The Effect of Aconitine on the Giant Axon of the Squid

W. H. HERZOG, R. M. FEIBEL, and S. H. BRYANT

From the Department of Pharmacology, University of Cincinnati College of Medicine, Cincinnati, Ohio, and the Marine Biological Laboratory, Woods Hole, Massachusetts

ABSTRACT In the giant axon of *Loligo pealii*, "aconitine potent" Merck added to the bath (10^{-7} to 1.25×10^{-6} gm/ml) (*a*) had no effect on resting membrane potential, membrane resistance and rectification, membrane response to subthreshold currents, critical depolarization, or action potential, but (*b*) on repetitive stimulation produced oscillations of membrane potential after the spike, depolarization, and decrease of membrane resistance. The effect sums with successive action potentials; it increases with concentration of aconitine, time of exposure, and frequency of stimulation. When the oscillations are large enough and the membrane potential is 51.6 ± SD 1.5 mv a burst of self-sustained activity begins; it usually lasts 20 to 70 sec. and at its end the membrane potential is 41.5 ± SD 1.9 mv. Repolarization occurs with a time constant of 2.5 to 11.1 min. Substitution of choline for external sodium after a burst hyperpolarizes the membrane to −70 mv, and return to normal external sodium depolarizes again beyond the resting membrane potential. The effect of aconitine on the membrane is attributed to an increase of sodium and potassium or chloride conductances following the action potential.

Aconitine is the active principle of an ancient poison of botanical origin. The chemical structure of this compound has been worked out in recent years (1). The material commercially supplied is a partially purified mixture of alkaloids from the plant *Aconitum napellus*. It has long been known to promote impulse production in the vertebrate heart and other excitable tissues. The nature of its action on the excitable membrane, however, has been little studied. Matsuda *et al.* (2) and Schmidt (3) have studied this problem in mammalian heart preparations, but no comparable studies in nervous tissues are at hand. In this paper the actions of commercial aconitine on the giant axon of the squid were studied.

MATERIALS AND METHODS

The last stellar nerve, containing the largest giant axon of *Loligo pealii*, with or without the stellate ganglion, was placed in a bath containing sea water. One of two electro-

physiological techniques was used: Technique 1, two 25 micron wires, scraped free of insulation for 1 mm on the ends and held together with sticky wax were inserted into the axoplasm. The cut ends of the wires were blunted with a small ball of sticky wax which made the total diameter of the tip 100 microns. One wire was connected to a stimulating circuit, and the other served as an internal lead for recording transients of membrane potential. A silver-chlorided bar in the external fluid served as a common indifferent electrode. Technique 2, a 60 micron glass tube filled with 0.53 M KCl was introduced into the axoplasm for measuring membrane potentials, and stimuli were delivered externally through platinum electrodes.

For measurement of accommodation, a circuit according to Solandt's (4) model delivered pulses with variable time constants of exponential rise, through a 2 megohm resistance, using technique 1. The minimum voltage required to produce the first action potential was determined for square waves (V_0) and for exponentially rising pulses (V). The ratios of V to V_0 were plotted against the corresponding time constants of rise (RC up to 24 msec.). The reciprocal of the slope of this straight line is Hill's time constant of accommodation. For measurement of the current-voltage relation, hyperpolarizing or depolarizing currents of 15 msec. duration were delivered through the membrane, using technique 1, and change in membrane potential from the resting level was measured just before the end of the pulse, at which time a stable level had been reached. The specific membrane resistance of the axon was calculated by derivations from the cable equations (5). Our values depend on the two assumptions that the current has a point-source and that the resistance of the membrane is the same at different distances from the source, neither of which is valid in our preparation. The calculated membrane resistances are therefore relative. Neither of the above measurements was made oftener than once per second. For measurements made while the axon was being stimulated with 50 μsec. shocks at a frequency of 10 per sec., pulses were introduced 20 to 40 msec. after the short shocks at intervals of several seconds.

The Merck's aconitine potent used in these experiments was converted to the hydrochloride and dissolved in sea water. The pH of all solutions by paper indicator was between 7 and 7.5. When a lower sodium concentration was required, an artificial sea water was used in which sodium chloride was replaced mole for mole by choline chloride. Solutions containing high potassium concentrations were prepared by adding potassium sulfate to either artificial or natural sea water. The experiments were carried out at room temperatures between 20 and 27°C. Results are expressed as mean ± standard deviation unless otherwise specified.

