tions of purified nuclear suspensions. The nuclei from one portion were dried to constant weight under vacuum over P_2O_5 and the total PSANP fraction was extracted from the second portion. Protein was determined by the method of Lowry *et al.* (*20*). For the G_2 period, the percentage of dry weight of nuclear material constituted by DNA (5.5 percent) was determined as above. Before DNA analysis the nuclear RNA was removed from the nuclear pellets with 0.4*N* NaOH, and the DNA was extracted as previously described [W. Sachsenmaier, H. P. Rusch, *Exp. Cell Res.* **36**, 124 (1964)]. The DNA content was determined by its optical density at 260 nm and by the diphenylamine test [K. Burton, *Biochem. J.* **62**, 315 (1956)].

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Snake Neurotoxins: Effect of Disulfide Reduction on Interaction with Electroplax

Abstract. *Reduction of disulfide bonds of cobra neurotoxin by dithiothreitol results in decreased activity on the electroplax preparation. Activity is restored completely after reoxidation by 3,3′-dithiobis[6-nitrobenzoic acid]. Reduction of the disulfide bonds in the vicinity of the receptor does not decrease the effect of cobrotoxin.*

A great deal of effort has been concentrated on purifying and elucidating the structure and toxicology of snake neurotoxins. The amino acid sequence (*1*) and the number and position of disulfide bonds (*2*) of several toxins have been determined, and some preliminary x-ray crystallography studies of erabutoxin have been reported (*3*). It was shown that the basic structure of erabutoxins, cobrotoxin, and bungarotoxins is the same and that only some amino acid positions are variable (*1*).

Changeux *et al.* showed that α-bungarotoxin acts somewhat like curare at the synapses of the electroplax, the only differences being its greater potency and its irreversibility (*4*). Miledi *et al.* (*5*) used bungarotoxin and Meunier *et al.* cobrotoxin (*6*) to isolate a protein fraction which showed AcCh-receptor binding characteristics. Earlier studies have shown that the tryptophan residue in position-29 (*7*) and the integrity of the disulfide bonds (*8*) are essential for activity. These experiments were done on whole animals and nothing was known about the tertiary structure of the toxins and the nature of receptors. Therefore, these experiments were repeated on the electroplax preparation (*9*) where much information about the receptor has been obtained during the last decade.

X-ray crystallography suggests that the erabutoxin may be a flattened, disk-shaped molecule with little helical structure (*3*). The tryptophan residue in position-29 and the disulfide linkage 3 to 24 are very likely at the surface of the molecule. The distance between the tryptophan residue and this disulfide bridge could be as little as 10 Å. Hence the tryptophan residue might possibly act as the affinity binding site and a disulfide exchange between the toxin and the disulfide bond of the receptor site may take place. Reduction of the receptor with dithiothreitol (DTT) has been shown to reduce the effects of carbamylcholine (*10*). However, the reduction of the receptor did not prevent the irreversible action of the cobrotoxin, and neither 3,3′-dithiobis[6-nitrobenzoic acid] (DTNB) nor cholinethiol could cause a reversal of the inhibitory effect of the neurotoxin. On the other hand, if the neurotoxin was treated with $5 \times 10^{-4}M$ DTT at *p*H 8.0 for 10 minutes before the application to the electroplax, the activity of the toxin was reduced considerably, and activity could be restored completely after reoxidizing the stock solution with $1 \times 10^{-3}M$ DTNB (Fig. 1). Although a disulfide interaction between toxin and receptor has not been ruled out completely by these experiments, it seems more likely that a complemen-

