Condylactis Toxin: Interaction with Nerve Membrane Ionic Conductances

Abstract. *A toxin from the Bermuda anemone* Condylactis gigantea *causes the early transient conductance change of crayfish giant axon membranes to persist without affecting the shape of its turning-on. The increase in late steady-state conductance is either not affected or slightly suppressed. The effect on the conductance components can adequately account for the prolonged action potential observed in the treated axon.*

This report describes the results of voltage clamp experiments with the crayfish giant axons poisoned with the toxin from the Bermuda anemone *Condylactis gigantea*. Shapiro (*1*) has found that *Condylactis* toxin (CTX) causes the action potential from the lobster giant axons or from the crayfish slow-adapting stretch receptors to be greatly prolonged, forming such a plateau as seen in cardiac tissues. He suggested that the slowing of the sodium-inactivation or the turn-off process of the membrane sodium conductance is responsible for the prolongation of action potential. The following experiments were undertaken to evaluate this hypothesis.

We first applied CTX to squid axons but found that their ionic conductances were not affected. We then moved to lobster and crayfish giant axons, and chose crayfish axons for use in the voltage clamp because (i) the action potential is similarly prolonged; (ii) they are larger in diameter and easier to handle; and (iii) the survival time is longer under sucrose-gap conditions.

The central nerve cord extending from the brain to the metathoracic ganglion was isolated from the crayfish *Procambarus clarkii*. The median giant axon was then isolated all the way through the nerve cord. The diameter of the giant axons ranged between 100 and 300 μ in different preparations, and usually became smaller as it passed through the ganglia. Most of the experiments were done with the axons with a diameter from 150 to 250 μ. The methods of the voltage clamp experiments with the sucrose-gap chamber were essentially the same as those previously used for squid or lobster giant axons (*2*). In short, a small portion (50 to 100 μ wide) of the isolated giant axon was insulated from the rest of the axon by means of two isotonic sucrose streams, and measurements of membrane potentials and membrane currents were made on this "artificial node." Van Harreveld solution (*3*) was used as the bathing medium. To completely depolarize one end of the axon preparation, we used 265 m*M* KCl solution (*4*). The concentration of the sucrose solution was 378 m*M*. All the experiments were conducted at a temperature of about 7°C. The CTX test solution was prepared as described (*1*).

When CTX was applied to the artificial node, at a concentration of 0.2 mg/ml, the rising phase and the peak magnitude of the action potential underwent little or no change, but the falling phase of the action potential was slowed enough to form a plateau phase. Under voltage clamp and in van Harreveld solution, the early transient (sodium) membrane currents were similar to those normally observed in squid and lobster axons. In about one-third of the axons, the late outward steady (potassium) current density was as large or larger than the early transient current, as was usually found in squid axons. However, the other two-thirds showed rather small late currents.

The steady-state current was depressed in magnitude after application of CTX, and within a few minutes converted into an inward steady-state current (Fig. 1A). The early transient current was slightly increased in its peak magnitude, associated with a slight increase of the time to the peak.

The change in the steady-state current might have been brought about by a reduction in the potassium conductance, or by a slowing of the sodium conductance decrease, or by both together. To choose between these alternatives, we used tetrodotoxin (TTX) to block the early transient conductance change without affecting the late steady-state conductance (*5*). The effect of TTX on the CTX-poisoned axon is shown in Fig. 1B. The early transient current was completely blocked by application of TTX, whereas the late steady-state inward current was brought back to the original outward current. Thus the difference between two membrane currents in Fig. 1B represents the sodium current under the influence of CTX, whereas the outward steady-state current remaining in axons treated with CTX and TTX represents the potassium current (and leakage current) under the influence of CTX. At a higher potential step near the equilibrium potential for sodium, the late current did not change appreciably upon application of CTX or CTX plus TTX. At still higher potential steps where the inside of the axon was made more positive than the sodium equilibrium potential, the late current was increased in axons with CTX but brought back to the original level when TTX was added to the CTX. In this particular experiment CTX does not affect the late potassium channel but only prolongs the early transient sodium current; that is, the sodium inactivation is effectively slowed by the action of CTX. However, in other experiments the late potassium current was sometimes decreased.

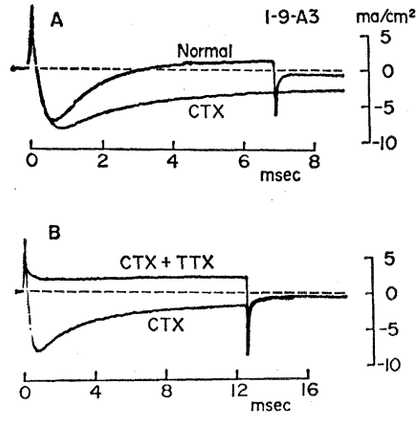

Fig. 1. Membrane currents associated with step depolarizations from the holding potential of −110 mv to 0 mv in a voltage-clamped crayfish giant axon. (A) Before (normal) and during a longer pulse applied after washing in *Condylactis* toxin (CTX) (0.2 mg/ml) for 10 minutes; (B) during application of CTX for 10 minutes and after application of CTX plus 3×10^{-7}*M* tetrodotoxin (TTX) for 2 minutes.

