Permeability Changes Associated with the Action Potential in Procaine-Treated Crayfish Abdominal Muscle Fibers

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ABSTRACT Permeability changes associated with prolonged action potentials have been analyzed in procaine-treated crayfish abdominal muscle fibers. The effect of external Ca indicates that the increase in membrane conductance observed during the rising phase of the action potential is primarily due to a permeability increase for Ca. A remnant of the permeability increase may cause the succeeding plateau as shown by its high conductance and by the effect of low Mn. A delayed increase in conductance precedes the termination of the plateau phase. This is due to a delayed increase in permeability, probably for K, that is observed when depolarizing electrogenesis is eliminated. High external Ca reduces the action potential duration, the falling phase starting at a higher depolarization. These changes may be related to an earlier onset of the delayed increase in permeability, induced by a larger inside positivity in the presence of higher Ca. No "anomalous rectification" is seen in early or late *I–V* curves for small depolarizations. Ba may replace Ca in its role in depolarizing electrogenesis, and the first action potential induced in Ba saline has a large overshoot and a long duration. In higher Ba salines, action potentials are greatly prolonged. Long term soaking in Rb-containing or K-free saline also augments and prolongs the action potential. These changes are assumed to be related to depression of the K permeability of the membrane.

INTRODUCTION

The electrically excitable membrane of arthropod muscle fibers differs in its electrophysiological and pharmacological properties from that which generates spikes in axons, vertebrate muscle fibers, and electroplaques (1, 12, 15, 16, 23, 24, 26, 48, 58, 59). Procaine and other anesthetics diminish Na and K conductances in squid axons (4, 34, 50, 56) or eliminate only the Na conduct-

ance component in eel electroplaques.[1] In arthropod muscle fibers, however, application of procaine converts the normal graded response into an all-or-none electrogenesis (8, 16, 17, 41, 46, 47).

In the present experiments the mechanisms underlying electrogenesis were examined in procaine-treated crayfish abdominal muscle fibers by applying inward currents during electrically evoked responses, or by varying the ionic content of the external medium. The results show that the electrogenesis is associated with two successive increases in membrane permeability, as in ordinary action potentials. The early permeability increase, which is mainly for Ca in normal saline, results in depolarizing electrogenesis as suggested or shown in other crustacean muscle fibers under different conditions (1, 15, 16, 24, 58). For reasons which will be described below this is referred to as increase in Ca permeability. The delayed permeability increase which terminates the prolonged depolarizing electrogenesis in normal saline is probably for K. This is tentatively referred to as increase in K permeability. The effect of external Ba is different from that of Ca in some respects. The results are discussed in connection with other prolonged action potentials. A preliminary note on some of the experiments has been published (51).

METHODS

All preparations were of crayfish belonging to unidentified species of the genus *Orconectes* collected in Wisconsin. Twitch fibers of the main abdominal flexor muscle, which had sarcomere lengths of about 2 μ (1, 31), were used for the study, preferably the caudal fiber group of anterior oblique muscle 4 (49), which is innervated from the third root of the third abdominal ganglion. The muscle was isolated by a ventral approach, leaving part of the caudal exoskeleton and part of the rostral fibers attached, and was placed dorsal side up on a paraffin block in a Lucite chamber. It was fixed with the aid of a pair of forceps and insect pins. The muscle fibers were slightly stretched on a layer of vaseline (Fig. 1). Although the muscle under investigation consisted of several layers of parallel cylindrical fibers, only the superficial fibers were used for microelectrode penetrations. The muscle fibers used were of fairly uniform length in any one preparation, ranging between 5 and 8 mm. The chamber contained about 15 ml of solution.

The preparation was usually kept in standard solution containing 10^{-8} g/ml procaine hydrochloride for several hours before starting electrical recordings. This procedure made the muscle fibers capable of generating all-or-none propagating action potentials in response to direct stimuli, and electrical recording was undisturbed, since contractions of the muscle fibers were diminished. In fact, most results included in this paper were obtained from fibers in which action potentials were not accompanied by contraction. In most cases, however, floating microelectrodes were used; i.e., a fine (110 μ diameter) flexible silver-silver chloride wire was inserted into a glass microelectrode and the other end of the wire was supported from a micromanipulator (31, 32).

[1] Nakamura Y., and Grundfest, H., personal communication.

Stimulating and recording equipment used was standard for the laboratory. To record *conducted* action potentials, which were the subject of the analysis, three microelectrodes were inserted into a fiber: one relatively close to one end for direct stimulation, and the others very close together, about 2.5 mm from the first and toward the middle of the fiber, for differential recording of the membrane potential and to polarize the membrane at the recording site, respectively. The recording microcapillary contained 3 M KCl and its resistance was about 10 M Ω. The stimulating and polarizing electrodes contained 2 M potassium citrate. The stimulating current was usually brief and was always terminated before the peak of the action potential.

To estimate membrane current during the action potential, a fire-polished glass capillary having an inside diameter of several microns and containing standard bathing solution was pressed to the outside of the fiber very close to the intracellular

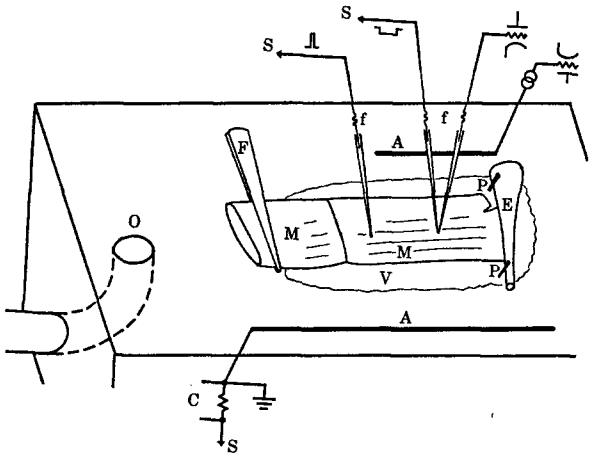

FIGURE 1. A schematic representation of the experimental arrangement. *A*, silver-silver chloride electrode; *C*, current recording; *E*, ventral exoskeleton; *F*, forceps; *f*, flexible silver-silver chloride electrode; *M*, muscle; *O*, outlet; *P*, insect pin; *S*, to isolator of stimulator; *V*, vaseline. Further explanation in text.

recording electrode. The potential change recorded differentially between this external electrode and an indifferent electrode immersed in the bath then reflects the current flowing across the small area of the membrane enclosed by the abutting external electrode. Positivity at this electrode indicates that the membrane current is outward and negativity indicates inward membrane current. To obtain action potentials under "semispace-clamped" conditions, a silver-silver chloride stimulating electrode (50 μ in diameter) was placed outside the fiber along almost its entire length. A brief current was passed between this and a distant parallel electrode. Action potentials evoked by this method of stimulation and recorded in the fiber at two distant sites arose nearly simultaneously, thus approximating the space-clamped condition.