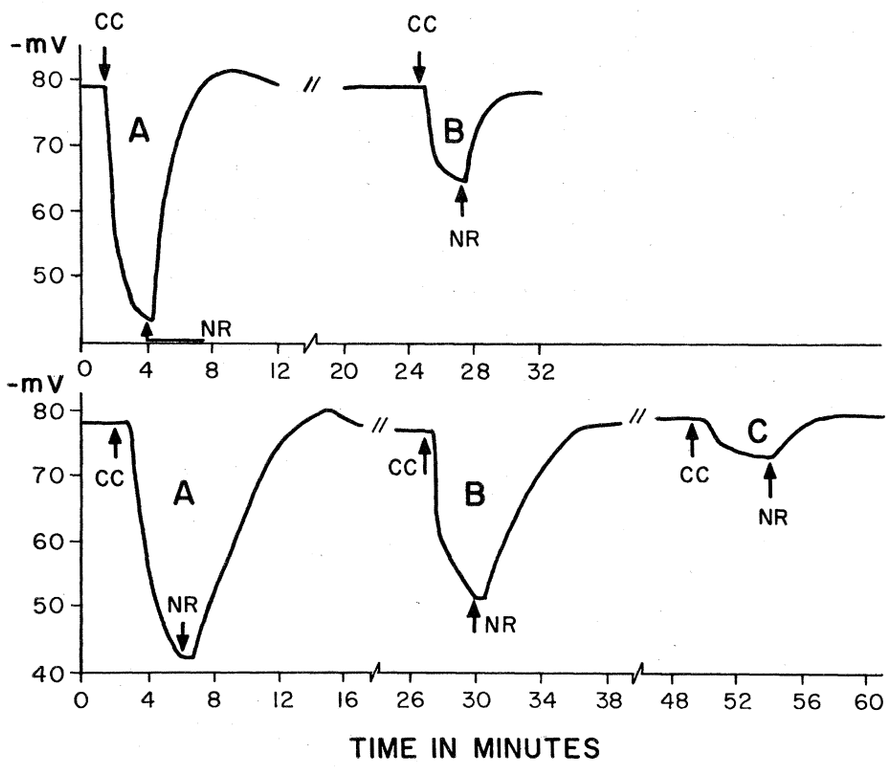

Fig. 1. Potential recording of the innervated membrane of a single electroplax from *Electrophorus electricus*. (Top A) Control response to $5 \times 10^{-5}M$ carbamylcholine (*CC*). (Top B) Reduced response to $5 \times 10^{-5}M$ carbamylcholine after the cell was exposed to cobrotoxin (0.001) mg/ml) for 10 minutes. (Bottom A) Control response to $5 \times 10^{-5}M$ carbamylcholine. (Bottom B) Response to carbamylcholine $5 \times 10^{-5}M$ after the cell was exposed to cobrotoxin (0.001 mg/ml) for 10 minutes which was incubated previously with $5 \times 10^{-4}M$ DDT as stock solution (0.1 mg/ml) for 10 minutes, *p*H 8.0. (Bottom C) Same as B, except that the cell was exposed to the same toxin which was reoxidized with $1 \times 10^{-3}M$ DTNB at *p*H 7.8. *NR*, normal Ringer solution.

tarity exists between the toxin and the receptor protein comparable to an antigen-antibody reaction, as suggested by Changeux *et al.* (*4*). The tertiary structure of the toxin, which is held together by the disulfide bonds, has to be intact for activity.

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Direct Test of the Positive Pressure Gradient Theory of Pseudopod Extension and Retraction in Amoebae

Abstract. *When one pseudopod of an amoeba is sucked into a capillary connected to a partial vacuum and subjected to a pressure reduction of 30 to 35 centimeters of water, extension of other pseudopods, exposed to atmospheric pressure, is not prevented. This result is interpreted to mean that cytoplasmic streaming cannot be the result of a positive pressure gradient generated along the length of the stream, for if it were, streaming would have reversed its direction under the applied pressure gradient of opposite sign and supposedly greater magnitude.*

The mechanisms of pseudopod extension and retraction in large, free-living amoebae have remained obscure and controversial despite much research.

According to the frontal contraction model of pseudopod extension (*1*), each advancing pseudopod tip is the source of a contraction drawing the viscoelastic endoplasm forward, where it becomes everted while contracting and solidifying to form the ectoplasmic tube. The advancing rim of the ectoplasmic tube is thought to consist of cytoplasm that has just contracted. The frontal contraction model received experimental confirmation when a weak photoelastic effect near pseudopod tips was predicted and observed (*2*).

Even if a motive force is applied at pseudopod tips, the bulk of endoplasmic streaming might still be attributed to a pressure gradient, as was postulated in the classical explanations of Pantin (*3*) and of Mast (*4*) and defended recently by Jahn (*5*). In fact, several investigators (*6–8*) have independently suggested that more than one force-producing mechanism could, at the same time or under different circumstances, cooperate to produce amoeboid movement.

We hope that the controversy regarding the possible role of hydrostatic pressure in pseudopod extension and retraction can be resolved in a direct and simple experiment. If a pressure gradient is responsible for flow into and out of pseudopods, then the reversal of that pressure gradient by suction on a pseudopod should cause the flow to be reversed.

Chaos carolinensis, a giant carnivorous amoeba, was cultured in Marshall's medium (*9*) and fed *Paramecium aurelia* every few days. Specimens for study were allowed to fast in fresh medium for 1 or 2 days to render them maximally active.

Amoeba proteus was raised in Prescott-James medium (*10*) and fed *Tetrahymena pyriformis* (*11*). Specimens were also allowed to fast 1 to 2 days before experiments.

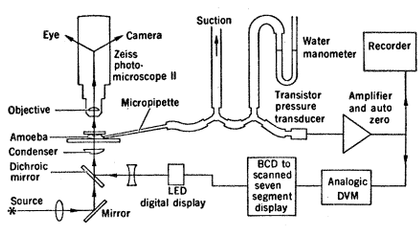

Fig. 1. Block diagram of the experimental arrangement. Abbreviations are: DVM, digital voltmeter; BCD, binary coded decimal output; LED, light-emitting diode. See text and notes for details.

Micropipettes were pulled from Vycor glass of 6.0 mm outer diameter in a Bunsen flame. Final inner diameters of 80 to 120 μm were used for *C. carolinensis* and 30 to 70 μm for *A. proteus*. Micropipettes were connected by Y tubes to a mouth pipette, a pressure transducer, and a water manometer for calibration (Fig. 1).

Amoebae were observed with either a $\times$6, 0.16 numerical aperture (n.a.) objective (for *C. carolinensis*) or a $\times$16, 0.32 n.a. objective (for *A. proteus*) and photographed by a Zeiss photomicroscope camera on Kodak Plus-X 35-mm film.

When suction was applied, the pressure difference was recorded directly in each film exposure by projection of the illuminated digital display into the object and image planes. The digital display itself was a three-digit light-emitting diode (*12*) connected to a circuit which scanned the binary-coded decimal output of the digital voltmeter (*13*). The voltmeter registered the amplified output of the pressure transducer (*14*). When the camera was activated, the pressure difference recorded by the pressure transducer was frozen until the exposure had ended. A chart recorder kept a complete record of suction applied and the times at which the pictures were taken.

Contact of the micropipette with the uroid (tail) of either species of amoeba did not elicit a detectable response. The likelihood of response seemed to increase when the point of contact came closer to advancing pseudopod tips. Contact at a tip either caused streaming to stop or changed the direction of pseudopod extension.

Suction applied to the tail or to retracting pseudopods of either species had little effect on streaming until the pressure exceeded the yield point of the ectoplasmic gel so that cytoplasm flowed easily into the tube. The pressure required to do this varied greatly. Once endoplasm had begun to flow into the tube, the suction required to keep it flowing decreased to 0.1 to 1.0 cm of water. At this point, when small changes in externally applied suction sensitively affected the rate of flow of