The Effect of Ciguatera Toxin on *Aplysia* Neurons[1] (34788)

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Ciguatera toxin is a pharmacologically active material, soluble in organic solvents, which may be extracted from a variety of fish inhabiting localized regions of the reefs in tropical waters (1). While this toxin was at one time considered to be an anticholinesterase, recent work suggests that, in mammals, its pharmacological activity is the result of a more widespread direct action on excitable membranes (2). Typically this involves an initial increase in excitability leading to blockade as dosage is further increased (3). It seems interesting, therefore, to look at its effects on invertebrate tissue even though the detailed mechanism of action of this toxin is not yet understood. The present work is a study of the effect of ciguatera toxin on certain electrophysiological characteristics of unidentified neurons in the pedal ganglion of *Aplysia juliana*.

Materials and Methods. The sea hare, *Aplysia juliana*, was collected on local beaches of the island of Oahu and maintained in circulating sea water at 22°. The animal was forced to extrude mucus and ink. It was then pinned out so that the foot was uppermost. The viscera were removed exposing the ganglia and connectives. The pedal ganglion was removed and placed in a plastic chamber containing 1.5 ml of sea water. A continuous flow system was attached to the chamber so that the solution bathing the cells could be washed out.

Only cells of the pedal ganglion were studied. The advantage of using this ganglion is that the connective tissue surrounding the cells may be easily slit to expose the cells. Thus, the cells are freely exposed in the bath and changes of solution act quickly on the cells without having to traverse connective tissue. Secondly, since the remaining connective tissue is minimal, easy penetration of the soma by the microelectrode is assured. At least three or four large cells are present into which microelectrodes can be easily inserted.

Toxin was isolated from the flesh of the moray eel *Gymnothorax javanicus* from Johnston Island using a modification of the procedure described by Scheuer *et al.* (4), which has yielded a pure substance giving an average death time of 120 min in mice after intraperitoneal injection at 0.1 mg/kg (5). The routine extraction procedure does not yield a product of constant toxicity, and the purified toxin is somewhat unstable even when stored at −20°; toxicity was therefore determined before each series of experiments. Since we have observed that a linear relationship exists between the logarithm of the ciguatera toxin dose and the reciprocal of the average death time, for each assay, groups containing four mice were injected intraperitoneally at four dose-levels giving average death times in the range 30–200 min. Toxicities, defined as the dose required to give a 120-min average death time, were determined from the resultant experimental curves. The dosages quoted in this paper have been adjusted to be consistent with the initial toxicity of the most highly purified extracts. A measured weight of toxin was dissolved in a small quantity of ethyl alcohol, and sea water was added to make the desired concentrations which ranged from 0.02 to 0.325 mg/100 ml. Control experiments showed that the solutions so made without toxin did not depolarize cells or cause a change in firing rate.

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Microelectrodes of 30–60 Mohms resistance filled with 2.5 *M* KCL by boiling were used to penetrate cells. Spontaneous potentials were continuously recorded through a Medistor DC amplifier connected to a Lockheed Electronics FM tape recorder (Model 1031A). Neural activity was monitored concomitantly with a 502 Tektronix oscilloscope. The results of each experiment were then written out by a Grass inkwriter so that alterations in pattern and dc shifts could be observed. Action potentials were photographed in order to measure changes in amplitude accurately—which is not possible with the inkwriter writeout. Changes in activity illustrated by inkwriter tracings involve shifts of baseline and the presence or absence of certain types of activity; the amplitudes of the spikes have no significance in these records.

After impalement of the cell, the preparation was allowed to stabilize for 10–15 min. A control series of recordings was then taken for about 5 min. Ciguatera toxin in 1.5 ml of solution was poured into the bath in which the pedal ganglion was being bathed in 1.5 ml of sea water. Thus, the stated doses include the dilution by the sea water already present in the bath. Control and ciguatera were always at the same termperature (between 18 and 20°). In some experiments it was possible to check for reversibility by washing out the toxin with sea water.

Results. Three types of cells showing spontaneous neural activity were investigated: (a) cells with pacemaker activity only, (b) cells with synaptic[3] activity only, and (c) cells with a mixture of pacemaker and synaptic activity. The latter are referred to as mixed units.

Ciguatera toxin at all doses caused a decrease in the transmembrane potential. Table I summarizes the data obtained from all units with respect to dose, type of unit, degree of depolarization, and transmembrane potential. The mean change in the transmembrane potential was 5.2 ± 1.1 mV. There was no significant correlation between the extent of depolarization and either dose or transmembrane potential.

