and taipoxin. Protective activity resided in amphiphilic drugs and correlated with the charge on the drug at physiological pH. Protection from lethality was maximal when the drugs were administered immediately after injection of the venom or toxin. Nifedipine, piracetam and reserpine provided no protection from any of the venoms or toxins tested.

INTRODUCTION

ANTIVENOMS are the agents currently used for treatment of intoxication due to snake venoms. Several factors, however, limit their usefulness. A given antivenom is effective against the venoms from only a small number of snake species, necessitating the availability of several antivenoms, and requiring the victim or physician to identify the guilty snake, which in many cases cannot be done. Furthermore, some people are hypersensitive to antivenoms. Finally, antivenoms require refrigeration, are sometimes needed in large quantities, and are expensive — three factors that limit their availability. Treatment of snake venom intoxication would be greatly enhanced if a drug could be found which would overcome these deficiencies of antivenoms.

Some snake venoms contain presynaptic toxins which constitute the most lethal components of the venoms (CHANG, 1985). These presynaptic toxins act by inhibiting the release of acetylcholine from neurons, thereby blocking muscle contraction, resulting in respiratory failure and death. These toxins have Ca^{2+}-dependent phosphatidate 2-acylhydrolase (EC 3.1.1.4) (trivial name: phospholipase A_2) activity, which may be implicated in their toxicity (CHANG, 1985). Venoms which contain such presynaptic toxins include those from the snakes *Bungarus caeruleus* (Indian krait), *Bungarus multicinctus* (many-

banded krait), *Crotalus durissus terrificus* (South American rattlesnake), *Notechis scutatus scutatus* (eastern tiger snake), and *Oxyuranus scutellatus* (taipan) (common names are from ROSENBERG, 1987). Some snake venoms also contain postsynaptic toxins (e.g. α-bungarotoxin) which work in concert with presynaptic toxins by binding to the acetylcholine receptor and also blocking muscle contraction.

I previously reported (CROSLAND, 1988; 1989*a*) that chloroquine, chlorpromazine and quinacrine were effective antagonists of the lethality in mice of *B. caeruleus* venom, *B. multicinctus* venom and the latter's presynaptic toxin, β-bungarotoxin. A salient feature of these drugs is their ability to inhibit phospholipase A_2 activity (AUTHI and TRAYNOR, 1979; JAIN and JAHAGIRDAR, 1985; BROEKMEIER *et al.*, 1985). Other drugs which inhibit phospholipase A_2 activity may be more efficacious or have a wider spectrum of action as antagonists of snake venom lethality than those drugs tested heretofore. An additional class of drugs which merits investigation is the Ca^{2+} antagonists. These drugs antagonize many Ca^{2+}-dependent processes (ORTEGA *et al.*, 1987; RADDINO *et al.*, 1987; ZERNIG, 1990) and could inhibit the Ca^{2+}-dependent phospholipase A_2 activity and thus the lethality of snake presynaptic toxins. Also of interest are reserpine and vesamicol, both of which inhibit transport of neurotransmitters into synaptic vesicles. ANDERSON *et al.* (1983) reported that chloroquine, chlorpromazine, quinacrine, reserpine and vesamicol inhibited transport of acetylcholine into synaptic vesicles from the electric organ of *Torpedo californica*. Since I found chloroquine, chlorpromazine and quinacrine to be effective antagonists of snake venoms, reserpine and vesamicol may also be effective.

MATERIALS AND METHODS

Materials

Bungarus caeruleus venom, *B. multicinctus* venom, *C. durissus terrificus* venom, *O. scutellatus* venom, α-bungarotoxin, β-bungarotoxin and crotoxin were purchased from Miami Serpentarium Laboratories, Salt Lake City, UT, U.S.A. *N. scutatus scutatus* venom and taipoxin were purchased from Ventoxin Laboratories, Frederick, MD, U.S.A. Lyophilized venoms and toxins (except *C. durissus terrificus* venom and crotoxin) were dissolved (1 mg/ml) in deionized water. *C. durissus terrificus* venom was dissolved (0.5 mg/ml) in 20 mM sodium phosphate, pH 7.4. Crotoxin was dissolved (1 mg/ml) in 10 mM sodium chloride + 10 mM sodium acetate. Venom and toxin solutions were stored in aliquots at −20°C and were not refrozen after thawing. On the day of the experiment, venoms and toxins were further diluted with gel-phosphate buffer (0.2% gelatin (w/v), 0.4% sodium phosphate (w/v), pH 6.2). Dexamethasone (9-fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione) 21-phosphate (disodium salt), diltiazem (*cis*-(+)-3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one) hydrochloride, nifedipine (1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl-3,5-pyridinedicarboxylic acid dimethyl ester), primaquine (8-[4-amino-1-methylbutylamino]-6-methoxyquinoline) diphosphate, reserpine (11,17α-dimethoxy-18β-[3,4,5-trimethoxybenzoyl)oxy]-3β,20α-yohimban-16β-carboxylic acid methyl ester) and verapamil (α-[3-[[2-(3,4-dimethoxyphenyl)ethyl]-methylamino]propyl]-3,4-dimethoxy-α-(1-methylethyl)(benzeneacetronitrile) hydrochloride were purchased from Sigma Chemical Co., St Louis, MO, U.S.A. Nicergoline (10-methoxy-1,6-dimethylergoline-8β-methanol 5-bromonicotinate) was the gift of Farmitalia Carlo Erba, Milan, Italy. Piracetam (2-oxo-1-pyrrolidineacetamide) was a gift from Dr HARVEY ALTMAN of the Lafayette Clinic, Detroit, MI, U.S.A. Vesamicol [(±)-2-(4-phenylpiperidino)-cyclohexanol] hydrochloride was purchased from Research Biochemicals, Inc, Natick, MA, U.S.A. Vesamicol analog 72 [(±)-*trans*-5-amino-2-hydroxy-3-(4-phenylpiperidino)tetralin] was the gift of Dr STANLEY PARSONS, University of California, Santa Barbara, CA, U.S.A. Chloroquine, chlorpromazine, dexamethasone, diltiazem, piracetam, primaquine and quinacrine were dissolved in 150 mM sodium chloride, 6 mM sodium phosphate (pH 7.2) (phosphate-buffered saline). Reserpine, nifedipine and vesamicol analog 72 were dissolved in dimethylsulfoxide and then diluted 1 → 50 with polyethylene glycol:water::1:1 (vol). Verapamil was dissolved in water. Nicergoline was dissolved in a given volume of 25 mM tartaric acid, diluted with 1.13 volumes of water, followed by addition of 0.45 volume of 25 mM sodium bicarbonate. The final pH was 4–5. Vesamicol was dissolved in 6.25 mM tartaric acid (pH 3.7). The appropriate vehicle without dissolved drug was the control for each experiment.

Methods

Female ICR mice (20–30 g; Harlan Sprague-Dawley, Inc., Frederick, MD, U.S.A.) were housed five per cage, maintained on a 12 hr light–dark (1800–0600) cycle, and allowed free access to food and water. Their use was in compliance with the Animal Welfare Act and the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. Venoms/toxins and drugs were injected i.p. into mice. All doses were adjusted for the weight of the animal and were administered in a volume of 10 ml/kg. The number of animals that died within 24 hr of the time of the injection of venom/toxin was used as the measure of lethality. All surviving mice were subsequently killed with gaseous carbon dioxide.

The effect of various doses of a particular drug was tested by injecting mice with approximately two times the LD_{50} of venom/toxin, immediately followed by a separate injection of the drug. If the drug provided significant protection from the venom/toxin, then further investigation of the drug's interaction with that venom/toxin was pursued. The ED_{50} and the LD_{50} of the dose-effect experiments were calculated by using the data of the rising and falling phases, respectively, of the dose-effect curve and refer to those doses of drug required to produce 50% of the maximal observed protective effect. The LD_{50} of the drug was that determined in the presence of venom/toxin, and was not the LD_{50} of the drug alone.