Changes in membrane conductance during an action potential were analyzed by measuring the membrane resistance. A train of pulses of constant inward current and

of brief and constant duration was applied to the membrane through the intracellular polarizing electrode. The equation for a cable model (30) was used to calculate the membrane resistance during a conducted action potential, i.e., $V/V_0 = \sqrt{x} \cdot erf\sqrt{t/x\tau}/erf\sqrt{t/\tau}$, where V_0 denotes the change in membrane potential induced at the end of each current pulse, having a duration t, which was applied to the resting membrane; V, the same quantity when the membrane resistance changes from its resting value by a factor x during the action potential; and τ, the time constant of the membrane at rest. The following assumptions have to be satisfied for the above equation to be applicable to the experimental results. (*a*) The repetitive application of current pulses does not modify electrical properties of the membrane at rest or in the active state. (*b*) Current-induced potential changes are related only to a variation in the passive electrical properties, and are free from electrogenic effects due to changes in permeability of the membrane. (*c*) During the action potential the membrane resistance changes, but membrane capacity and internal resistance remain constant (13, 40), and the change in membrane resistance during single current pulses is negligible. (*d*) The reduction of the membrane resistance during the action potential is spatially uniform within the area of the membrane where the reduction affects the recorded potential change. (*e*) The distance between the current and potential electrodes is negligible. Since the rising phase of the action potential did not satisfy some of these assumptions, it was omitted and the analysis was made for the rest of the action potential. Assumptions (*a*) and (*b*) were fully satisfied in the present experimental conditions. Repeated application of the brief pulses did not alter the form or amplitude of the induced potential changes in the resting membrane. If, however, much larger and longer inward pulses were applied, the induced potential change was gradually reduced during repetition until it attained a new steady level. Since the recorded current was constant throughout this change, the effect must have involved hyperpolarizing activation of the membrane conductance (see below). The electrogenic effect of the delayed increase in K permeability, that tended to repolarize the membrane and induced a larger potential change towards the end of the action potential if long lasting pulses were used (see below), was apparently absent when using brief pulses (Fig. 4 *B*). This is also supported by the time-dependent nature of the delayed conductance increase (Figs. 12 and 13). Assumption (*c*) was postulated to be approximately satisfied. Although assumption (*d*) was not satisfied in the strict sense, it may be approximated for these conducted action potentials due to the short length constant of the fibers (less than 1 mm) and to the slow rate of the late conductance change compared with the conduction velocity of the action potential (10 cm/sec). Assumption (*e*) was approximately satisfied under the present conditions. The value for the membrane conductance, expressed as $\frac{1}{x}$, was thus obtained by calculating x for each current pulse, and interpolating from the experimentally measured values of V_0, V, t, and τ. Although the conductance values obtained are approximations due to the smaller degree of change in $\frac{V}{V_0}$ relative to that of x, they are useful for the present rather qualitative analysis.

The standard bathing solution consisted of (in mM) NaCl, 208; KCl, 5.25; $CaCl_2$,

14; $MgCl_2$, 2.8; $NaHCO_3$, 2. Test solutions of the various ionic compositions used were isosmotic. The bathing solution was changed by draining it off through an outlet and then carefully introducing new saline. The old solution was washed out by replacing the new saline several times. During long soaking in Rb-containing or K-free salines, preparations were kept in a refrigerator. All the various solutions used for the experiments always contained 10^{-3} g/ml procaine hydrochloride. Experiments were done at room temperatures of 20–23°C.

RESULTS

Action Potential

Normally graded responses induced by direct stimulation were converted into all-or-none action potentials when the muscle fibers were bathed in the procaine-containing standard solution for about one-half hour. The configuration of the action potential during the early period was irregular and complicated with secondary peaks. With time the response became stable and a smooth conducted action potential was produced (16). The conduction velocity was usually uniform along the length of the fiber and ranged between 6 and 22 cm/sec (in 10 fibers). After conduction had been blocked by repetitive stimulation, large local depolarizations were observed, the amplitude of which at the stimulating site was comparable to that of the propagating action potential. The amplitudes of the local depolarization and current-induced hyperpolarizations measured at 0.5 mm from the current electrode were attenuated to one-sixth and to one-fifth, respectively, at 1.5 mm from the current source, and to one-thirtieth and to one-twentieth at 2.5 mm. At about 2.5 mm from the stimulating site (see Methods), therefore, the recorded potential change was almost undisturbed by such local events. Nevertheless, complications in the recorded action potential were sometimes encountered. These might be at least partly due to interaction between adjacent fibers (43). To avoid such complications only fibers which showed single smooth action potentials were selected for the present study.

The conducted action potential of the procaine-treated fibers consisted of a fast rising phase (10–20 v/sec, in 10 fibers), a plateau phase of slow repolarization (lasting up to several hundred milliseconds), and then a steep falling phase (0.6–6 v/sec, except for very old preparations). The action potential usually terminated with an after-depolarization. An overshoot of 10 mv was seen unless the preparation became old.