TABLE I. Effect of Toxin on Neural Activity.

Unit	Concentration (mg /100 ml)	Type	Depolarization (mV)	Transmembrane potential (mV)
1	0.02	Mixed	6.2	38
2	0.13	Synaptic	3.0	—
3	0.1	Pacemaker	2.0	44
4	0.2	Mixed	5.0	42
5	0.3	Pacemaker	8.3	46
6	0.1	Mixed	1.6	60
7	0.15	Synaptic	8.0	35
8	0.15	Mixed	14.0	40
9	0.25	Mixed	3.0	33
10	0.1	Synaptic	4.7	38
11	0.325	Synaptic	4.7	38

The two cells exclusively showing pacemaker activity behaved identically after application of ciguatera toxin. Figure 1 shows the effect of ciguatera toxin (0.3 mg/100 ml) on pacemaker cell 5. Panel A exhibits a portion of the record at slow speed. Toxin was applied at the arrow. About 40 sec later a maintained slowing of activity appeared. Activity before application of toxin consisted exclusively of pacemaker activity as shown on the left side of Fig. 1B. The right side of Fig. 1B is a part of the record at faster speed 1 min after application of toxin. A depolarization of about 8 mV is clearly evident in the record. At the instants marked by dots, pacemaker activity has been replaced by synaptic excitation. A prolongation of the interval between pacemaker firing has also occurred. Measurements of the peak-to-peak amplitude of the action potentials from oscilloscopic photographs indicate that there has been no change in this quantity. Later portions of this record show that almost complete disappearance of pacemaker activity occurs. The remaining unit activity is clearly synaptic, rising sharply from a flat baseline.

Figure 2 shows the effect of a lower dose of ciguatera toxin (0.1 mg %) on pacemaker cell 3. Toxin applied at the arrow causes a small

[3] Pacemaker activity is distinguishable as a slow depolarization endogenous to the cell which reaches a critical firing level. Synaptic activity is distinguishable by potentials such as the epsp, ipsp, ild, etc., which indicate activation through excitatory or inhibitory synapses.

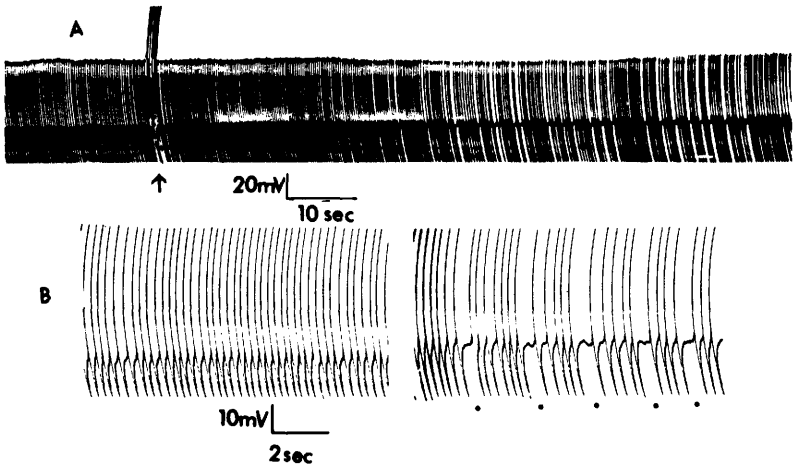

FIG. 1. The effect of ciguatera toxin (0.3 mg/100 ml) on a pacemaker cell. The arrow indicates the time when the toxin is applied in this and other figures.

depolarization and a diminution of pacemaker activity about 50 sec later. A section of the record taken 2.7 min after toxin application is shown expanded on the right side of Fig. 2B. Only one pacemaker response is visible in the record at the time indicated by the dot. In this unit synaptic activity replaced pacemaker activity completely within a short time.

Figure 3 illustrates the effect of toxin on a cell which initially showed a mixture of pacemaker and synaptic activity (unit 8). After the application of toxin (0.15 mg/100 ml) a maximum depolarization of 14 mV occurred. Upper panel A shows the great reduction in firing rate of this cell. This slowing resulted from a disappearance of pacemaker activity, as shown in the lowest panel C. The left side shows an expanded part of the control period 15 to 9 sec before toxin was applied. Mixed pacemaker and synaptic activity is evident. The right side of panel C taken 44 sec after toxin application shows complete absence of pacemaker activity. Nevertheless considerable synaptic activity is observable even between action potentials. Note also in panel A the appearance of inhibitions of long duration during which unit firing is absent although the baseline has not been hyperpolarized back to the control period level. In panel B a