The optimal time of injection of a drug was determined by injecting the most protective dose of the drug at different times before (−60, −30, −15 min) or after (+0, +15, +30, +60 min) the injection of approximately two times the LD_{50} of the venom/toxin. Control animals received an injection of venom/toxin which was either preceded (−45 min) by an injection of vehicle alone (one-half of controls) or followed (+45 min) by an injection of vehicle alone (one-half of controls).

Each experiment was repeated at least once, and the data were combined. Each data point represents at least five mice. A P value associated with a change in the LD_{50} of a venom/toxin due to drug treatment refers to the drug's effect on the dose–response curve as calculated using logit analysis. Other tests of significance were calculated using contingency or regression analysis. Statistical tests were considered significant when $P < 0.05$.

RESULTS

Dexamethasone

Dexamethasone protected mice from the lethality of *B. multicinctus* venom, β-bungarotoxin, crotoxin, *O. scutellatus* venom and taipoxin, while providing no protection from *B. caeruleus* venom, *C. durissus terrificus* venom, α-bungarotoxin or *N. scutatus scutatus* venom (Fig. 1). In the cases of *B. multicinctus* venom, β-bungarotoxin and crotoxin protection increased with increasing doses of dexamethasone (the optimal doses were 15 μmoles/kg, 75 μmoles/kg, and 6.2 μmoles/kg, respectively) and then declined with further increasing doses. With *O. scutellatus* venom and taipoxin, however, protection increased with increasing doses of dexamethasone (100% protection at 90 μmoles/kg and 60 μmoles/kg, respectively) and remained at 100% with further increases in dosage. The ED_{50} of dexamethasone was 5.8 μmoles/kg, 7.2 μmoles/kg, 0.17 μmoles/kg, 51 μmoles/kg, and 22 μmoles/kg with respect to *B. multicinctus* venom, β-bungarotoxin, crotoxin, *O. scutellatus* venom and taipoxin. The LD_{50} with respect to *B. multicinctus* venom, β-bungarotoxin and crotoxin was 44 μmoles/kg, 87 μmoles/kg and 30 μmoles/kg, while the corresponding therapeutic indices were 7.6, 12 and 176. There was no declining phase to the remaining dose–response curves, so no LD_{50}s or therapeutic indices could be calculated for them.

Dexamethasone increased the LD_{50} of *O. scutellatus* venom 3.5-fold from 22 μg/kg to 76 μg/kg ($P < 0.0005$). It completely protected mice from a dose of venom that was lethal to 86% of the untreated mice. Dexamethasone also increased the LD_{50} of taipoxin 4.0-fold from 2.5 μg/kg to 10 μg/kg ($P = 0.001$), completely protecting mice from a dose of the toxin that was lethal to all of the untreated mice. At 66 μmoles/kg, dexamethasone had no significant effect, however, on the LD_{50} of *B. multicinctus* venom, increasing it from 90 μg/kg to 120 μg/kg ($P = 0.10$); or, at 6.2 μmoles/kg, on the LD_{50} of crotoxin, increasing it from 58 μg/kg to 90 μg/kg ($P = 0.88$). An injection of gel-phosphate buffer followed

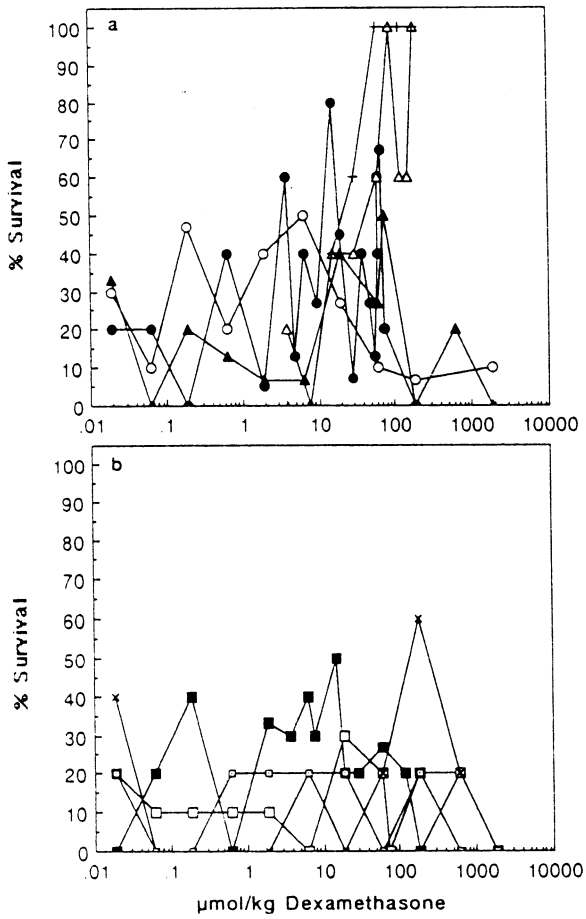

FIG. 1. DOSE-EFFECT OF DEXAMETHASONE ON THE LETHALITY OF VENOMS AND TOXINS.
(a) Mice were injected with 80 μg/kg of *B. multicinctus* venom (●), 30 μg/kg of β-bungarotoxin (▲), 150 μg/kg of crotoxin (○), 50 μg/kg of *O. scutellatus* venom (△) or 5 μg/kg of taipoxin (+), followed immediately by a separate injection of various doses of dexamethasone. Test of overall significance, % of control mice surviving: *B. multicinctus* venom, 0.0001, 0%; β-bungarotoxin, 0.032, 17%; crotoxin, 0.038, 12%; *O. scutellatus* venom, 0.0011, 0%; taipoxin, 0.0001, 10%. (b) Mice were injected with 350 μg/kg α-bungarotoxin (X), 50 μg/kg of *B. caeruleus* venom (■), 200 μg/kg of *C. durissus terrificus* venom (□), or 200 μg/kg of *N. scutatus scutatus* venom (⊡), followed immediately by a separate injection of various doses of dexamethasone. Test of overall significance, % of control mice surviving: α-bungarotoxin, 0.084, 10%; *B. caeruleus* venom, 0.15, 10%; *C. durissus terrificus* venom, 0.73, 7%; *N. scutatus scutatus* venom, 0.82, 25%.

immediately by an injection of 90 μmoles/kg of dexamethasone made 20 mice lethargic and sleepy for several hr. After 24 hr all of the mice recovered.

Diltiazem

Diltiazem protected mice from the lethality of *B. caeruleus* venom, *B. multicinctus* venom, and β-bungarotoxin, while not protecting them from α-bungarotoxin, *C. durissus terrificus* venom, crotoxin, *N. scutatus scutatus* venom, *O. scutellatus* venom, or taipoxin