The time course of the action potential during successive responses was generally the same in a given muscle fiber if the stimulus frequency was fixed. If stimuli were delivered every few minutes, action potentials could be elicited for a long time. Fibers stimulated every 5 sec lost responsiveness after some 10 to 30 stimuli, but regained responsiveness after a rest. In Fig. 2 *A*, the action potential with the longest duration represents the steady response to stimuli

given at 5 min intervals. When the frequency was raised to once every 5 sec, the action potential duration gradually shortened until it attained a new steady value. With the decrease in duration, the rate of repolarization of the plateau phase increased. However, there was no appreciable change (at the sweep speed used) in the rate of repolarization of the steep part of the falling phase. Thus stimulation frequency differentiates between the behavior of these two phases, suggesting that the mechanisms involved in the two are different. Although the transition to the falling phase was gradual initially, as reflected in the time course of the membrane conductance (Fig. 4 *A*), the steep falling phase usually started at an approximately constant level of membrane potential (about 10 mv inside negative in the standard bathing solution; for other

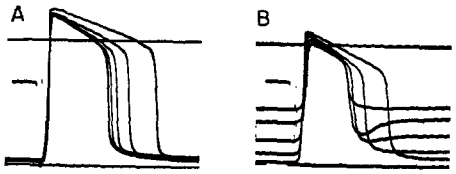

FIGURE 2. *A*, conducted action potentials of a procaine-treated fiber. The first response was after a rest of 5 min and six successive responses elicited every 5 sec are superimposed. The action potentials gradually shortened and stabilized so that the time courses of the fifth and sixth were identical. *B*, five similar responses in another fiber. Contractions caused depolarization, disclosing an undershoot. Upper lines register external reference potential. Calibration at left, 50 msec; 50 mv (in *B*, with reference to the lowest trace).

salines see below), in spite of variations in the action potential duration (Fig. 2 *A*). This constancy of the transition level of the membrane potential suggests that the activation of the repolarizing mechanism is voltage-dependent.

Sometimes, as shown in Fig. 2 *B*, a reduction in (resting) membrane potential revealed an undershoot at the end of the falling phase of the action potential. In this experiment the membrane potential was reduced after every response as a result of muscle contraction. The undershoot indicates that the permeability of the membrane increases during the falling phase and that its equilibrium potential is located at a more inside-negative level than the membrane potentials at which the undershoot appears.

Membrane Current during the Action Potential

Fig. 3 shows the extracellular records used to estimate membrane current during action potentials (see Methods). The membrane current for the conducted action potential (Fig. 3 *A*) and that obtained under a semispace-clamped condition (Fig. 3 *B*) were qualitatively similar. Generally the records showed an initial outward current at the very beginning of the rising phase of the action potential, which suddenly shifted to a large inward surge that lasted

for the rest of the rising phase. After the inward surge was over there was a phase of outward current which was maintained during the plateau phase of the action potential. The falling phase of the action potential was preceded by an associated change in membrane current.

The early inward surge of current may imply a momentary increase in membrane permeability for Ca (see below). The inward surge was briefer and larger than the change in the current associated with the falling phase of the action potential. The increasing phase of the early inward surge coincided with the steep rising phase of the action potential and its decreasing phase coincided with the following less steep rising phase. The end of the inward surge approximately coincided with the peak of the action potential.

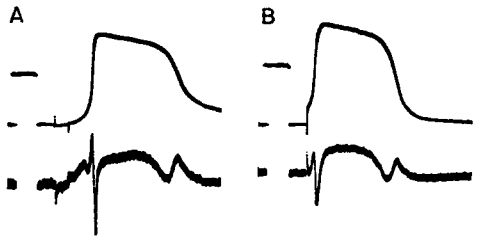

FIGURE 3. Extracellular records for estimation of membrane current (lower traces, positivity at active electrode upward) registered simultaneously with intracellular action potential (upper traces). *A*, for conducted action potential; *B*, for semispace-clamped condition in another fiber. Calibration, 50 mv (for upper trace); 20 msec in *A*, 50 msec in *B*.

Conductance Change during the Action Potential

Fig. 4 *A* demonstrates the early and delayed increase in membrane conductance during the action potential, which was measured with brief pulses (see Methods). The potential change V_0 used was 19 mv, the pulse duration t was 0.6 msec, and τ, the time required for the recorded potential change (an error function) to reach 0.84 of the final steady value on applying a constant current, was 8.2 msec. A pulse frequency of about 100/sec was used. The early increase in the membrane conductance, which was deduced from the results of membrane current measurement (dotted line), was associated with the rising phase of the action potential. The conductance gradually decreased during the early plateau phase but remained elevated above the resting value. A slower second increase in the conductance started late in the plateau phase, reached a maximum during the early falling phase of the action potential, and then decreased. Thus the delayed conductance increase was associated with the termination of the plateau phase and its initial effect was reflected in the increased rate of repolarization at the late plateau phase. Fig. 4 *B* shows a measurement of the delayed conductance increase. The membrane

conductance during the action potential was always higher than the resting value. Five experiments of this kind were performed and the results were generally similar. However, less decrease in the conductance was observed in some fibers at the plateau phase, and the delayed increase in conductance was less prominent in older preparations. These results suggest that the plateau

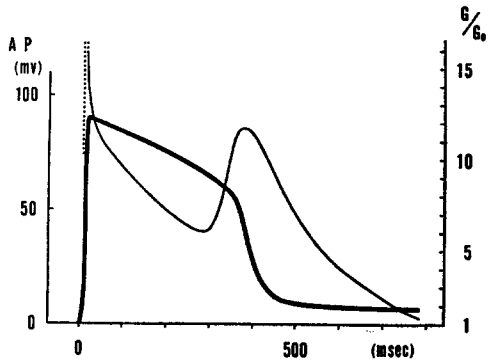

FIGURE 4 *A*. Membrane conductance change during the action potential. Ordinate, thin line, $\frac{G}{G_0}$; where G is the membrane conductance during action potential and G_0 denotes that at rest. Dotted line represents a portion deduced from the results of Fig. 3, but not measured directly. Thick line, action potential. Abscissa, time.

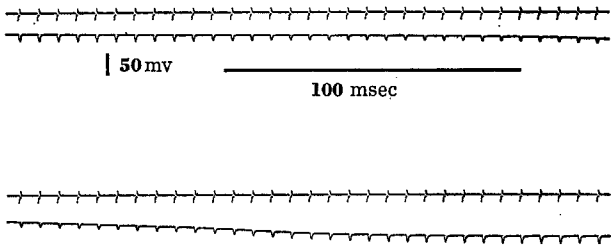

FIGURE 4 *B*. Reduction in current-induced potentials due to delayed conductance increase. Only the period from late in the plateau to the end of the action potential is shown from another fiber. The last brief pulse in the upper record is identical with the first in the lower record. Upper trace, current record (after a capacitative artifact the trace reached a steady level at the end of each pulse). Lower trace, potential record.

phase of the action potential is due to a remnant of the early increase in Ca permeability which initiated the action potential (see also below). The transition from the plateau to the falling phase may follow when the remaining increase in Ca permeability is overcome by the delayed increase in K permeability. Since the rate of repolarization of the falling phase was considerably slower than the passive decay due to the membrane time constant (see Fig. 4 *B*), some of the early increase in Ca permeability might possibly have

remained during the falling phase and caused the after-depolarization of the action potential.