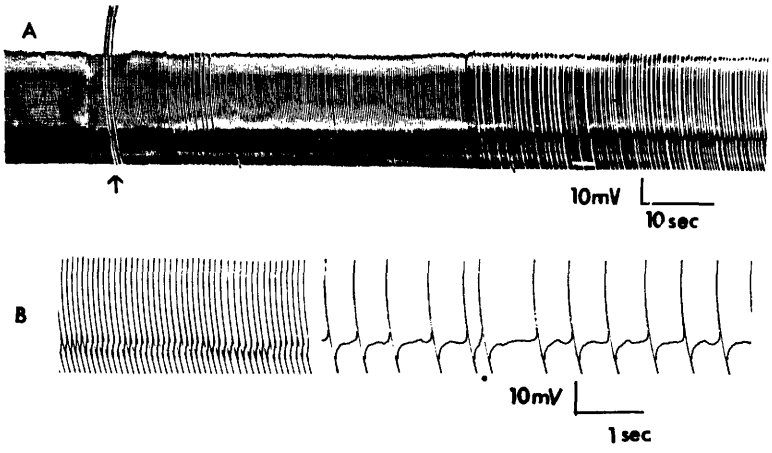

FIG. 2. The effect of ciguatera toxin (0.1 mg/100 ml) on a pacemaker cell.

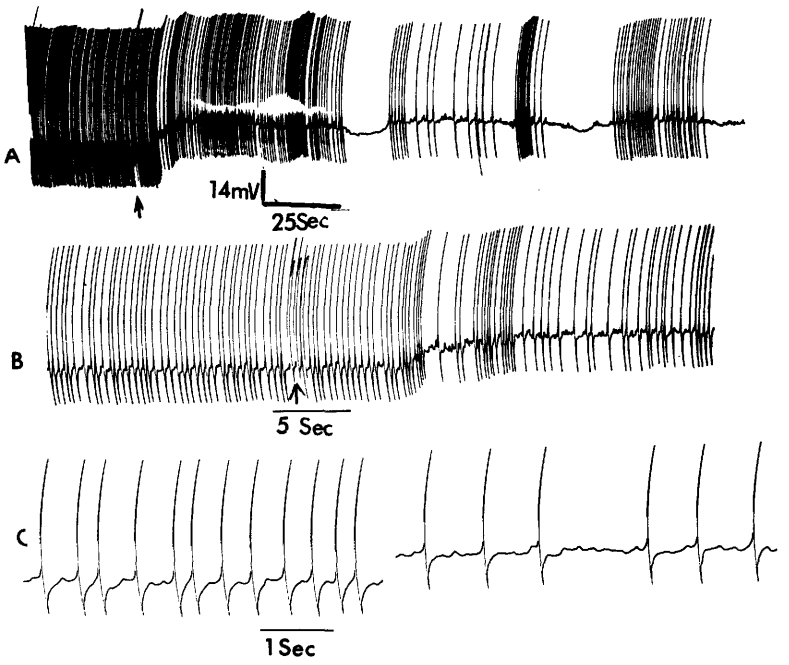

FIG. 3. The effect of ciguatera toxin (0.15 mg/100 ml) on a cell with both pacemaker and synaptic activity.

short portion of the record is shown in which a brief rapid firing occurred during depolarization at the dot.

The effect of ciguatera toxin on a cell exhibiting only synaptic activity (unit 11) is shown in Fig. 4. Toxin (0.325 mg/100 ml) applied at the arrow caused a brief depolarization accompanied by an increased firing. In the course of time, however, there is a slowing of activity although the cell does not become repolarized to the initial transmembrane potential. A recovery of activity oc-