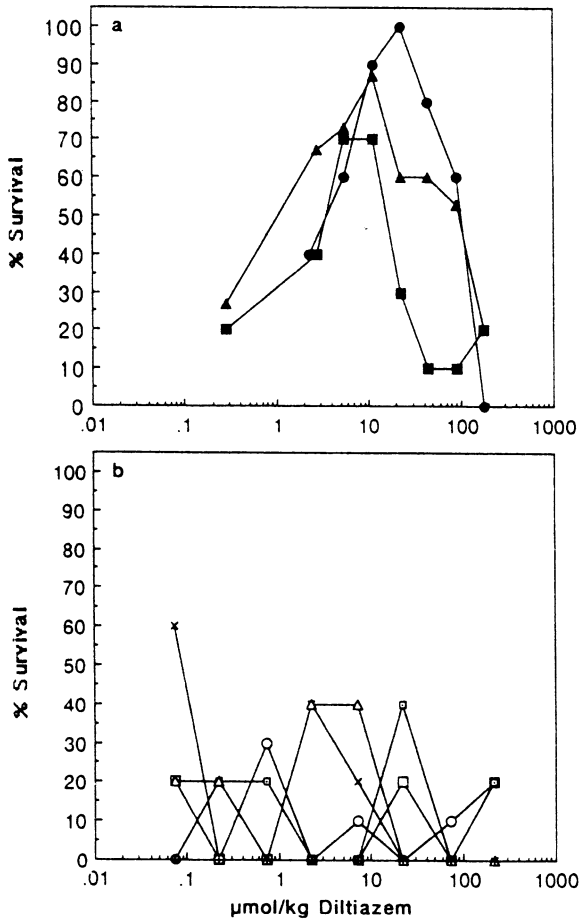

FIG. 2. DOSE-EFFECT OF DILTIAZEM ON THE LETHALITY OF VENOMS AND TOXINS.
(a) Mice were injected with 80 μg/kg of *B. caeruleus* venom (■), 80 μg/kg of *B. multicinctus* venom (●), or 50 μg/kg of β-bungarotoxin (▲), followed immediately by a separate injection of various doses of diltiazem. Test of overall significance, % of control mice surviving: *B. caeruleus* venom, 0.0005, 5%; *B. multicinctus* venom, 0.0001, 0%; β-bungarotoxin, 0.0001, 13%. (b) Mice were injected with 350 μg/kg of α-bungarotoxin (X), 150 μg/kg of *C. durissus terrificus* venom (□), 150 μg/kg of crotoxin (○), 200 μg/kg of *N. scutatus scutatus* venom (⊡), 20 μg/kg of *O. scutellatus* venom (△), or 4 μg/kg of taipoxin (+) followed immediately by a separate injection of various doses of diltiazem. Test of overall significance, % of control mice surviving: α-bungarotoxin, 0.094, 10%; *C. durissus terrificus* venom, 0.60, 0%; crotoxin, 0.19, 0%; *N. scutatus scutatus* venom, 0.26, 0%; *O. scutellatus* venom, 0.39, 20%; taipoxin, 1.00, 0%.

(Fig. 2). Protection increased with increasing amounts of diltiazem up to 5.5 μmoles/kg in the case of *B. caeruleus* venom, 22 μmoles/kg in the case of *B. multicinctus* venom, and 11 μmoles/kg in the case of β-bungarotoxin. Higher doses of diltiazem resulted in a decline in effectiveness. The ED_{50} of diltiazem with respect to *B. caerulus* venom, *B. multicinctus* venom and β-bungarotoxin was 2.4 μmoles/kg, 4.1 μmoles/kg and 2.0 μmoles/kg, respectively. The LD_{50}s were 31 μmoles/kg, 89 μmoles/kg and 72 μmoles/kg, resulting in therapeutic indices of 13, 21 and 36.

Diltiazem increased the LD_{50} of *B. caeruleus* venom 2.2-fold from 51 μg/kg to 110 μg/kg ($P = 0.010$). It also increased the LD_{50} of *B. multicinctus* venom 7.4-fold from 23 μg/kg to

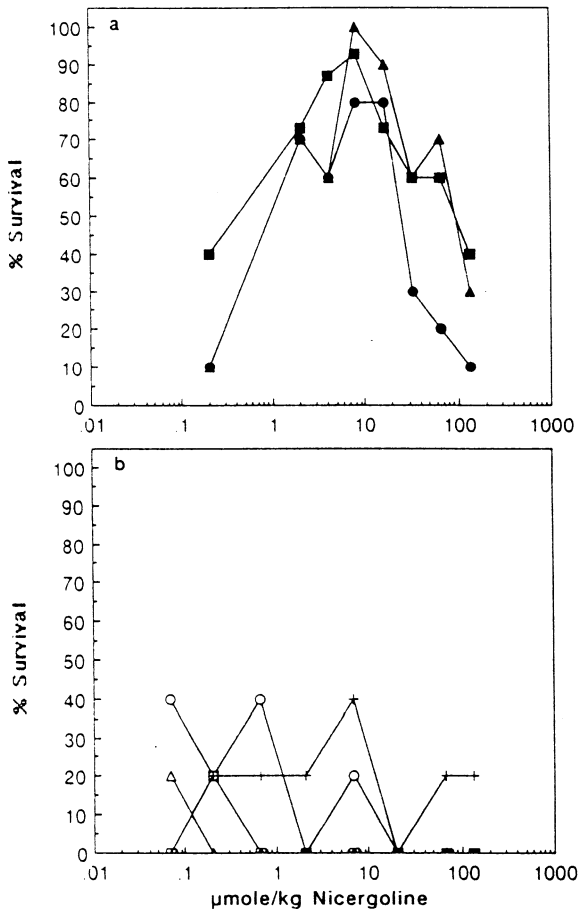

FIG. 3. DOSE-EFFECT OF NICERGOLINE ON THE LETHALITY OF VENOMS AND TOXINS.
(a) Mice were injected with 50 μg/kg of *B. caeruleus* venom (■), 50 μg/kg of *B. multicinctus* venom (●), or 30 μg/kg of β-bungarotoxin (▲), followed immediately by a separate injection of various doses of nicergoline. Test of overall significance, % of control mice surviving: *B. caeruleus* venom, 0.0003, 20%; *B. multicinctus* venom, 0.0003, 20%; β-bungarotoxin, 0.0001, 0%. (b) Mice were injected with 200 μg/kg of *C. durissus terrificus* venom (□), 100 μg/kg of crotoxin (○), 20 μg/kg of *O. scutellatus* venom (△), or 2 μg/kg of taipoxin (+), followed immediately by a separate injection of various doses of nicergoline. Test of overall significance, % of control mice surviving: *C. durissus terrificus* venom, 0.33, 0%; crotoxin, 0.40, 20%; *O. scutellatus* venom, 0.33, 0%; taipoxin, 0.71, 20%.

170 μg/kg ($P = 0.0010$). In fact, it completely protected mice from two times a lethal dose of *B. multicinctus* venom. Diltiazem also increased the LD_{50} of β-bungarotoxin 1.9-fold from 19 μg/kg to 37 μg/kg ($P = 0.014$). An injection of gel-phosphate buffer followed immediately by an injection of 22 μmoles/kg of diltiazem caused no overt effects in 20 mice.

Nicergoline

Nicergoline protected mice from the lethality of *B. caeruleus* venom, *B. multicinctus* venom and β-bungarotoxin, while providing no protection from *C. durissus terrificus*

venom, crotoxin, *O. scutellatus* venom, or taipoxin (Fig. 3). Protection increased with increasing amounts of nicergoline up to 8.3 μmoles/kg in all cases. Higher doses of nicergoline resulted in a decline in effectiveness. The ED_{50} of nicergoline was 2.2 μmoles/kg, 1.8 μmoles/kg, or 1.9 μmoles/kg with respect to *B. caeruleus* venom, *B. multicinctus* venom, or β-bungarotoxin. Combining these values with LD_{50}s of 71 μmoles/kg, 33 μmoles/kg and 76 μmoles/kg resulted in therapeutic indices of 32, 18 and 40 for *B. caeruleus* venom, *B. multicinctus* venom and β-bungarotoxin, respectively.

I tested nicergoline (8.3 μmoles/kg,) for its ability to increase the LD_{50} of *B. caeruleus* venom, *B. multicinctus* venom, α-bungarotoxin and β-bungarotoxin. It increased the LD_{50} of *B. multicinctus* venom 4.6-fold from 24 μg/kg to 110 μg/kg ($P < 0.0005$) and the LD_{50} of β-bungarotoxin 4.0-fold from 9.6 μg/kg to 38 μg/kg ($P < 0.0005$). It had no significant effect, however, on the LD_{50} of *B. caeruleus* venom or α-bungarotoxin, increasing the former's LD_{50} by 1.8-fold from 35 μg/kg to 62 μg/kg ($P = 0.073$) and increasing the latter's LD_{50} by 1.0-fold from 200 μg/kg to 210 μg/kg ($P = 0.39$). An injection of gel-phosphate buffer followed immediately by an injection of 8.3 μmoles/kg of nicergoline had no overt effect on 20 mice observed for 48 hr.