Effect of Inward Current

As already described (see Methods) brief current pulses may not modify the time courses of Ca and K permeability changes during the plateau and falling phases of the action potential. Relatively large and long lasting constant inward currents, however, induced larger potential changes toward the end of the plateau phase. A similar effect also occurs in other prolonged action potentials (references 16 and 57). Since the late plateau phase is accompanied by an increase in membrane conductance in the present preparations, the

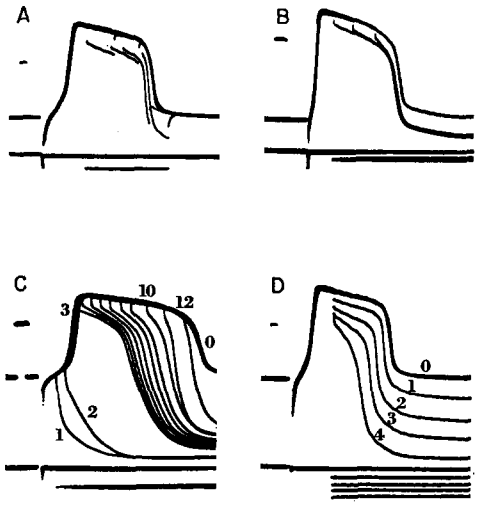

FIGURE 5. Effects of inward currents on action potential. Constancy of superimposed action potentials was assured by a fixed stimulus interval and also by the identity of first and last responses without current. Potential (upper) and current (lower) records with various gains and from various fibers. Calibrating pulses, 50 mv; 10 msec in *A*, *C*, *D*; 20 msec in *B*. Explanation in text.

augmentation in the induced potential changes must involve a repolarizing electrogenesis which resulted from modification of the relative magnitudes of Ca and K permeabilities that occurred during the long lasting potential changes. Nevertheless, when the inward current was terminated, the potential returned to its original level when the current was delivered during the plateau phase (Fig. 5 *A*). The complete restoration of the potential implies that the original relative magnitudes of Ca and K permeabilities are restored after the termination of current, even under the spatially nonuniform current distribution of the present experiments.

To eliminate transient effects due to break of the current, longer inward currents were used which lasted until the action potential was over (Fig. 5 *B–D*). When a weak inward current was started at the plateau phase of the action potential an initial rapid repolarization occurred, the time course of which was determined by the membrane capacity and conductance at the

moment. It was followed by a slower repolarization during the rest of the plateau phase (Fig. 5 *B*). The action potential was terminated a little earlier than normal (abolition: references 53, 54, 57). When the initiation time of the applied current was varied, the time course of the later part of the action potential was identical. The earlier termination of the action potential implies that the increase in K permeability dominated the Ca permeability earlier, as the result of the current-induced repolarization.

When strong inward current was started at the plateau phase (Fig. 5 *C*), the earlier the onset of the current, the earlier the termination of the action potential. The inflection point between the plateau and falling phase of these action potentials appeared to fall at approximately the same membrane potential (which, of course, was more inside negative than without the current), as in the case of repetitive stimulation (Fig. 2 *A*). The later the onset of the current the less the membrane was hyperpolarized after the action potential was terminated while the current still continued.

For inward currents initiated at a fixed time early in the plateau phase (Fig. 5 *D*), the stronger the applied current, the earlier the termination of the action potential, and the more accelerated the rate of repolarization of the plateau phase. However, the rate of repolarization of the falling phase was accelerated most in trace 2 and was less accelerated in traces 1 and 3. The rate in trace 4 was the same as that in the action potential without the current (trace 0). The stronger the current the more inside negative was the membrane potential at which the transition occurred from the plateau to the falling phase.

In Fig. 6 the changes in membrane potential in the records of Fig. 5 *D* are plotted against the intensities of the applied currents at various times during (lines 0–10) and after (line 20) the action potential. The linearity of the early *I–V* relation (line 0) implies that the current-induced potential changes are at that time without effect on the electrogenic processes. The linearity was almost regained after the action potential was over (line 20). The slope for line 0 was about one-third of that for line 20, indicating that the (specific) membrane conductance for the early plateau phase (line 0) was about nine times higher than that for the resting state (line 20). The nonlinear *I–V* relations for the intermediate lines represent electrogenic effects due to relative changes in Ca and K permeabilities. The earlier start of the nonlinearity for stronger currents indicates that the relative change in the permeabilities is voltage-dependent.

Effects of Ca

Figs. 7 and 8 show the effects of increases in external Ca concentration (substituted for Na) on the action potential. For the highest concentration of Ca used, NaCl was totally replaced with $CaCl_2$. The $NaHCO_3$ normally used was

omitted in the series, and the solution was thus Na-free. Nevertheless, action potentials were generated (Fig. 7 *D*). The overshoot values increased linearly with the logarithm of the Ca concentration, and the slope for the increase was about 23.5 mv for a tenfold increase in Ca (Fig. 8 *A*, line *0*). The action

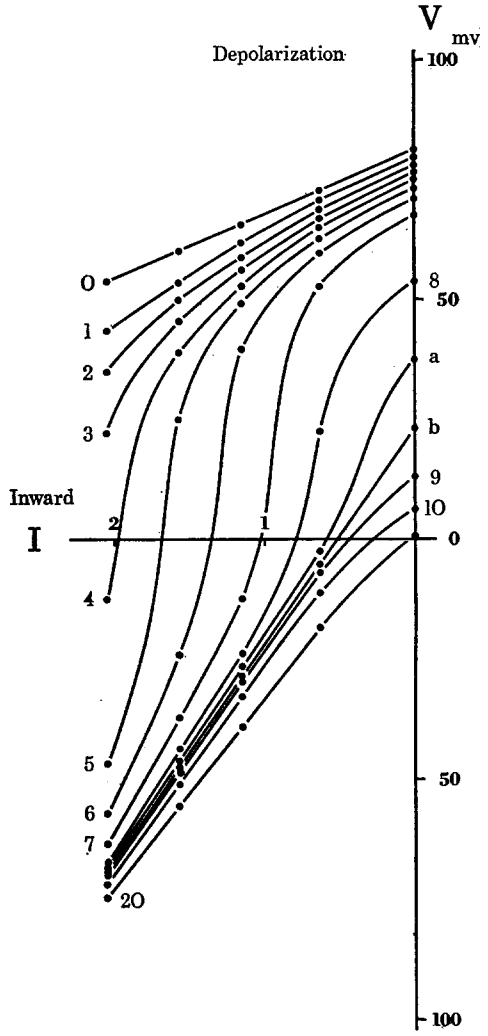

FIGURE 6. Change in membrane potential during action potential (resting potential as zero of ordinate) plotted against the intensity of applied inward current (abscissa, in arbitrary units). Data from Fig. 5 *D*. Lines follow time sequences. Line 0, values of step change on starting currents early at plateau; lines 1–10, at successive 12.5 msec intervals thereafter; line 20, 125 msec after line 10; lines *a* and *b*, between lines 8 and 9. In each line the potential at the right end represents the level of the action potential without applied current.