RESULTS

A. *Control Observations*

Resting potentials (technique 2) usually exceeded 53.5 mv. On stimulation with short shocks (technique 1 or 2), action potentials exceeded 100 mv in most of our axons. Oscillations of potential after the action potential (6) occurred only when the latter was less than 100 mv, in which case the resting potential was less than 50 mv. Long subthreshold currents produced a local

potential followed by a series of damped oscillations. With higher currents, action potentials arose from the local potential, and from the oscillations when they were great enough (7). With increasing current strength, the number of action potentials increased to a maximum of six and then decreased. Repetitive firing to prolonged constant currents was absent from axons with low resting potentials and disappeared in axons studied with technique 1 after about 3 hours of experiment. The average time constant of accommodation for 28 axons was 0.98 ± 0.14 msec. This value may be compared with Fig. 1 of LeFevre (8), in which the constant was about 4 msec. The straight line from which the time constant of accommodation is derived has an intercept on the time axis at 0.18 ± 0.26 msec. In LeFevre's figure the intercept is at 0.2 msec. The average membrane resistance for sixteen axons was 437 ± 82.6 ohms cm^2. Rectification was comparable to that of the uncorrected curves of Cole (9).

B. *Observations after Drug Application*

Under appropriate conditions after aconitine the squid axon can give self-sustained bursts of repetitive firing. This occurred after repetitive stimulation at concentrations between 10^{-7} and 1.25×10^{-6} gm/ml. When 10^{-8} gm/ml were used, no effect was seen. In five experiments with 3×10^{-6} to 10^{-4} gm/ml the threshold started to rise immediately and within 5 to 10 min. exceeded the voltage output of the stimulator. In three of these experiments in which technique 2 was used, with the rise of threshold there was a depolarization. This was not reversed even after washing the axon in normal sea water for 1 hour. A similar lack of reversibility was seen at lower concentrations of aconitine for the phenomena of oscillation and repetitive firing.

Even with concentrations between 10^{-7} and 10^{-6} gm/ml repetitive firing never occurred if the axons were stimulated at rates less than one per second for many minutes, nor were the parameters measured reliably altered. The resting potentials and the height of the action potentials showed no change. The average change in the time constant of accommodation of thirteen axons for drug concentrations of 3×10^{-7} and 10^{-6} gm/ml was -0.85 per cent (the minus sign indicating an increase of accommodation), with a range of 27.4 to -18.0 per cent. In three of the above axons in which the accommodation was measured 5 min. after the application of aconitine without making other measurements, the changes were less than 10 per cent. Aconitine itself therefore probably has no effect on the accommodation constant under these conditions. Among thirteen axons in which membrane resistance was measured, ten showed no change and three increased by 17, 19, and 45 per cent respectively.

When axons treated with aconitine in concentrations between 10^{-7} and 10^{-6} gm/ml were driven at higher frequencies, a new set of phenomena

appeared. After a number of responses there developed just after each action potential oscillations which, when they reached a critical magnitude, resulted in a self-sustained burst of action potentials. At the end of the burst, the axon was depolarized and underwent a slow repolarization.

1. VARIABLES ASSOCIATED WITH DEVELOPMENT OF OSCILLATIONS AND INITIATION OF A REPETITIVE BURST

Beginning 5 to 10 min. after aconitine was applied, the axons were tested at 10 to 15 min. intervals by stimulating at rates of 5 to 125 per sec. Several rates were tested for 1 min. each, in ascending order. The onset of bursts of self-sustained firing was dependent on the concentration of aconitine, the rate of stimulation, and the time after application of aconitine to the axon. With lower concentrations, higher frequencies of stimulation and longer periods of exposure were required. As the period of exposure increased, the rate and duration of stimulation necessary to produce the bursts decreased. For example, an axon in 1.25×10^{-6} gm aconitine per ml fired repetitively during the first 20 per sec. stimulation, whereas axons in 10^{-7} aconitine fired repetitively only after 20 to 112 min. of test sequences at rates up to 125 per sec.

The following phenomena are associated with the development of a burst. After a number of responses, oscillations begin to appear after each action potential (in axons in which action potentials exceed 100 mv). With continued stimulation, these oscillations grow until there suddenly occurs a train of repetitive impulses arising from the crests of these waves (Fig. 1). At this time the membrane potential just before stimulation is 51.6 ± SD 1.5 mv, representing a depolarization of about 3.5 mv (Table I, columns 1, 2, and 4).

Even in axons treated with suitable concentrations of aconitine and tested at suitable intervals, the oscillations and repetitive firing could not be elicited by stimulating with subthreshold shocks at 500 per sec. In this case the local potentials were normal.

In axons which had low resting or action potentials, or both, the oscillations which were seen before aconitine increased in height after aconitine, but did not give rise to trains of repetitive impulses unless the oscillations before the drug were less than approximately 3 mv in amplitude.