Nifedipine, piracetam, reserpine

Nifedipine, piracetam and reserpine failed to protect mice from the lethality of any of the venoms/toxins tested, and no further investigation of the drugs' interactions with the venoms/toxins was pursued. An injection of gel-phosphate buffer followed immediately by an injection of 29 μmoles/kg of nifedipine or 7000 μmoles/kg of piracetam had no overt effect on 20 mice that were observed for 24 hr. An injection of gel-phosphate buffer followed immediately by an injection of 5.1 μmoles/kg of reserpine sedated 20 mice for 24 hr. All of the reserpinized mice recovered after 48 hr.

Primaquine

Primaquine protected mice from the lethality of *B. caeruleus* venom, *B. multicinctus* venom and β-bungarotoxin, while not protecting them from *C. durissus terrificus* venom, crotoxin, *O. scutellatus* venom, or taipoxin (Fig. 4). Protection increased with increasing amounts of primaquine up to 44 μmoles/kg in the case of *B. caeruleus* venom, 11 μmoles/kg in the case of *B. multicintus* venom, and 22 μmoles/kg in the case of β-bungarotoxin. Higher doses of primaquine resulted in a decline in effectiveness. The ED_{50} of primaquine was 2.1 μmoles/kg, 1.9 μmoles/kg, or 7.2 μmoles/kg with respect to *B. caeruleus* venom, *B. multicinctus* venom, or β-bungarotoxin. Combining these values with LD_{50}s of 98 μmoles/kg, 78 μmoles/kg and 49 μmoles/kg, resulted in therapeutic indices of 47, 41 and 6.8 for *B. caeruleus* venom, *B. multicinctus* venom and β-bungarotoxin, respectively.

Primaquine increased the LD_{50} of *B. caeruleus* venom 2.9-fold from 27μg/kg to 79 μg/kg ($P < 0.0005$) of *B. multicinctus* venom 6.0-fold from 35 μg/kg to 210 μg/kg ($P < 0.0005$), and of β-bungarotoxin 3.9-fold from 8.8 μg/kg to 34 μg/kg ($P = 0.002$). In fact, primaquine completely protected mice from a dose (about three times the LD_{50}) of *B. multicinctus* venom that killed 100% of the control mice. Primaquine (11 μmoles/kg) had no significant effect on the LD_{50} of α-bungarotoxin, increasing it by 1.2-fold from 320 μg/kg to 390 μg/kg ($P = 0.12$). An injection of gel-phosphate buffer followed immediately by an injection of 11 μmoles/kg of primaquine had no overt effect on 20 mice observed for 48 hr.

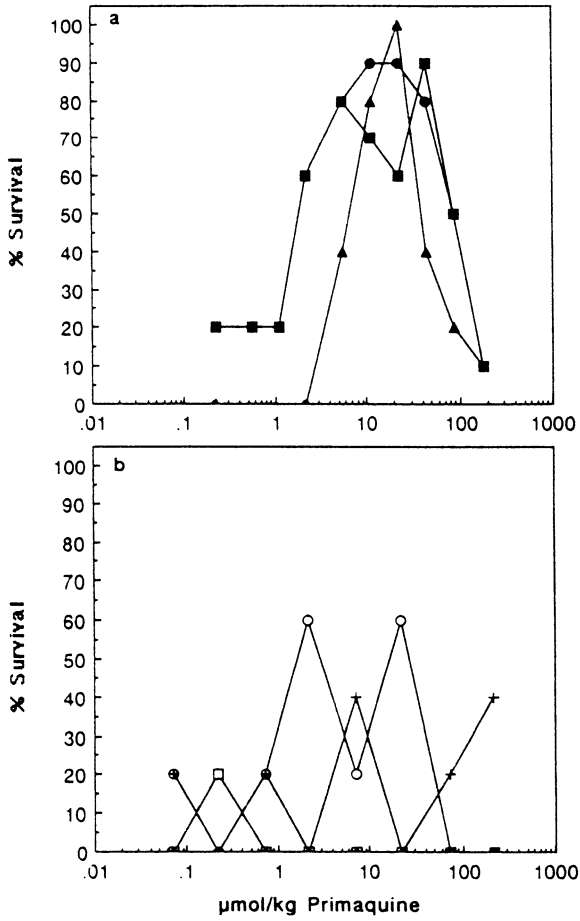

FIG. 4. DOSE-EFFECT OF PRIMAQUINE ON THE LETHALITY OF VENOMS AND TOXINS.
(a) Mice were injected with 50 μg/kg of *B. caeruleus* venom (■), 100 μg/kg of *B. multicinctus* venom (●), or 30 μg/kg of β-bungarotoxin (▲), followed immediately by a separate injection of various doses of primaquine. Test of overall significance, % of control mice surviving: *B. caeruleus* venom, 0.0006, 30%; *B. multicinctus* venom, 0.0066, 20%; β-bungarotoxin, 0.0001, 0%. (b) Mice were injected with 200 μg/kg of *C. durissus terrificus* venom (□), 100 μg/kg of crotoxin (○), 20 μg/kg of *O. scutellatus* venom (△), or 2 μg/kg of taipoxin (+), followed immediately by a separate injection of various doses of primaquine. Test of overall significance, % of control mice surviving: *C. durissus terrificus* venom, 0.33, 0%; crotoxin, 0.14, 30%; *O. scutellatus* venom, 1.00, 0%; taipoxin, 0.26, 0%.

Verapamil

Verapamil protected mice from the lethality of *B. caeruleus* venom, *B. multicinctus* venom and β-bungarotoxin, while providing no protection from α-bungarotoxin, *C. durissus terrificus* venom, crotoxin, *N. scutatus scutatus* venom, *O. scutellatus* venom, or taipoxin (Fig. 5). Protection increased with increasing amounts of verapamil up to 5.1 μmoles/kg in all cases. Higher doses of verapamil resulted in a decline in effectiveness. The ED_{50} of verapamil with respect to *B. caeruleus* venom, *B. multicinctus* venom and β-bungarotoxin was 1.5 μmoles/kg, 1.0 μmole/kg and 1.4 μmoles/kg, respectively. The LD_{50}

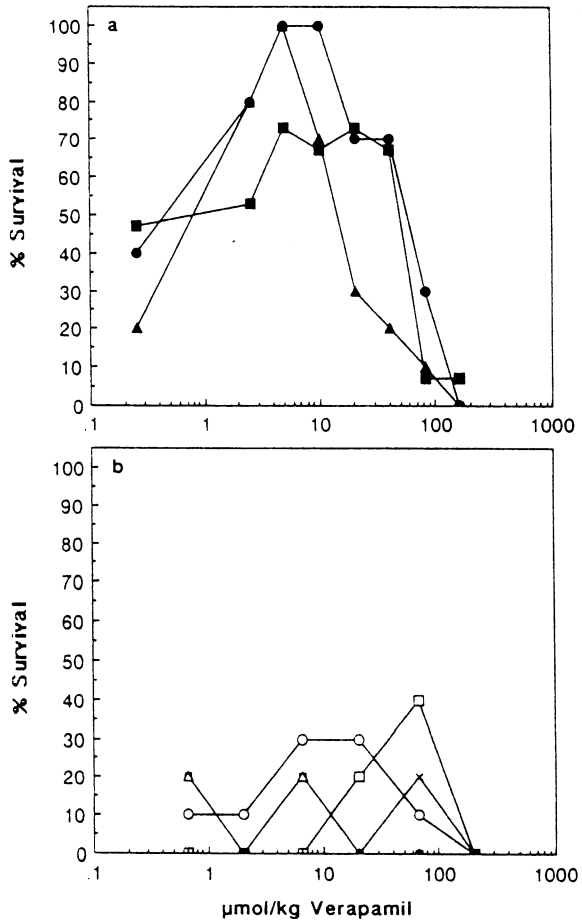

FIG. 5. DOSE-EFFECT OF VERAPAMIL ON THE LETHALITY OF VENOMS AND TOXINS.
(a) Mice were injected with 60 μg/kg of *B. caeruleus* venom (■), 60 μg/kg of *B. multicinctus* venom (●), or 50 μg/kg of β-bungarotoxin (▲), followed immediately by a separate injection of various doses of verapamil. Test of overall significance, % of control mice surviving: *B. caeruleus* venom, 0.0001, 7%; *B. multicinctus* venom, 0.0010, 20%; β-bungarotoxin, 0.0001, 0%. (b) Mice were injected with 350 μg/kg of α-bungarotoxin (X), 150 μg/kg of *C. durissus terrificus* venom (□), 150 μg/kg of crotoxin (○), 200 μg/kg of *N. scutatus scutatus* venom (⊡), 20 μg/kg of *O. scutellatus* venom (△), or 4 μg/kg of taipoxin (+), followed immediately by a separate injection of various doses of verapamil. Test of overall significance, % of control mice surviving: α-bungarotoxin, 0.68, 10%; *C. durissus terrificus* venom, 0.40, 20%; crotoxin, 0.24, 10%; *N. scutatus scutatus* venom, 0.74, 10%; *O. scutellatus* venom, 0.59, 0%; taipoxin, 1.00, 0%.

was 51 μmoles/kg, 53 μmoles/kg and 17 μmoles/kg, respectively, resulting in therapeutic indices of 34, 53 and 12.