potentials became briefer on increasing the Ca concentration (Fig. 7). The membrane potential at which the falling phase started increased linearly toward more inside-positive values with the logarithm of the Ca concentration (Fig. 8 *A*, line *F*). This potential as plotted in Fig. 8 *A* was represented for convenience by the extrapolated intersection of the linear portions of the plateau and falling phases. Similar linear relations were obtained when other

landmarks of the potential were plotted; e.g., the value at the midpoint between the two phases. The durations of the action potentials measured at their half-amplitude decreased linearly with the logarithm of the Ca concentration (Fig. 8 *A*, line *D*). The rates of repolarization of the plateau (Fig. 8 *B*, line *P*) and of the falling phase (Fig. 8 *B*, line *F*) were not linearly related to the logarithm of the Ca concentration, but increased more rapidly with higher Ca.

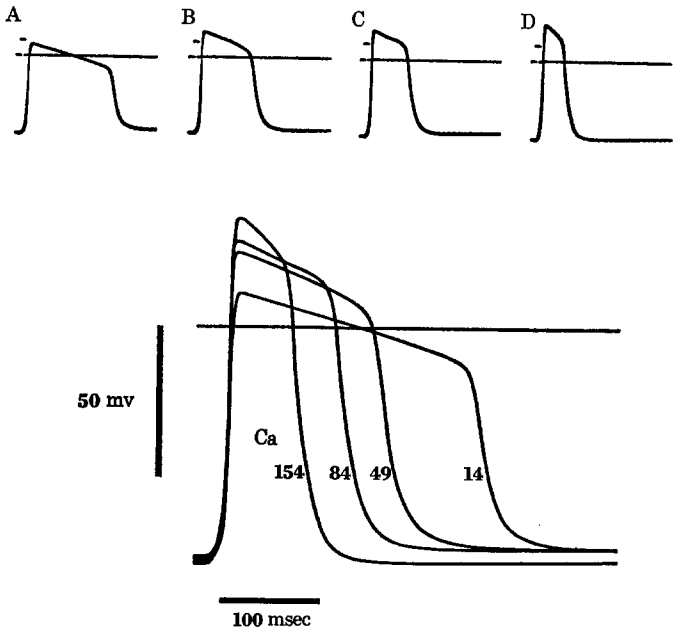

FIGURE 7. Action potentials of a fiber recorded in salines in which various levels of Ca were substituted stoichiometrically for Na. *A–D*, records for Ca concentration (in mM) of 14 (normal), 49, 84, and 154 (Na-free). Upper trace registers external potential and stimulating current. Lower figure, superimposed traces of the records so as to make the rising phase of the action potentials coincide. Ca concentrations are indicated.

In Fig. 9 total substitution of sodium salts in the standard solution with sucrose, keeping the Ca concentration constant, caused a small reduction in the resting potential (from 77 to 72 mv). The action potential produced in this Na-free saline showed a decrease in the overshoot (by 9 mv) and in the rates of potential change of the rising and falling phases (Fig. 9 *B*). These changes are probably at least partly related to the reduction in external Cl and to the consequent decrease in resting potential, which might result in some inactivation of the depolarizing electrogenesis. At any rate, the slight decrease in action potential amplitude resulted from drastic reduction of the external Na (from 210 to 0 mM). Thus, Na apparently is not the ion which

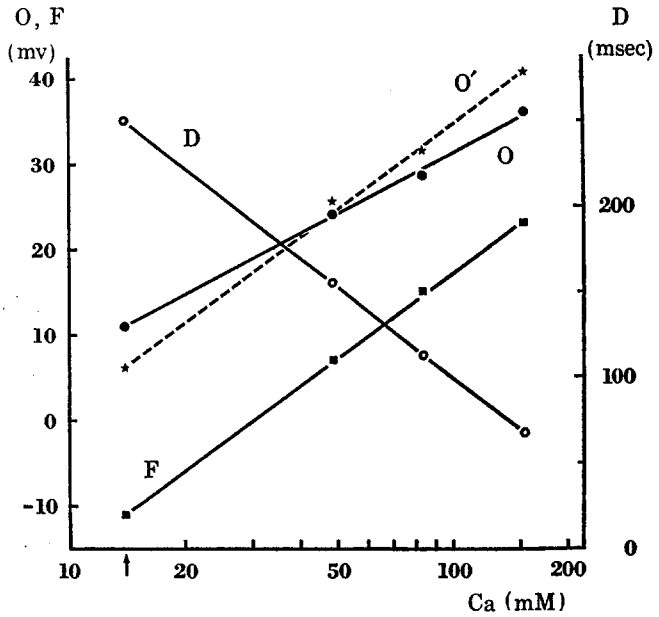

FIGURE 8 *A*. Action potential overshoot (line *O*, filled circles), action potential duration (line *D*, open circles), and the membrane potential at which falling phase started (line *F*, squares) plotted (in ordinate) against logarithm of the external Ca concentration (abscissa). Data from Fig. 7. Arrow on abscissa indicates normal Ca concentration. Line *O'* (broken line, stars) shows the overshoot in Na-free series (substituted with sucrose) in another fiber.