2. THE BURST AND ITS SEQUELAE

As the burst begins the axon fires 300 to 500 times per sec. The shape and size of the successive action potentials do not change appreciably at first, but eventually the height (Fig. 2) and the rate of rise and fall of the action potentials and the rate of firing decrease. The bursts usually lasted between 20 and 70 sec., but in two cases the firing continued for 8 min.

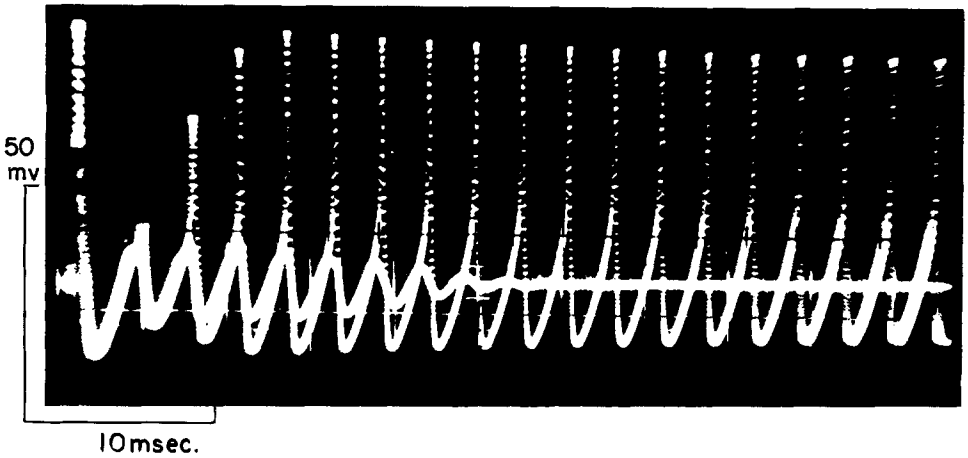

FIGURE 1. Initiation of a repetitive burst in an aconitine-treated axon. Responses to two successive stimuli are superimposed, the first showing only the damped oscillations, and the second the beginning of the self-sustained burst. Recording through internal nichrome wire electrode (slow changes of potential not shown).

TABLE I

EFFECT OF STIMULATION ON RESTING POTENTIAL OF ACONITINE-TREATED AXONS

Axon No.	(1) Before stimulation	(2) Immediately before repetitive burst	(3) End of repetitive burst	(4) Depolarization before burst (1)-(2)	(5) Depolarization during burst (2)-(3)	(6) Total depolarization (1)-(3)
	mv	*mv*	*mv*	*mv*	*mv*	*mv*
75	53.5	51.5	43	2	8.5	10.5
71	54	50	39	4	11	15
	57	50	38	7	12	19
	54	50	42	4	8	12
74	58	53	43	5	10	15
	55.5	54	40	1.5	14	15.5
70	53.5	52	43	1.5	9	10.5
	53.5	52	42	1.5	10	11.5
	55	51.5	43.5	3.5	8	11.5
77	58.5	53.5	42	5.0	11.5	16.5
78	54	50	41.5	4.0	8.5	12.5
Average, ±SD	55.1±1.9	51.6±1.5	41.5±1.9	3.5±1.8	10±1.9	13.6±2.7

At the end of the burst, damped oscillations occur (Fig. 2) and the membrane potential is 41.5 ± SD 1.9 mv, representing 10 mv depolarization during the burst or 13.6 mv from the resting state (Table I). Differences between axons, the length of time the axons are bathed in aconitine, and the concentration of aconitine probably determine the number of impulses that will