Verapamil (10.2 μmoles/kg) increased the LD_{50} of *B. caeruleus* venom 5.2-fold from 21 μg/kg to 110 μg/kg ($P = 0.001$). It provided almost complete protection from 30 μg/kg *B. caeruleus* venom, a lethal dose. Verapamil (5.1 μmoles/kg) also increased the LD_{50} of *B. multicinctus* venom 3.8-fold from 36 μg/kg to 135 μg/kg ($P = 0.001$) and increased the LD_{50} of β-bungarotoxin 5.0-fold from 30 μg/kg to 150 μg/kg ($P = 0.001$). An injection of

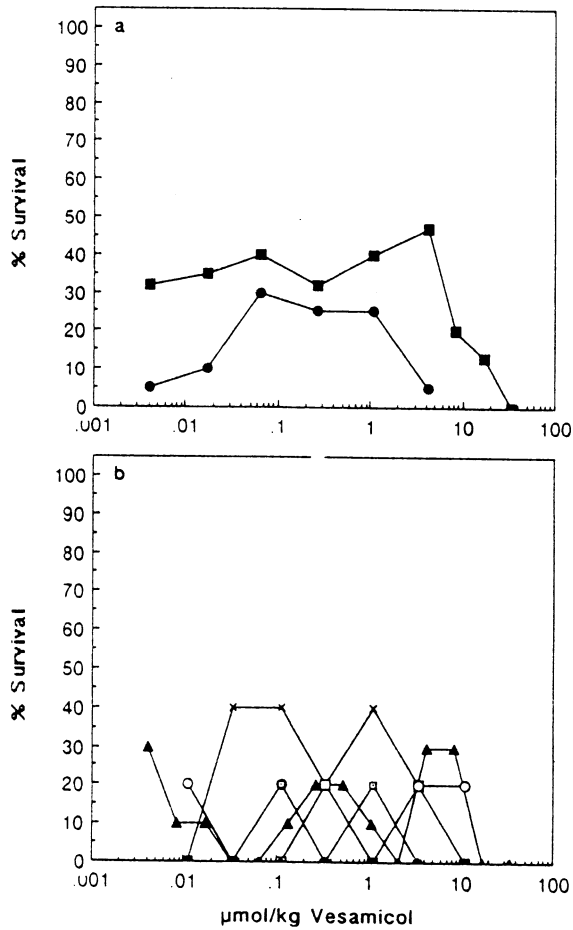

FIG. 6. DOSE-EFFECT OF VESAMICOL ON THE LETHALITY OF VENOMS AND TOXINS.
(a) Mice were injected with 80 μg/kg of *B. caeruleus* venom (■), or 60 μg/kg of *B. multicinctus* venom (●), followed immediately by a separate injection of various doses of vesamicol. Test of overall significance, % of control mice surviving: *B. caeruleus* venom, 0.0005, 8%; *B. multicinctus* venom, 0.0012, 2.5%. (b) Mice were injected with 350 μg/kg of α-bungarotoxin (X), 40 μg/kg of β-bungarotoxin (▲), 150 μg/kg of *C. durissus terrificus* venom (□), 150 μg/kg of crotoxin (○), 200 μg/kg of *N. scutatus scutatus* venom (⊡), 20 μg/kg of *O. scutellatus* venom (△), or 4 μg/kg of taipoxin (+), followed immediately by a separate injection of various doses of vesamicol. Test of overall significance, % of control mice surviving: α-bungarotoxin, 0.55, 10%; β-bungarotoxin, 0.059, 3.3%; *C. durissus terrificus* venom, 0.59, 20%; crotoxin, 0.65, 10%; *N. scutatus scutatus* venom, 0.69, 10%; *O. scutellatus* venom, 0.40, 20%; taipoxin, 1.00, 0%.

gel-phosphate buffer followed immediately by an injection of 5.1 μmoles/kg of verapamil caused no overt effects in 20 mice observed for 24 hr.

Vesamicol

Vesamicol protected mice from the lethality of *B. caeruleus* venom and *B. multicinctus* venom, while providing no protection from α-bungarotoxin, β-bungarotoxin, *C. durissus terrificus* venom, crotoxin, *N. scutatus scutatus* venom, *O. scutellatus* venom, or taipoxin

(Fig. 6). I observed protection over a broad range of doses, especially in the case of *B. caeruleus* venom. This might have been due to the presence of both entantiomers of vesamicol. The 'optimal' dose of vesamicol was 4.2 μmoles/kg in the case of *B. caeruleus* venom and 0.066 μmole/kg in the case of *B. multicinctus* venom. Higher doses of vesamicol resulted in a decline in effectiveness. The ED_{50} of vesamicol with respect to *B. caeruleus* venom and *B. multicinctus* venom was 0.045 μmole/kg and 0.027 μmole/kg, respectively. The LD_{50}s were 7.7 μmoles/kg and 0.93 μmole/kg, respectively, resulting in therapeutic indices of 171 and 34. Vesamicol analog 72 (a more potent inhibitor of acetylcholine transport than vesamicol) at 0.0000011 μmoles/kg to 3.4 μmoles/kg provided no protection from β-bungarotoxin and was not tested with any other venoms or toxins.

Vesamicol had no effect on the LD_{50} of *B. caeruleus* venom or *B. multicinctus* venom. At 0.0011 μmoles/kg, it increased the LD_{50} of *B. caeruleus* venom 1.5-fold from 28 μg/kg to 42 μg/kg ($P = 0.49$), and at 0.26 μmoles/kg, it increased the LD_{50} of *B. multicinctus* venom 2.0-fold from 18 μg/kg to 36 μg/kg ($P = 0.13$). An injection of gel-phosphate buffer followed immediately by an injection of 4.2 μmoles/kg of vesamicol caused no overt effects in 20 mice observed for 24 hr.

Optimal time of injection of drugs

I tested the effective drug and venom/toxin combinations to determine the optimal time of injection of the drug relative to that of the venom/toxin. All drugs were maximally effective when they were injected immediately after injection of venom/toxin. Protection decreased rapidly when any drug was administered 15 min either before or after intoxication. No drug provided protection when administered more than 30 min before or after intoxication.

DISCUSSION

Table 1 summarizes the effects of twelve drugs on the lethality of venoms and neurotoxins from selected snakes. Examination of the table reveals interesting patterns in both the venoms/toxins and the drugs.