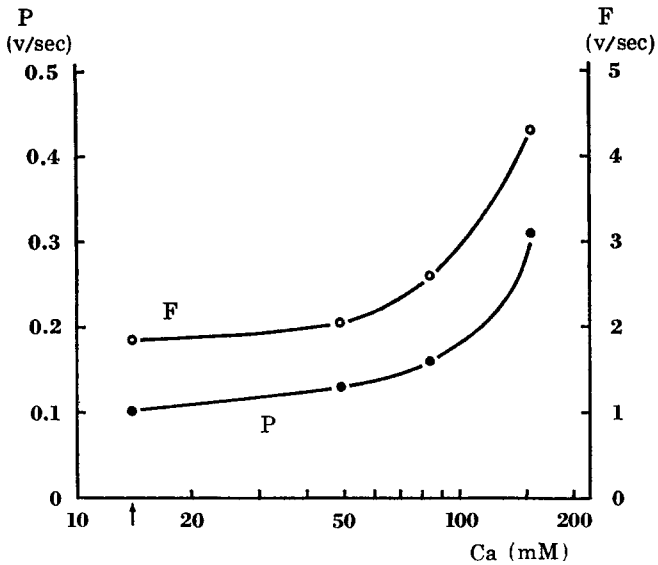

FIGURE 8 *B*. Rate of repolarization of the plateau (line *P*, filled circles) and of the falling phase (line *F*, open circles) of action potentials plotted (ordinate) against logarithm of external Ca concentration (abscissa). Data from Fig. 7.

is responsible for the depolarizing electrogenesis. The results obtained on increasing the $CaCl_2$ concentration (substituting it for sucrose) in Na-free saline were similar to those already described when Ca was substituted for Na in the standard solution. The slope for the change in action potential overshoot for a tenfold increase in the Ca concentration was 33 mv in the Na-free series (Fig. 8 *A*, line *O'*). This value of the slope slightly larger than that for a theoretical Ca electrode could be due to the effect of change in the Cl concentration as $CaCl_2$ replaced sucrose. The comparable slope of this fiber with Ca substituted for Na was 24 mv. The earlier start of the falling phase (and subsequent shortening of the action potential) on increasing the Ca concentration may be related to the larger overshoot reached with higher Ca concentrations, which induced an earlier increase in K permeability (see Figs. 12 and 13).

When the external Ca was reduced to one-half of normal by replacing it with Mg, the threshold for the action potential rose and the overshoot of the

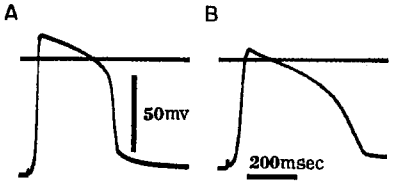

FIGURE 9. Action potentials of a fiber recorded in standard saline (*A*) and in a Na-free saline (*B*) with reduced Cl (Na salts substituted with sucrose). Upper line registers external potential.

action potential was reduced, with a slope of 29 mv change for a tenfold change in the Ca concentration (Fig. 10 *B*). The rate of depolarization of the rising phase and the action potential duration also decreased. When the external Ca was totally replaced with Mg, no conducted action potential could be elicited by the strongest stimuli available, even when their duration was much prolonged (Fig. 10 *C*). In Fig. 10 *C* the small electrotonic depolarization due to stimulating current was recorded at about 2.5 mm from the current electrode so that the depolarization induced at the stimulating site must have been very large (over 150 mv). All these results obtained by changing the external Ca concentration indicate that an increase in permeability of the membrane mainly to Ca is responsible for the depolarizing electrogenesis of the procaine-induced action potentials.

Effect of Tetrodotoxin

10^{-5} g/ml tetrodotoxin added to the standard solution did not affect the configuration of the conducted action potentials of the procaine-treated fibers. Similarly, no effect of tetrodotoxin has been observed on crayfish limb muscles (41, 42) and on the Ca spike of the barnacle muscle (26).

Effect of Mn

The addition of manganese depresses action potentials in other crustacean muscles (15, 26). Substitution of 4 mM Mn for Na in the saline caused a failure to generate conducted action potentials in the crayfish abdominal muscle fibers within 10 min. Just before the block, the rate of depolarization of the rising phase and the action potential amplitude were markedly reduced (Fig. 11 *B*). However, the falling phase was affected very little if at all. On returning the preparation to the standard saline the effect of the Mn was gradually re-

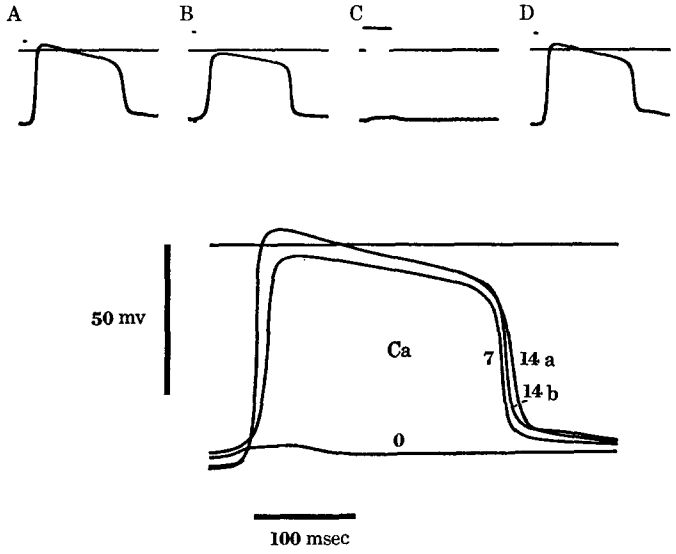

FIGURE 10. Effects of reduction in external Ca (by substitution with Mg) on action potential. *A–D*, records for Ca concentrations (in mM) of 14 (normal), 7, 0, and after return to 14. Upper trace registers external potential and stimulating current. Lower figure, superimposed tracings of the records. Ca concentrations are indicated. 14 *a* is record *A*, 14 *b* is record *D*.

versed (Fig. 11 *C*). The results suggest that the effect of Mn at this concentration is mainly to block the increase in Ca permeability, with relatively little effect on the change in permeability which causes repolarizing electrogenesis. The Mn affected equally both the rising and the plateau phases of the action potential (Fig. 11), unlike the case in frog cardiac fibers (25, 26). The results are consistent with the view that the permeability increase for Ca is responsible both for the rising and plateau phases of the action potential in the abdominal muscle fibers.