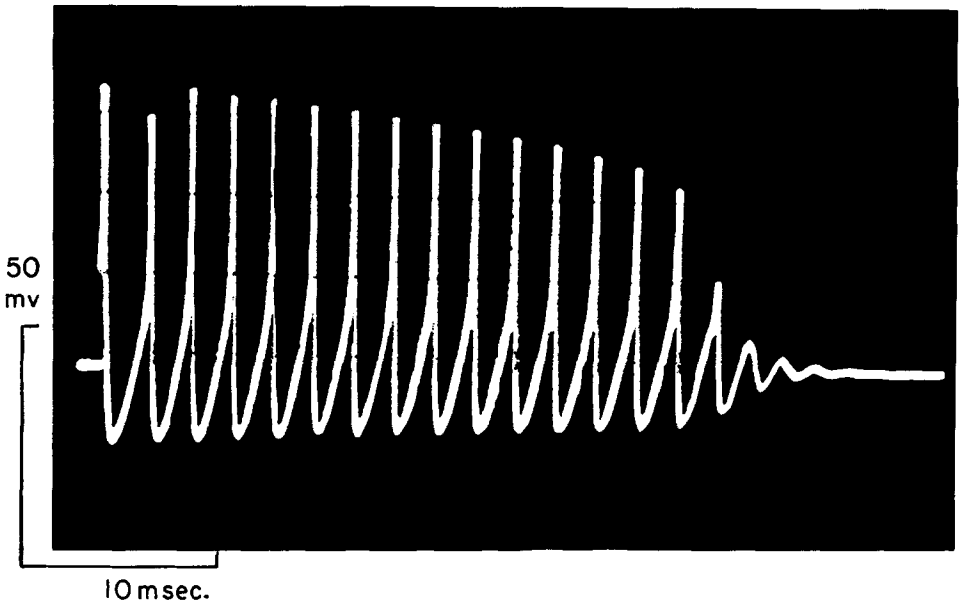

FIGURE 2. Repetitive burst to a single 50 μsec. shock soon after a previous burst in an aconitine-treated axon, recorded internally with nichrome wire electrodes.

TABLE II

TIME CONSTANT OF REPOLARIZATION AFTER A REPETITIVE BURST IN ACONITINE-TREATED AXONS (10^{-7} TO 1.25×10^{-6} GM/ML)

Normal sea water		Low sodium sea water		Normal Na / Low Na	Room temperature
Trial 1	Trial 2	Trial 1	Trial 2		
min.	*min.*	*min.*	*min.*		°C
4.2	3.8				27
2.5	3.4				25
3.0					22
5.3	6.8				21.0
10.3		3.7	1.6	2.8; 6.4	No record
8.0*		3.3		2.6	21.0
3.7*		2.1		1.7	20.5
6.4*		1.3		4.9	20.5
11.1*		2.0		5.5	20

* In these experiments the low sodium trial occurred before the control.
$0.012 > P > 0.005$ for difference between means for low and high sodium, by t-test.

produce the level of depolarization. Repolarization occurs slowly and is exponential, with time constants varying between 2.5 and 11.1 min. (Table II, Fig. 4). This variation may be related to differences in room temperature, as noted in Table II.

Within 15 sec. after the end of the first burst, a few stimuli, or even a single shock, sufficed to set off another burst (Fig. 2). The longer the wait after the burst, the more stimuli were required. Bursts elicited successively at intervals of about a minute after the end of the previous burst were successively shorter. In one axon the oscillations occurring at the end of a burst increased in height and a new burst of self-sustained repetitive firing followed.

3. EFFECT OF HYPERPOLARIZATION ON THE OSCILLATIONS AND BURSTS

In aconitine-treated axons stimulated at a rate which produced oscillations after each action potential, the axons were hyperpolarized by a 25 to 30 msec. current and 50 μsec., suprathreshold, depolarizing currents were applied 10 to 15 msec. after the beginning of the hyperpolarization. The height of the resulting oscillations was reduced proportionally to the strength of the hyperpolarizing current. Abolition of the oscillation occurred when the membrane was hyperpolarized to a level at which the positive potential disappears.

When the action potentials elicited by short shocks were followed by oscillations, the break of hyperpolarizing pulses was followed by an oscillation or action potential. This did not occur with comparable hyperpolarizations in normal axons, or in non-oscillating aconitine-treated axons. Hyperpolarizing pulses also interrupt the repetitive self-sustained burst of action potentials. At the break of the hyperpolarizing pulse the burst continues.

4. TIME CONSTANT OF ACCOMMODATION AND MEMBRANE RESISTANCE DURING AND AFTER THE BURST

Time constants of accommodation were determined during stimulation of axons treated with 10^{-6} gm/ml of aconitine at frequencies of 1 to 10 per sec., sufficient to induce oscillation of the membrane potential after each action potential. Usually there was a slight decrease in the time constant, indicating slightly increased accommodation (Table IIIA). When time constants of accommodation were determined after the occurrence of self-sustained bursts of repetitive firing, the result was similar (Table IIIB).

Current-voltage curves were also constructed for axons undergoing stimulation which induced oscillation. In eleven aconitine-treated axons the resistance of the membrane decreased by an average factor of 2.56 (range 1.7 to 3.4) (Fig. 3A). The values though returning had not reached the prestimulation levels by 5 min. after termination of the stimulation. In six axons not exposed to aconitine similar experiments showed that 10 per sec. stimulation does not affect membrane resistance. When the current-voltage relation was tested in aconitine-treated axons within 1 min. of the end of a burst, membrane resistance was found to have decreased by an average factor

of about 21 (range 3.5 to 39) in five axons (Fig. 3B). 5 min. later the resistance had returned to more than 50 per cent of the prestimulation value.