The peak of the early transient current is increased and slightly delayed by application of CTX. This effect was observed in most of the experiments, and is possibly related to the slowing of the sodium inactivation, the slower development of inactivation permitting the sodium current to reach a slightly higher value than observed in the control (*6*). However, repolarization to the holding potential apparently cuts off this conductance almost immediately. This can be gleaned from the fact that the current "tail" at the end of the pulse in the CTX curve is identical to the CTX plus TTX curve (Fig. 1B) in which only potassium and leakage currents flow.

Other experimental agents which have prolonged the early sodium conductance are the insecticide allethrin

(7), cesium applied internally (8), sodium fluoride perfused internally (9), internal media of low ionic strength (10), and scorpion venom (11). However, some difference is noted between CTX and the other agents. For the other agents, it was concluded that the magnitude of the steady-state potassium current was decreased along with the prolongation of the early transient current. This may suggest a coupling between these two processes. It remains to be seen whether CTX is consistent in its lack of effect on the potassium channel and therefore different from the other agents in its action on the possible coupling process.

Condylactis toxin is a rather large molecule (molecular weight 10,000 to 15,000) (12), and it is interesting to see how such a large molecule specifically slows the turn-off without affecting the turn-on of the early transient conductance. Because CTX did not affect the turn-off of the transient conductance in squid giant axons, there may be some species specificity of the CTX action, requiring a special configuration of membrane molecular structure.

TOSHIO NARAHASHI
JOHN W. MOORE
Department of Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina
BERT I. SHAPIRO
Biological Laboratories, Harvard University, Cambridge, Massachusetts

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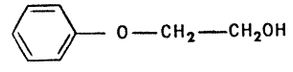
Phenoxyethanol: Protein Preservative for Taxonomists

Abstract. *Pieces of chicken heart or skeletal muscle were placed in a dilute solution of the antimicrobial agent 2-phenoxyethanol and stored at room temperature. Under these conditions, the serum albumin, lactate dehydrogenase, and malate dehydrogenase in these tissues survived in easily detectable amounts for at least 2 weeks. The surviving proteins appeared to be identical with those of fresh tissues in physical, catalytic, and immunological properties. Phenoxyethanol also preserved heart and muscle proteins of representatives of other vertebrate classes. Tissue samples collected in the field may be thus preserved for later analysis by biochemical taxonomists.*

Biochemical methods are used more and more to classify species (1) and elucidate their evolutionary history (2). To simplify the collection and transportation of samples collected in the field, we sought a preservative that prevents bacterial and fungal growth and yet permits the survival of native proteins at room temperature for several weeks (3).

The compound 2-phenoxyethanol, also known as phenoxetol or ethylene glycol monophenyl ether, appears to be such a preservative.



It is a colorless, nonvolatile, nonflammable, stable, and nearly odorless liquid. Moreover, it is low in price and relatively nontoxic. Phenoxyethanol has received limited clinical and industrial use both as an antibacterial and antifungal agent (4). It has been employed at the British Museum as a preservative for animals and plants, after fixation with formaldehyde or Bouin's fluid, but invertebrates that have not been first fixed in formaldehyde disintegrate within a few weeks when stored in 2-phenoxyethanol (5). Thus, to be of use to the biochemical taxonomist, selected tissues and not whole organisms have to be placed in the 2-phenoxyethanol solution.

The preservative properties of phenoxyethanol are illustrated by our experiment with chicken tissues. A piece of heart muscle (1 g) was put in 5 ml of a 2 percent (by volume) solution of phenoxyethanol in distilled water and stored in a screw-capped glass vial (15-ml capacity) at room temperature (22° to 28°C) on a laboratory bench. After 2 weeks, the contents of the vial were homogenized. The homogenate was clarified by centrifugation at 30,000*g* for 10 minutes. Some of the resulting supernatant fluid was assayed spectrophotometrically (6, 7) for lactate dehydrogenase activity. The total number of enzyme units (8) in the vial was calculated to be 600; in comparison, 1 g of fresh heart muscle contained about 650 units of lactate dehydrogenase activity.

With crystalline chicken H_4-lactate dehydrogenase (9) and a freshly prepared extract of chicken heart muscle as controls, we examined the following properties of the surviving lactate dehydrogenase: (i) electrophoretic mobility in starch gel at *p*H 7; (ii) sensitivity to thermal denaturation under standard conditions; (iii) Michaelis constant (K_m); (iv) sensitivity to inhibition by pyruvate; and (v) reactivity in immunodiffusion and microcomplement fixation tests with rabbit antiserum directed against pure chicken H_4-lactate dehydrogenase (Fig. 1A). These properties of the fresh enzyme have been described (6). The enzyme from the phenoxyethanol-treated tissue appeared identical with the pure enzyme and with that from fresh tissues.

A similar experiment was conducted with a 1-g portion of chicken breast muscle, which contains M_4-lactate dehydrogenase, an enzyme that is quite distinct chemically from the H_4 enzyme (6, 9). After 2 weeks, all of the original M_4 enzyme activity was still present. The surviving enzyme had normal electrophoretic properties.

The two isoenzymes of malate dehydrogenase also survived in these experiments with chicken heart and breast muscle. The recovery of malate dehydrogenase activity at 2 weeks was 20 percent. Electrophoretic analysis revealed that the mitochondrial isoenzyme survived to a lesser extent than did the supernatant isoenzyme. The mobilities of the surviving isoenzymes were normal (10).

Serum albumin, which occurs in readily detectable amounts in excised muscle tissues, also survived in the above experiments. This protein was detected by electrophoresis as well as by immunological tests with rabbit