I-V Relation

The current-voltage (*I–V*) characteristic was studied (Fig. 12) in the presence of Mn, so as to block depolarizing electrogenesis. The characteristic developed a downward curvature for potentials more positive than about −10mv. The change was markedly time-variant in the presence of normal Ca, and the values of the peak potential (open circles) and of the late state, measured 200 msec after onset of the current (filled circles), formed two branches. The decrease in the potential started earlier with larger outward currents (inset records). The latency was linearly related to the peak depolarization and was

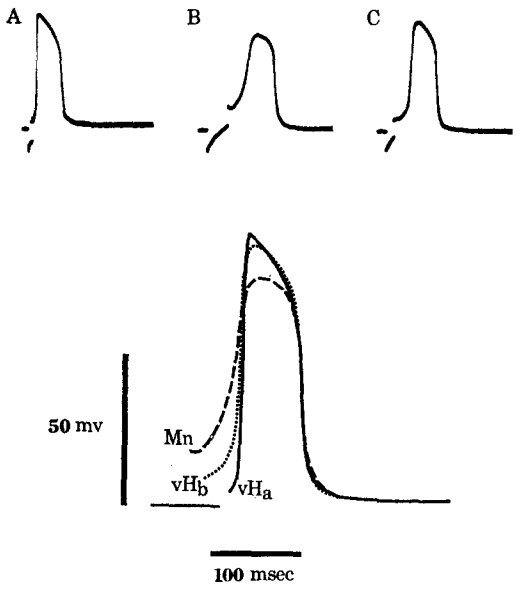

FIGURE 11. Effects of low Mn on action potential in a fiber. *A*, in standard solution; *B*, with 4 mM Mn substituted for Na; *C*, after return to standard saline. Lower figure, superimposed traces of the records; solid line, of record *A*; broken line, of record *B*; dotted line, of record *C*.

still 5.3 msec for a depolarization of 132 mv (Fig. 13). The curvature and the branches of Fig. 12 indicate the occurrence of delayed rectification (14) which is probably due to depolarizing K activation (19). By superimposing brief pulses delivered through a third microelectrode a conductance increase could be shown to be associated with this nonlinearity of the characteristic. An upward curvature which developed with large inward currents (Fig. 12) is presumably similar to the hyperpolarizing activation which is observed in other crustacean muscle fibers (20, 23, 41 *a*, 44).

The dotted line of Fig. 12 shows the measurements made prior to the time-variant reduction of the potential. The early and also the late *I–V* characteristics were nearly linear for small depolarizations. Thus, depolarizing K inactivation (18) which is a prominent feature in claw and walking leg muscles

of crayfish (9, 41, 41 *a*, 44), in cardiac muscle (18, 28), and in other cells (21) appears to be negligible in the abdominal muscle fibers.

The triangles and broken line of Fig. 12 describe the *I–V* characteristic of the same fiber in high Ca (105 mM substituted for Na). A high concentration of Mn (49 mM, also substituted for Na) was required to block the depolarizing

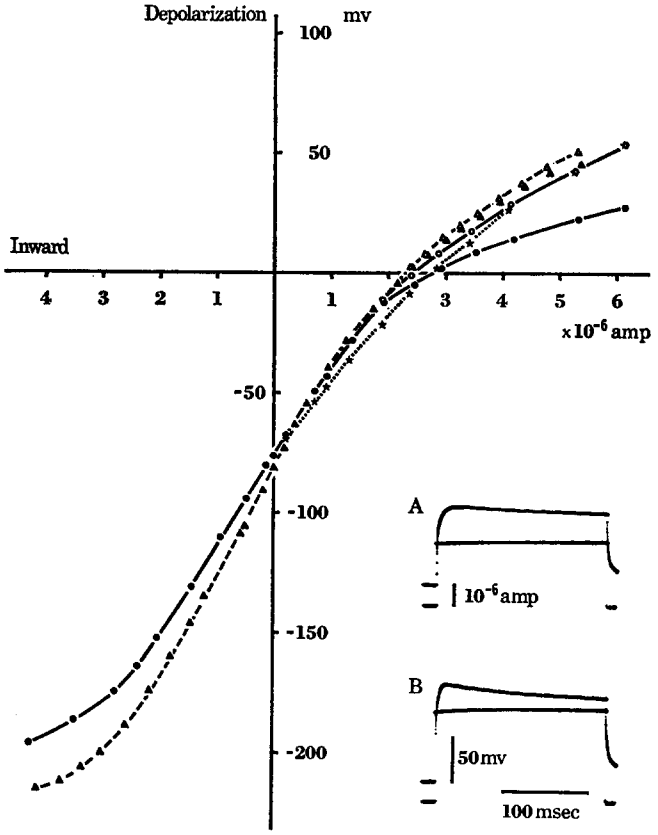

FIGURE 12. *I–V* relations, peak (open symbols) and late (200 msec from onset of current, closed symbols) potentials in saline containing 4 mM Mn (circles, solid lines) and in high (105 mM) Ca, high (49 mM) Mn saline (triangles, broken lines). Stars (dotted line) indicate early *I–V* relation (6.7 msec from onset of current) in the former saline. Inset *A* and *B*, outward current (lower trace) and potential (upper trace) records in the low Mn saline. Current was increased in *B*.

electrogenesis. The resting potential increased by 5 mv in this saline. In the hyperpolarizing quadrant the chord resistance was increased somewhat, indicating a degree of pharmacological K inactivation (19), due to the high Ca and/or Mn. The time variance of the delayed rectification was also affected. Nevertheless, the essential features of the *I–V* characteristic were similar to those of the relation in the normal Ca low Mn saline.

Influence of Cl

On reducing the external Cl (from 247 to 39 mM) action potentials of a slightly modified form were produced as shown previously (Fig. 9). The reduction in the Cl caused a small transient decrease in the resting potential. Similar transient reductions in the resting potential, which indicate permeability of the fibers to Cl, were also observed in crayfish limb muscle (45), but to a lesser extent than in frog muscle fibers (29). The present results suggest that the permeability to Cl does not vary when the delayed increase in conductance that terminates the action potential occurs.

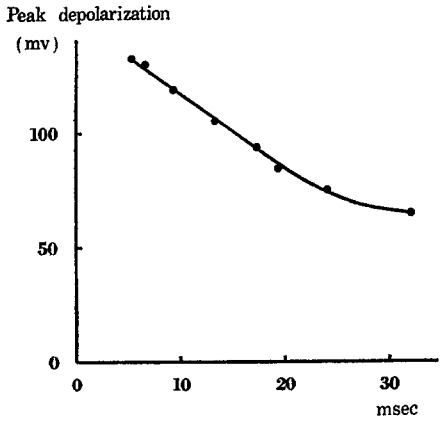

FIGURE 13. Latency (from onset of outward current) of the time-variant reduction in depolarization (abscissa) plotted against peak depolarization (ordinate). From records of the experiment in Fig. 12, in saline containing low Mn and normal Ca.