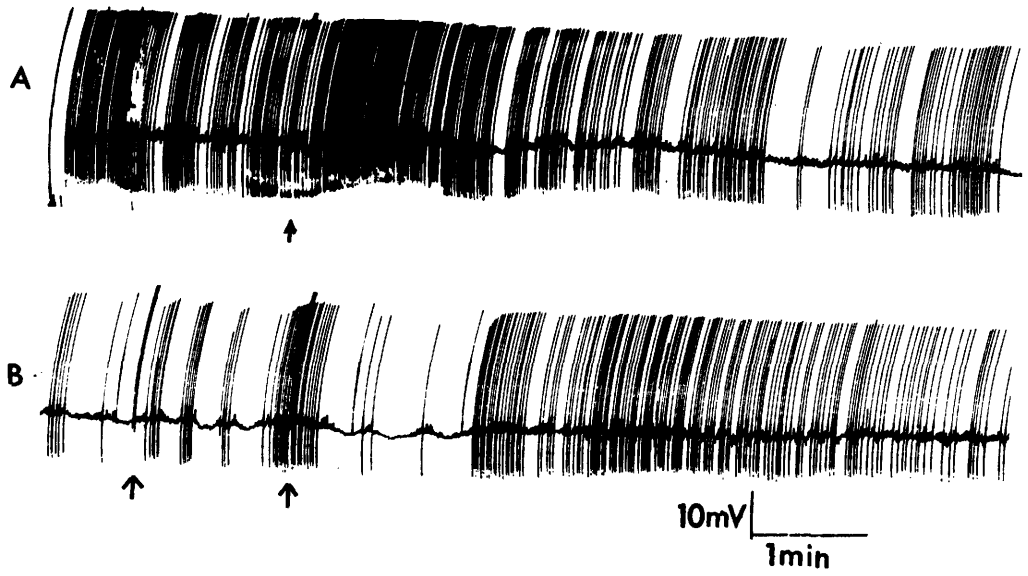

FIG. 4. The effect of ciguatera toxin (0.325 mg/100 ml) on a cell with synaptic activity only.

curred after the wash with sea water begun at the second arrow and terminated at the third arrow.

Discussion. The ability of the membrane potential to increase the firing rate of pacemaker neurons has been demonstrated in experiments in which slow depolarizations were followed by an increased firing rate of *Aplysia* neurons (6). Similarly it has been shown that hyperpolarization of these neurons will depress the firing rate (7).

On the other hand, pacemaker activity can be dissociated from membrane potential level by raising the temperature of the ambient solution so that membrane hyperpolarization occurs at the same time as increased pacemaker activity (8). The work reported here shows that dissociation of pacemaker firing and membrane potential can also take place in depolarized neurons. Ciguatera toxin causes a depolarization which is not accompanied by increased firing. Instead, the activity of pacemaker cells vanishes and synaptic firing appears. Apparently the pacemaker mechanism can be altered in either direction independent of the level of membrane potential. We note also in this regard that high calcium can reduce pacemaker activity without substantial changes in membrane potential (9). A mechanism whereby calcium affects those parameters of the membrane which control rhythmic activity has been put forward (10). An interaction between ciguatera toxin and this calcium mechanism seems possible; high calcium levels have been shown to inhibit the effects of the toxin on sodium transport across the isolated frog skin (11).

No decision can be made at this time with regard to whether the action of the toxin is on the soma or on subsynaptic sites. But pacemaker activity has been shown to persist in the soma when it is physically separated from synaptic regions (12). This would suggest that the abolition of pacemaker activity very probably occurs by inactivation of somatic sites. In the present experiments ciguatera toxin did not appear to act with much effect on synaptic sites since synaptic activity remained relatively unchanged.

Summary. Ciguatera toxin causes a depolarization of the pedal ganglion cells of *Aplysia juliana*. Accompanying the depolarization is a reduction in the firing rate of the cell and a replacement of pacemaker by synaptic activity. This observation provides additional evidence that pacemaker activity can be dissociated from membrane potential changes in particular experimental situations.

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1. Banner, A. H., Scheuer, P. J., Sasaki, S., Helfrich, P., and Alender, C. B., Ann. N. Y. Acad. Sci. **90**, 770 (1960).

2. Rayner M. D., Baslow, M. H., and Kosaki, T. I., J. Fish. Res. Bd. Can. **26**, 2208 (1969).

3. Rayner, M. D., Proc. Food–Drugs from the Sea Conference, Univ. Rhode Island (in press).

4. Scheuer, P. J., Takahashi, W., Tsutsumi, J., and Yoshida, T., Science **155**, 1267 (1967).

5. Takahashi, W., and Scheuer, P. J., Rep. sem. Icthyosarcotoxism, WP/19. S. Pac. Comm., Noumea, New Caledonia (1968).

6. Tauc, L., Colloq. Int. Centre Nat. Rech. Sci. No. 67, p. 91 (1957).

7. Tauc, L., C. R. Soc. Biol. **154**, 17 (1960).

8. Carpenter, D. O., J. Gen. Physiol. **50**, 1469 (1967).

9. Geduldig, D., and Junge, D., J. Physiol. **199**, 347 (1968).

10. Huxley, A. F., Ann. N. Y. Acad. Sci. **81**, 221 (1959).

11. Setliff, J. A., Rayner, M. D., and Hong, S. K., Physiologist **12**, 353 (1969).

12. Alving, B. O., J. Gen. Physiol. **51**, 29 (1968).

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