With the exception of dexamethasone, all of the effective drugs were so only against *B. caeruleus* venom, *B. multicinctus* venom and β-bungarotoxin. In addition (again with the exception of dexamethasone) any drug which was ineffective against *B. caeruleus* venom was also ineffective against *B. multicinctus* venom and β-bungarotoxin. I summarized these observations using two methods of correlation. As a qualitative correlation of the drugs' effects on the lethality of *B. multicinctus* venom vs the drugs' effects on the lethality of β-bungarotoxin, I utilized Spearman's rank-order method (corrected for ties), using a value of one to represent a significant drug effect and a value of zero to represent no drug effect ($\rho = 0.82$, $P = 0.0068$). (If vesamicol were considered an effective drug against β-bungarotoxin [$P = 0.059$, Fig. 6], the correlation would be 1.00.) As a quantitative correlation I used Pearson's product–moment method to describe the relationship between the drug's effects on the LD_{50} of *B. multicinctus* venom vs the drugs' effects on the LD_{50} of β-bungarotoxin (Fig. 7a) ($r = 0.47$, $P = 0.15$). (I chose to utilize the fold change in LD_{50} caused by a drug as the quantitative measure of efficacy because the fold change has no upper limit. For drugs which had no significant effect on lethality in the dose-effect experiments I assigned a fold change in LD_{50} of 1.0.) The significant qualitative correlation demonstrates that a drug which protected mice from β-bungarotoxin was likely to protect

TABLE 1. SUMMARY OF EFFECTS OF DRUGS ON VENOMS AND TOXINS

Parameter Venom/Toxin	Chlrqn	Chlrprmzn	Dxmthsn	Dltzm	Ncrgln	Prmqn	Qncrn	Vrpml	Vsmcl
Optimal Drug Dose (μmol/kg)									
B. caeruleus venom	78	2.8	none	5.5	8.3	44	4.9	5.1	4.2
B. multicinctus venom	78	2.8	15	22	8.3	11	9.8	5.1	0.066
α-Bungarotoxin	none	none	none	none	nd	nd	none	none	none
ß-Bungarotoxin	39	1.4	75	11	8.3	22	2.0	5.1	none
C. durissus venom	none	none	none	none	none	none	none	none	none
Crotoxin	none	none	6.2	none	none	none	none	none	none
N. scutatus venom	nd	nd	none	none	nd	nd	nd	none	none
O. scutellatus venom	none	none	90	none	none	none	none	none	none
Taipoxin	none	none	60	none	none	none	none	none	none
Maximal % Survival									
B. caeruleus venom	80	90	na	70	93	90	80	73	47
B. multicinctus venom	72	100	80	100	80	90	80	100	30
ß-Bungarotoxin	100	100	50	87	100	100	100	100	na
C. durissus venom	na	na	na	na	na	na	na	na	na
Crotoxin	na	na	50	na	na	na	na	na	na
O. scutellatus venom	na	na	100	na	na	na	na	na	na
Taipoxin	na	na	100	na	na	na	na	na	na
Fold Change in LD50 of Venom/Toxin									
B. caeruleus venom	15	8.7	nd	2.2	1.8 (ns)	2.9	5.7	5.2	1.5 (ns)
B. multicinctus venom	5.0	2.6	1.7 (ns)	7.4	4.6	6.0	11	3.8	2.0 (ns)
α-Bungarotoxin	1.4 (ns)	0.9 (ns)	nd	nd	1.0 (ns)	1.2 (ns)	1.1(ns)	nd	nd
ß-Bungarotoxin	17	3.8	nd	1.9	4.0	3.9	8.6	5.0	nd
C. durissus venom	nd	nd	nd	nd	nd	nd	nd	nd	nd
Crotoxin	nd	nd	1.6 (ns)	nd	nd	nd	nd	nd	nd
O. scutellatus venom	0.9	nd	3.5	nd	nd	nd	nd	nd	nd
Taipoxin	nd	nd	4.0	nd	nd	nd	nd	nd	nd

Data for chloroquine, chlorpromazine, and quinacrine are from CROSLAND, 1989a; 1989b. Nifedipine, piracetam, and reserpine had no effect on any of the venoms/toxins tested. na = not applicable, nd = not determined, ns = not significant.

mice from *B. multicinctus* venom. The non-significance of the quantitative correlation was largely due to to the substantial change in the LD_{50} of β-bungarotoxin (17-fold) caused by chloroquine. Excluding this point raises the quantitative correlation to 0.79 ($P = 0.0060$). It appears that a drug's effect on the lethality of β-bungarotoxin was reflected in its effect on the lethality of *B. multicinctus* venom. This is not surprising since β-bungarotoxin is the most lethal component of *B. multicinctus* venom and contributes the majority of the venom's lethality (CHANG, 1985). Any drug which reduces the lethality of β-bungarotoxin would be expected to reduce the lethality of *B. multicinctus* venom also.

Correlations similar to those between the drugs' effects on β-bungarotoxin and *B. multicinctus* venom were observed between the drugs' effects on *B. caeruleus* venom and *B. multicinctus* venom. The qualitative correlation was 0.75 ($P = 0.012$) while the quantitative correlation was 0.31 ($P = 0.32$) (Fig. 7b). *B. caeruleus* venom not only comes from a snake of the same genus as *B. multicinctus*, it also contains presynaptic toxins with potencies similar to that of β-bungarotoxin (ABE *et al.*, 1977; LEE *et al.*, 1976). Although the relative contribution of these neurotoxins to the lethality of the whole venom has not been thoroughly studied, it would seem reasonable that this contribution is similar to that of β-bungarotoxin's contribution to the lethality of *B. multicinctus* venom. Thus, we could expect that any drug which reduces the lethality of *B. multicinctus* venom would also reduce the lethality of *B. caeruleus* venom. This, indeed, appears to be the case.

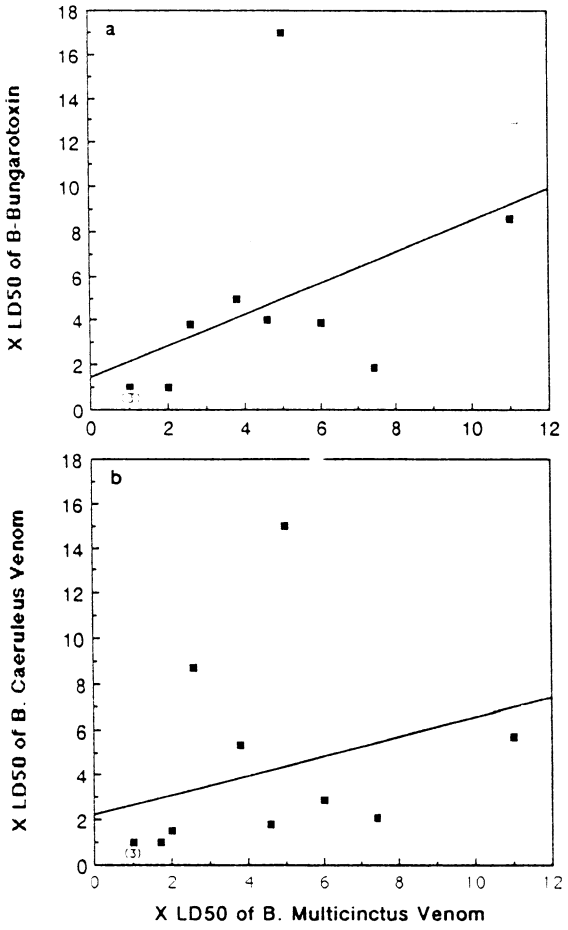

FIG. 7. CORRELATIONS OF FOLD CHANGES IN LD_{50}.
(a) Plot of the drug-induced fold changes in the LD_{50} OF β-bungarotoxin vs the drug-induced fold changes in the LD_{50} of *B. multicinctus* venom. (b) Plot of the drug-induced fold changes in the LD_{50} of *B. caeruleus* venom vs the drug-induced fold changes in the LD_{50} of *B. multicinctus* venom.