5. SUBSTITUTION OF CHOLINE FOR SODIUM

When the sodium chloride is replaced by choline chloride in artificial sea water, the membrane potential of untreated axons increases by about 10 mv. In untreated axons depolarized by high external potassium (28 mEq/liter) to a membrane potential of 40 mv, *i.e.*, the same depolarization found after the burst in aconitine-treated axons, membrane potential increases by

TABLE III

TIME CONSTANTS OF ACCOMMODATION OF STIMULATED ACONITINE-TREATED AXONS

	A. Stimulated at *rates* which induce oscillations		
Axon No.	Before	During	After
	msec.	*msec.*	*msec.*
69	1.6	1.5	—
68	1.4	1.4, 1.3	1.6
	1.6	1.2	1.8
49	1.2	0.9	1.0
61	0.83	0.87	—

	B. Effects of a self-sustained repetitive burst		
Axon No.	Before	1 min. after	5 min. after
	msec.	*msec.*	*msec.*
14	1.4	0.89, 1.0	2.2
18	1.1	1.0	—
68	1.4	1.2	1.6

8 to 10 mv when the sodium is replaced by choline. This is in agreement with the small potential changes observed by Hodgkin and Katz (10) on changing the external sodium concentration bathing giant axons.

In aconitine-treated axons which had just finished self-sustained bursts, the membrane repolarized from its characteristic level of about 41.5 mv more rapidly when sodium was replaced by choline than did the same axon under similar circumstances with normal sodium, the time constant of repolarization being 1.7 to 6.4 times less than in normal sea water (Table II, Fig. 4). The choline sea water was substituted within 15 sec. following the burst. The membrane potential approached −70 mv, a value higher than the normal resting potential of aconitine-treated or untreated axons and closer to the theoretical potassium equilibrium potential. An experiment in which these procedures were carried out is diagrammed in Fig. 5.

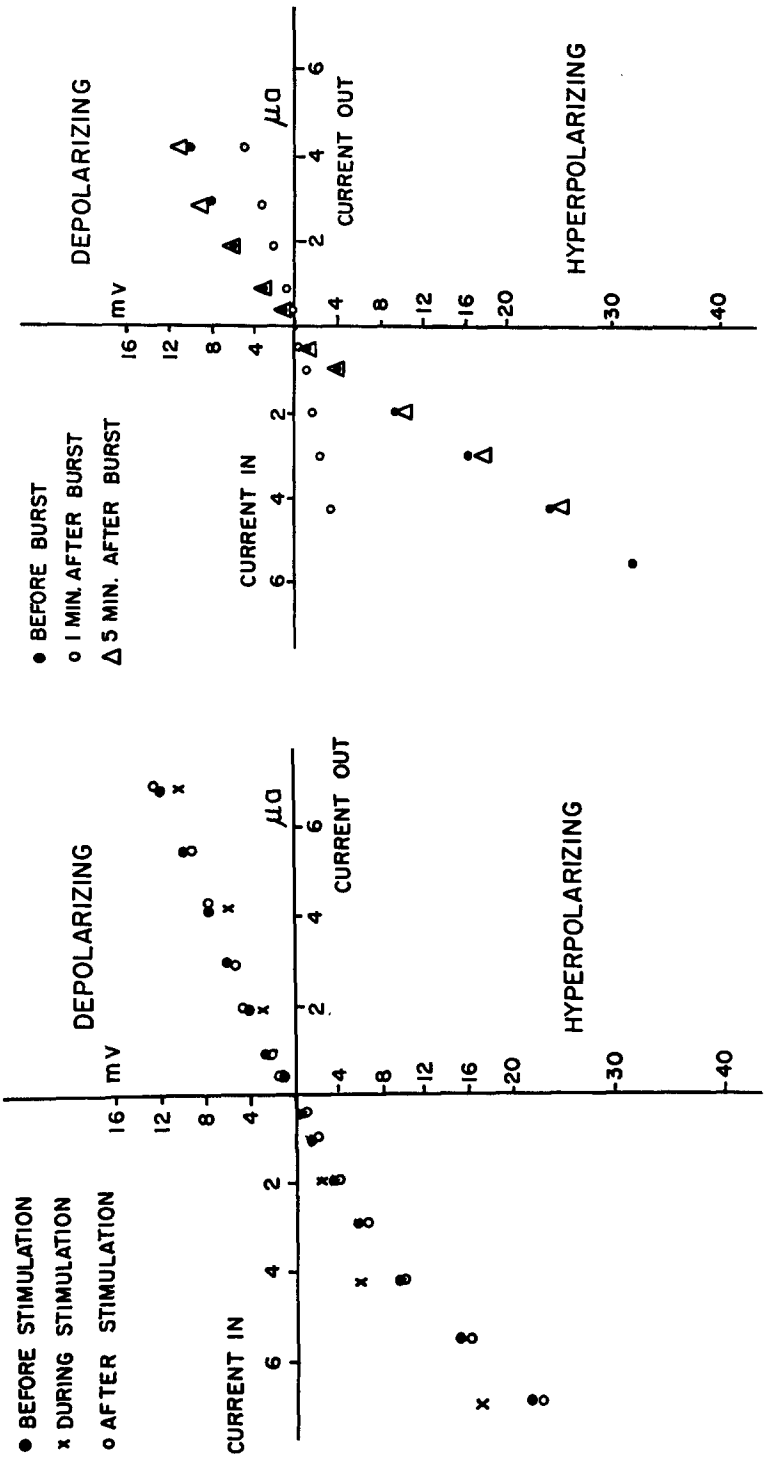

FIGURE 3. Current-voltage curves of two aconitine-treated axons. Axon A, before, during, and 5 min. after stimulation sufficient to induce oscillations. Axon B, before, and 1 and 5 min. after a self-sustained repetitive burst.

DISCUSSION

The results reported here confirm and extend for the giant axon of the squid the effects of aconitine found by Matsuda *et al.* (2) in ventricular fibers of the dog's heart and, with certain differences, by Schmidt (3), in canine Purkinje

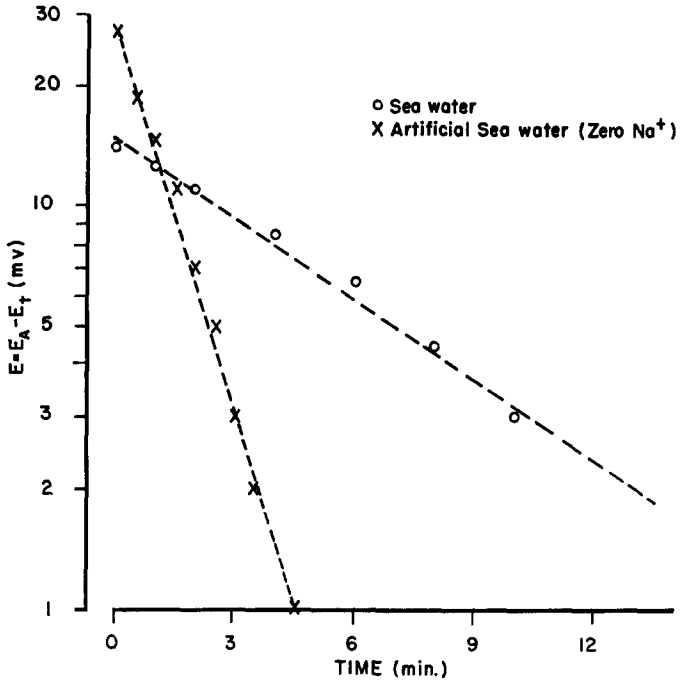

FIGURE 4. Repolarization of the aconitine-treated squid axon after a repetitive burst in normal and low sodium sea water. E_A is the final potential which the membrane reached after recovery from the repetitive burst. E_t is the potential at time (t) after the end of a repetitive burst.

fibers. For the characteristic irreversible effects of aconitine to develop, the axon requires a concentration about ten times that required by the heart (2). These concentrations do not affect the resting preparation. This is true not only of the resting membrane potential, the membrane response to subthreshold currents, critical depolarization, and the rising phase of the action potential, as described by Matsuda *et al.* (2) in the ventricular cells, but also of resting membrane resistance and rectification, and the falling phase of the action potential in the squid axon. In both preparations the characteristic effects of aconitine are seen following the action potential and wax with repeated activation. Since, in the aconitine-treated axon, subthreshold stimulation at a rapid rate elicits normal local potentials, it is clear that

these characteristic effects result, not from the stimulus or from the local potential which it initiates, but from the action potential.