The same drugs (except dexamethasone) which protected mice from the two *Bungarus* venoms and β-bungarotoxin did not protect mice from *C. durissus terrificus* venom or its presynaptic toxin crotoxin, or *O. scutellatus* venom or its presynaptic toxin taipoxin. β-Bungarotoxin, crotoxin and taipoxin have similar effects on neuromuscular transmission, have phospholipase A_2 activity, and are thought to act through similar biochemical mechanisms (CHANG, 1985). With respect to the action of the majority of the drugs tested, however, β-bungarotoxin was quite distinct from crotoxin and taipoxin. The discriminatory action of the drugs may be related to one or more of the salient differences among the toxins. β-Bungarotoxin, for example, consists of two polypeptide chains linked by a disulfide bond, whereas crotoxin and taipoxin are composed of two and three subunits, respectively. Also, β-bungarotoxin has a basic isoelectric point (9.1) (OTHMAN *et al.*, 1982), while both crotoxin and taipoxin have an acidic isoelectric point (5.0) (KARLSSON, 1979). The difference in isoelectric points, however, may not account entirely for the differential

action of the drugs because all of the drugs were ineffective against the lethality of α-bungarotoxin, which also has a basic isoelectric point (9.2) (ELDEFRAWI and FERTUCK, 1974). Finally, there is evidence that β-bungarotoxin, crotoxin, and taipoxin bind at different sites on the presynaptic membrane (CHANG and SU, 1980; REHM and BETZ, 1982). The drugs may act to inhibit differentially the binding of the toxins, thus providing selective protection from the toxins and their respective venoms. Whatever its cause, the differential effect of the drugs on the lethality of the three toxins is further evidence of distinctions among the toxins.

Several observations can be made concerning the drugs used in these studies. One is that all but vesamicol have been used clinically in humans (BARNHART, 1989; BILLUPS and BILLUPS, 1989). Also, the effective drugs were generally so in doses which approximated those used clinically, suggesting that the drugs acted through a clinically relevant mechanism. The effective agents did not, however, belong to a single therapeutic group of drugs, precluding correlation of venom/toxin antagonism with the primary therapeutic action of an agent. Diltiazem, nicergoline, nifedipine and verapamil are vasodilators, and all but nifedipine were effective antagonists of the lethality of the *Bungarus* venoms and β-bungarotoxin. All of the antimalarial drugs tested — chloroquine, primaquine and quinacrine — were likewise effective against the *Bungarus* venoms and β-bungarotoxin. Also effective in varying degrees were the tranquilizer chlorpromazine, the anti-inflammatory agent dexamethasone and the non-clinically utilized acetylcholine transport inhibitor vesamicol. The cerebral stimulant piracetam and the antihypertensive reserpine were ineffective.

On another level, diltiazem, nifedipine and verapamil are Ca^{2+} antagonist drugs which could act to inhibit presynaptic, toxin-related, Ca^{2+}-dependent phospholipase A_2 activity and perhaps thereby the lethality of the venoms/toxins (CHANG, 1985). The drugs bind at different sites on a protein component of the L-type, voltage-dependent Ca^{2+} channel, reducing Ca^{2+} permeation. They also affect other Ca^{2+}-related processes (ZERNIG, 1990). Diltiazem and verapamil inhibited the lethality of the *Bungarus* venoms and β-bungarotoxin, whereas nifedipine failed to inhibit the lethality of any of the venoms/toxins. It does not appear that being a Ca^{2+} antagonist drug *per se* guaranteed effectiveness against the venoms/toxins. Nevertheless, other selected members of this class of drugs may effectively antagonize the lethality of snake venoms.

Chloroquine, chlorpromazine, quinacrine, reserpine, vesamicol and vesamicol analog 72 are inhibitors of acetylcholine transport into synaptic vesicles prepared from the electric organ of *Torpedo californica* (ANDERSON *et al.*, 1983; ROGERS *et al.*, 1989) (Table 2). Since chloroquine, chlorpromazine and quinacrine were shown to be antagonists of the lethality of the *Bungarus* venoms and β-bungarotoxin (CROSLAND, 1988; 1989*a*,*b*), other transport inhibitors could have been also. The results, however, suggested otherwise because the fold changes in the LD_{50} of β-bungarotoxin due to the above drugs did not correlate ($r = -0.33$, $P = 0.52$) with their IC_{50}s with respect to acetylcholine transport, implying that their antagonism of the lethality of β-bungarotoxin was not related to their acetylcholine transport inhibitory activity.

The initial criterion for choosing a drug for this series of studies was that it inhibits phospholipase A_2 activity. The investigated venoms/toxins have phospholipase A_2 activity which may be implicated in their toxicity (CHANG, 1985), suggesting that inhibitors of this activity could reduce that toxicity. Two immediate problems with this hypothesis, however, are the observations that nifedipine and piracetam were ineffective against the lethality of any of the venoms/toxins and that all of the drugs (except dexamethasone)

TABLE 2. PROPERTIES OF DRUGS

Drug	Charge (a) pH 7.2	IC50 ACh (b) Transport (μM)	MW	Solubility (M) (c)	Ki PLA2 (μM)	Reference, Ki
Chloroquine	1.9	0.5	320	0.48	165	AUTHI & TRAYNOR, 1979
Chlorpromazine	1.0	3.0	319	1.4	27	JAIN & JAHAGIRDAR, 1985
Dexamethasone	-1.5		392	0.23	1	PILTCH et al., 1989
Diltiazem	1.0		415	1.1	100	BROEKMEIER, et al., 1985
Nicergoline	0.9		484	0.00026	0.1	NIKOLOV & KOBUROVA, 1984
Nifedipine	0.05		346	0.00036	50	CHANG, et al., 1987
Piracetam	0.0		142	3.5	20	NIKOLOV & KOBUROVA, 1984
Primaquine	1.9		259	0.15	17	AUTHI & TRAYNOR, 1979
Quinacrine	1.8	0.4	400	0.019	400	BROEKMEIER, et al., 1985
Reserpine	0.2	8.0	608	0.000082		
Verapamil	1.0		455	0.0041	200	BROEKMEIER, et al., 1985
Vesamicol	1.0	0.04	259	0.011		
Vesamicol 72	0.1					

(a) The charge of a drug at pH 7.2 was calculated from the measured pKa(s) when available (PERRIN, 1965; PERRIN, 1972). Otherwise, I estimated it using the methods in PERRIN *et al.* (1981). (b) Values are from ANDERSON *et al.* (1983) and ROGERS *et al.* (1989). (c) The solubility of a drug in phosphate-buffered saline was determined by diluting the drug in 2-fold steps from 1 g/ml to 0.125 mg/ml. The concentration (in molarity) at which the drug completely dissolved was taken as its solubility. Note that this value could have almost a 2-fold error. Nicergoline, nifedipine, and reserpine did not completely dissolve at 0.125 mg/ml, but I used this value to calculate their solubilities.

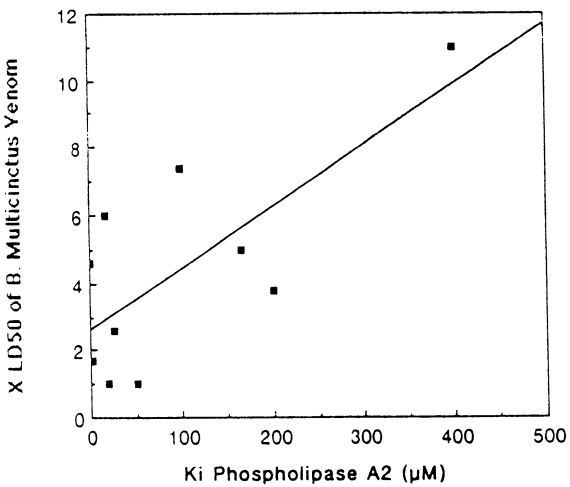

FIG. 8. CORRELATION OF FOLD CHANGES IN LD_{50} OF *B. multicinctus* VENOM AND K_i OF DRUGS TOWARD PHOSPHOLIPASE A_2.