The effects of aconitine are (*a*) augmentation of oscillations of the membrane potential, (*b*) depolarization, and (*c*) decrease of membrane resistance. The data presented suggest that (*a*) each action potential elicits a change

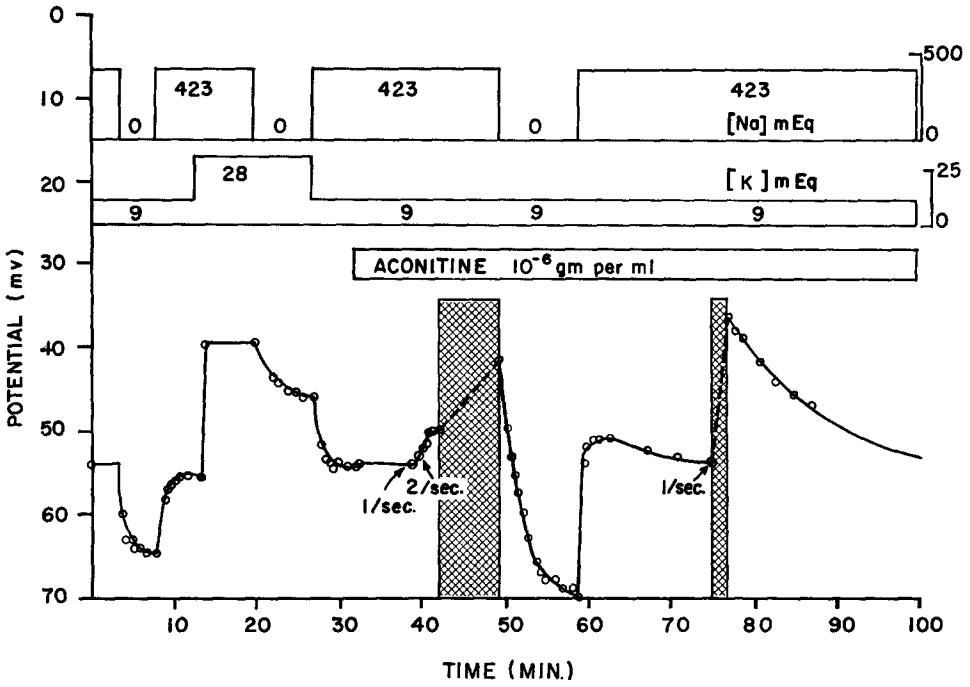

FIGURE 5. Effects of changes in external sodium and potassium concentrations on membrane potential of an axon. From left to right, replacement of $[Na^+]_0$ by choline at normal $[K^+]_0$. Elevation of $[K^+]_0$ at normal $[Na^+]_0$. Replacement of $[Na^+]_0$ by choline at high $[K^+]_0$. At 27 min. return to normal $[Na^+]_0$ and $[K^+]_0$. Addition of aconitine; stimulation; hatched area is repetitive burst (after discharge). At end of burst, replacement of $[Na^+]_0$ by choline. At 59 min. return to normal $[Na^+]_0$. Stimulation; hatched area is repetitive burst (after discharge).

(*e.g.*, of membrane permeability) which disappears slowly and therefore can sum with succeeding impulses, and (*b*) the amount of the change elicited per impulse increases with accumulation of aconitine in the axon, up to some limiting quantity of aconitine (achieved rapidly at a concentration of 10^{-4} in the bath) at which block occurs. With a suitable rate of stimulation, the oscillations of aconitine-treated squid axons occur with a period of 2 to 3 msec. In the ventricular cell the period is about one hundred times longer (2). In both preparations, self-sustained activity begins when, as a result of repeated activation, the oscillations become large enough to elicit an action

potential, which then produces an oscillation large enough to elicit the next action potential. The characteristics of the last few action potentials at the end of a burst (Fig. 2) suggest that the cutoff of a burst occurs when membrane potential is low enough to be unfavorable for action potentials; *i.e.*, when sodium inactivation is too great. In Purkinje fibers, Schmidt (3) did not find oscillations after the action potential. Instead, there was a negative afterpotential from which a new spike arose, at first in the manner of a coupled beat and then in salvos.

In both the ventricular cells and the axon, the depolarization which accumulates during repetitive stimulation is as striking a feature of the aconitine effect as is the shorter lived oscillation. In Purkinje fibers it is the principal change (the negative afterpotential). Several features of this depolarization in the axon suggest that it plays an important role: its uniformity just before the burst, and again just after the burst, its slow disappearance after the burst, and the increasing number of stimuli required to produce a burst as the depolarization disappears. It is possible that the fundamental action of aconitine is to produce a negative afterpotential, as noted by Graham and Gasser (11). Even in the untreated axon, depolarization promotes oscillation whereas hyperpolarization counteracts oscillation. Matsuda *et al.* (2) argue, however, that in the heart aconitine exaggerates the oscillatory behavior characteristic of a given degree of depolarization. Our data do not exclude this interpretation in the squid axon.

Shanes (6) notes that repetitive firing is usually associated with decreased accommodation. Whatever the basis of the oscillations and depolarization in the aconitine-treated squid axon, the effect is correlated with a slight increase of accommodation (Table III). The same effect (decrease of time constant of accommodation) occurs in aconitine-treated toad nerve (12).