Plot of the drug-induced fold changes in the LD_{50} OF *B. multicinctus* venom vs the inhibitory constants (K_i) of the drugs with respect to phospholipase A_2 activity.

which provided protection from the *Bungarus* venoms and β-bungarotoxin were completely ineffective against *C. durissus terrificus* venom, crotoxin, *O. scutellatus* venom and taipoxin. In the latter instance it is possible, though unlikely, that the effective drugs acted by inhibiting a *Bungarus*-specific phospholipase A_2 activity. *Apropos* of this possibility,

TABLE 3. CORRELATION (r) OF FOLD CHANGE IN LD_{50}S WITH DRUGS' PROPERTIES

Venom/Toxin	Molecular Weight	Solubility	Charge pH 7.2
B. caeruleus venom	0.10	-0.02	0.62 (a)
B. multicinctus venom	0.10	-0.25	0.76 (b)
ß-Bungarotoxin	-0.02	-0.18	0.71 (c)

Values are the correlation coefficients between the fold changes in the LD_{50} of a venom/toxin caused by the various drugs and the particular property of those drugs. (a) $p = 0.041$ (b) $p = 0.0068$ (c) $p = 0.014$

there was a significant correlation ($r = 0.73$, $P = 0.016$) between the fold change in LD_{50} of *B. multicinctus* venom and the K_is of the drugs with respect to phospholipase A_2 activity (Fig. 8). A large part of this correlation, however, was contributed by the 11-fold increase in LD_{50} caused by quinacrine. Removal of this point from consideration reduced the correlation to 0.32 ($P = 0.39$), which was similar to that ($r = 0.39$, $P = 0.26$) between the fold change in the LD_{50} of *B. caeruleus* venom and K_i and the correlation ($r = 0.51$, $P = 0.16$) between the fold change in the LD_{50} of β-bungarotoxin and K_i. It should also be noted that the correlations were positive. *A. priori* I would expect that a phospholipase A_2 inhibitor with a low K_i would cause a large increase in LD_{50}, i.e. the correlation would be negative. Moreover, an important *caveat* to this analysis is the determination of the K_is of the drugs with respect to phospholipase A_2 activity. The values that I used (Table 2) were the lowest that I found in the literature and were determined from assays that used different sources of phospholipase A_2, different substrates and different detection methods. The pitfalls of comparing results from different studies of phospholipase A_2 activity have been well documented, particularly the problem of using non-physiological substrates (CHANG, 1985; ROSENBERG, 1979). Unfortunately, due to their small relative mass, it is not possible to detect any β-bungarotoxin-stimulated phospholipid hydrolysis at the presynaptic terminals of the phrenic nerve-diaphragm (GHASSEMI *et al.*, 1988), making it impossible to compare directly the relevant anti-phospholipase A_2 activity of the drugs with their protective activity. From the preponderance of the available data, however, I cannot conclude that the phospholipase A_2 inhibitory activity of the tested drugs was a significant factor in their ability to afford protection from the lethality of the venoms/toxins.

I examined the quantitative relationships between the protective ability of a drug and its mol. wt, solubility and charge. There was no correlation between either the mol. wt or the solubility of a drug in phosphate-buffered saline and the fold change in LD_{50} for either of the *Bungarus* venoms or β-bungarotoxin (Table 3). There was, however, for both venoms and β-bungarotoxin a significant correlation between the protective ability of a drug and its positive charge at pH 7.2. Figure 9 illustrates this relationship for *B. multicinctus* venom. (Dexamethasone was the only drug with a negative charge and was omitted from the correlation.) It appears that a drug needed a positive charge in order to antagonize the lethality of the venoms or β-bungarotoxin, and the higher the charge, the more protection the drug afforded. Since the charge of a molecule is a major factor in its ability to bind to a receptor or enzyme, the sucessful antagonists may compete with the presynaptic toxins for binding to a receptor or they may compete with some substrate for binding to the

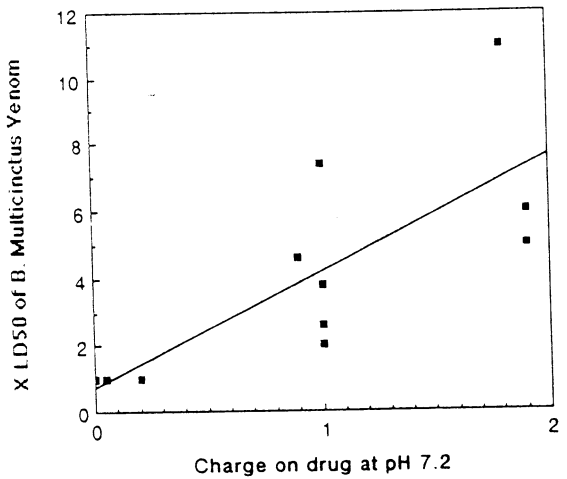

FIG. 9. CORRELATION OF FOLD CHANGES IN LD_{50} OF *B. multicinctus* VENOM AND CHARGE ON DRUGS AT pH 7.2.
Plot of the drug-induced fold changes in the LD_{50} of *B. multicinctus* venom vs the charge on the drugs at pH 7.2.

toxins or for binding to some enzyme activated by the toxins. Whatever the mechanism may be, it seems to be limited to the *Bungarus* venoms and β-bungarotoxin.

Dexamethasone was the exceptional drug throughout this study. It was the only drug which was an effective antagonist of the lethality of venoms/toxins other than those from the *Bungarus* snakes (Table 1). It antagonized five of the nine venoms/toxins tested and almost antagonized α-bungarotoxin and *B. caeruleus* venom (Fig. 1). It was particularly effective (100%) against *O. scutellatus* venoms and taipoxin, with which it did not exhibit declining effectiveness at high doses of drug (the only effective drug and venom/toxin combinations not to do so). Dexamethasone was the only drug in this study to have a negative charge (−1.5) at physiological pH (due to the phosphate group attached to the parent molecule), also making it the only drug without a positive charge to antagonize effectively the lethality of *B. multicinctus* venom and β-bungarotoxin. This could mean that *any* charge will serve to antagonize the venoms/toxin or that some other characteristic(s) of dexamethasone overcame the lack of a positive charge. The K_i (1 μM) of dexamethasone with respect to phospholipase A_2 activity was the second lowest of all the drugs tested. Molecular weight and solubility were in the midrange of the group.

Others have reported mixed results when corticosteroids were used to treat envenomation by snakes. BENYAJATI *et al.* (1961) found that prenisolone significantly enhanced the survival of dogs that had been injected with *Naja tripudians* (cobra) venom. They also found that cortisol or prednisolone was very beneficial in the treatment of three known and three presumed cobra-bite victims. REID (1964), on the other hand, reported that prednisolone was not beneficial in the cases of four humans bitten by cobras (*Naja naja*). In addition, REID *et al.* (1963) reported that prednisolone had no beneficial action on human envenomation by Malayan vipers (*Agkistrodon rhodostoma*). Although the above results and my results with dexamethasone are not rigorously comparable, considered *in toto* they suggest that corticosteroids or derivatives thereof could provide protection from the lethal venoms of several species of snakes.

The time of injection of a drug relative to the time of injection of the venom/toxin was an important factor in the drug's efficacy. All of the time-tested drugs were maximally effective when they were injected immediately after the venom/toxin was injected. Injection of the drug either 15 min before or 15 min after injection of the venom/toxin greatly reduced or, in some cases, eliminated effectiveness. None of the drugs was effective when it was injected 30 min prior to the injection of venom/toxin, and only chloroquine (CROSLAND, 1989*b*) and dexamethasone were even partially effective when they were injected 30 min after the injection of venom/toxin. The requirement for temporal proximity of injection of drug and venom/toxin may suggest that the drugs were protecting mice from the lethality of the venoms/toxins by interrupting some initial step(s) in intoxication such as transport and/or binding to the target organ.

In summary, the drugs utilized in this study can be grouped into three categories: (1) drugs which antagonized the lethality of only the *Bungarus* venoms and β-bungarotoxin; (2) dexamethasone; (3) drugs which were ineffective. All effective drugs were amphiphilic and carried a charge at physiological pH. Future research to delineate the properties of a drug which make it an antagonist of the lethality of snake venoms and toxins could lead to the development of drugs which are even more effective and have a broader spectrum of action than those investigated to date.

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