Membrane resistance, on the other hand, shows variations parallel to the oscillations and depolarization of the aconitine-treated squid axon. Whereas resistance is normal without stimulation, it is lower during stimulation which causes the oscillations and depolarization, and even lower after the burst; furthermore, after a burst it slowly returns toward normal, as does the depolarization. The decrease of membrane resistance suggests that ionic conductances are increased after the action potential in the aconitine-treated squid axon. Two findings suggest a contribution of increased sodium permeability to the increased membrane conductance and the depolarization: (*a*) reducing external sodium after a burst restores membrane potential toward the resting level and (*b*) subsequent replacement of sodium depolarizes beyond the resting potential (Table II and Fig. 5, *cf.* the 50 to 59 min. record with that of 77 to 100 min.). Repolarization is too slow to be accounted for by charging the membrane capacity and cannot be related to the Hodgkin-Huxley parameters, which have time constants of a few milliseconds. The rate

of repolarization doubtless depends upon movements of ions across the membrane. These in turn depend upon electrochemical gradients, membrane potentials, ion concentrations, permeabilities, etc. Some of these variables are dependent upon one another. Accelerated repolarization in low sodium could result from increased chloride and potassium permeabilities. If during a burst sodium has accumulated inside the cells, its outflow in the presence of low external sodium would accelerate repolarization and account for temporary hyperpolarization.

We have calculated the consequences of attributing all of the increased conductance to an increase in sodium permeability, using the Goldman equation as elaborated by Hodgkin and Katz (10), the quantities P_{Na}, P_K, and P_{Cl} having their usual significance and being proportional to our membrane conductances g_{Na}, g_K, and g_{Cl}. If the 21-fold increase in membrane conductance after a typical aconitine burst were due solely to an increase of sodium conductance, the ratio P_{Na}/P_K would be about 20 as compared to 0.04 in the untreated axon. This 500-fold increase is the same as that calculated for maximum sodium activation during the spike and would lead to a reversed membrane potential of +30 to +40 mv. Since the membrane potential is about −41.5 mv after a burst (Table I), we must postulate increased conductance to other ions in addition to Na.

Further assumptions permit us to assess the increases in g_K and g_{Cl} by substitution into the Goldman equation. At least in the absence of aconitine, P_K increases with depolarization (13). We do not know what happens to P_{Cl}, but the normal gradients make changes in P_K and P_{Cl} less important to membrane potential than change in P_{Na}. We assumed that P_{Cl}/P_K remains 0.45 and supposed the internal concentrations to be like those described by Hodgkin and Katz (10). The postburst membrane potential of −41.5 mv then requires an increase of P_{Na}/P_K from 0.04 to 0.14. This represents a 69-fold increase in g_{Na} and a 20-fold increase in g_K and in g_{Cl}. Despite the larger change in g_{Na}, the increases in g_K and g_{Cl} would account for 91 per cent of the observed increase of membrane conductance. At this value of P_{Na}/P_K (0.14), reducing external sodium would bring the membrane potential to −65 mv. This potential is slightly greater than that calculated for a similar reduction in the untreated axon (to −63 mv) and close to that which we observed (−70 mv) (Fig. 5, 59 min.).

Aconitine-treated Purkinje fibers are similar to squid axon in that low external sodium decreases the augmented negative afterpotential; and in this case a change of chloride conductance can be ruled out because low external chloride has little effect (3). The augmented afterpotential of veratrinized tissues is also accompanied by decreased membrane resistance, and here, too low external sodium decreases the afterpotential (14).

In the veratrine-treated squid axon, the negative afterpotential after a

single spike may be small, yet sufficient repetitive stimulation leads to increasing oscillation and depolarization, which culminate in a burst (6). The similarity between these actions of aconitine and veratrine is clear. In the squid axon, the time constants of decay of the accumulated negative afterpotential are 49 msec. for cevadine, 397 msec. for veratridine (15), and 2.5 to 11.1 min. for aconitine. The slower decay of depolarization in the aconitine-treated nerve would promote the summation of negative afterpotentials.

In line with the views of Matsuda *et al.* (2) and Schmidt (3) we propose that (*a*) a certain prolonged increase of sodium conductance follows each action potential in the aconitine-treated axon, (*b*) that this effect disappears with the time constant noted after the burst and accounts for the slowly disappearing accumulated negative afterpotential, (*c*) that the summation of the oscillations and the depolarization reflects the summation of this increased conductance to sodium, and (*d*) that increase in conductance may also involve chloride and potassium, partly from the changes in membrane potential (accumulated negative afterpotential).

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