Effect of Ba

All the Ca in the external medium was replaced with Ba in this series, and the concentration of Ba was raised by substituting for Na. $NaHCO_3$ was omitted. The action potentials that were evoked in Ba left behind a prolonged refractoriness and a rest of 30 min was insufficient to restore the full amplitude of the response. Therefore, only the first responses of fibers which had not previously been subjected to stimulation were analyzed. The responses produced in different muscle fibers after about 1 hr of immersion in various Ba salines, following about 6 hr of previous soaking in the standard procaine-containing saline (procaine saline), are shown in Fig. 14 A_1, and *B–D*. The general form of these action potentials was reproducible in different fibers for each Ba concentration used. The response sometimes included one or more small "abortive" spikes after the falling phase of the action potential as seen in Fig. 14 A_1 (59). The overshoot of the action potential produced in 14 mM Ba saline (Fig. 14 A_1) was much larger than that in normal (14 mM) Ca saline (Fig. 14 *E*), and increased in the higher Ba salines (Fig. 14 *B-D*). The duration of the action potentials was much prolonged in the Ba salines (15, 24, 59) and

increased with the Ba concentration. The rate of repolarization of the plateau phase was reduced with increase of Ba. Thus, these effects of Ba on the action potential duration and rate of repolarization of the plateau phase were opposite to those seen on varying the Ca concentration (Fig. 7).

The action potential in Fig. 14 A_2 was obtained after only several minutes bathing in the same saline as in A_1, but with longer previous soaking (9 hr) in procaine saline. The action potential was similar in shape to that in A_1, but the overshoot was much larger and rather comparable to that in the high Ba salines of the above series. Fig. 14 *E* shows, for comparison, the first response of a fiber recorded after long soaking in procaine saline. The amplitudes

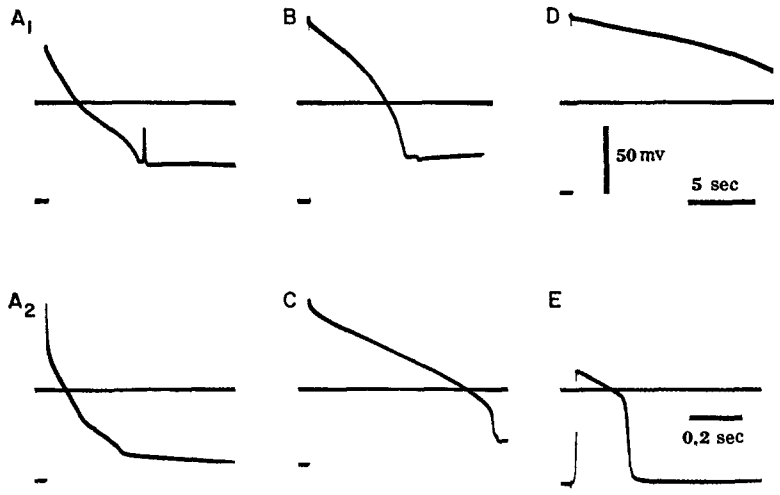

FIGURE 14. Conducted action potentials recorded in salines containing 14 mM Ba (A_1, A_2), 49 mM Ba (*B*), 84 mM Ba (*C*), 154 mM Ba (*D*), and 14 mM Ca (*E*). Upper line registers external potential. 5 sec calibration in *D* applies to *A–D*. Explanation in text.

(see Fig. 15, squares) and configurations of such action potentials were similar to those described above. When the muscles were transferred to the high Ca (instead of Ba) salines, however, the action potentials recorded after about 1 hr immersion were very irregular in form. Thus these action potentials simulated the depolarizations observed during transition from graded to all-or-none responses after a short period of immersion in procaine saline.

In Fig. 15, the overshoot of the action potentials was plotted against the logarithm of the Ba concentration from a series of experiments like that shown in Fig. 14 A_1, *B-D*. The slope for the mean values was 35 mv for a tenfold increase in the Ba for low Ba concentrations, and was reduced for high Ba concentrations. The mean resting potentials of the fibers were, in ascending order for the four Ba concentrations used: 80 (SD, ±5) mv, 79 (±6) mv, 63 (±6) mv, and 68 (±9) mv, respectively. Thus the reduction in the slope might be

related to the depolarization of the fibers in the higher Ba salines. The slope value observed for low levels of Ba was larger than that of a theoretical Ba electrode. This deviation could have resulted from a little longer previous soaking in procaine saline of the muscles used for the higher Ba salines, which might augment the responses in the higher Ba salines provided there was no effect of reduction in the resting potential. The larger overshoot obtained in the 14 mM Ba saline by longer previous soaking in procaine saline (Fig. 15,

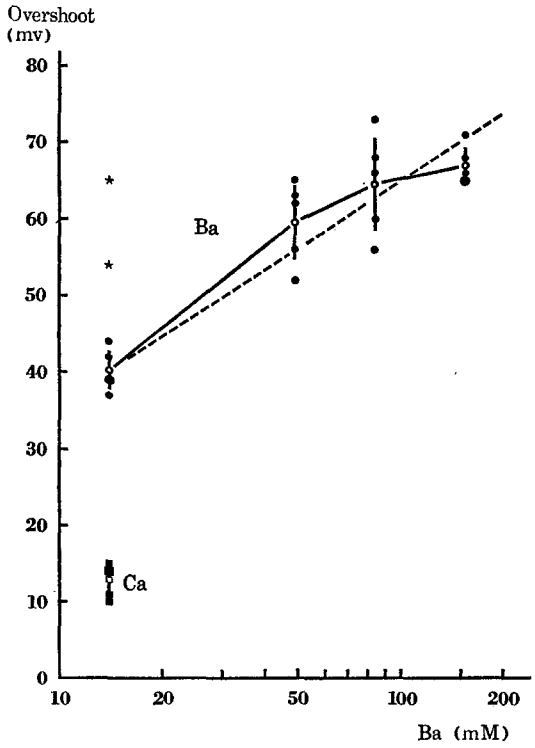

FIGURE 15. Overshoot of action potentials (ordinate) plotted against logarithm of Ba concentration (abscissa). Filled circles, values from five fibers for each concentration in a series as in Fig. 14 A_1, *B-D*; open circles, mean values; vertical lines, standard deviation; broken line, a slope of 29 mv for a tenfold increase in Ba concentration. Stars, values in another series as in Fig. 14 A_2. Squares, overshoots in standard solution as in Fig. 14 *E*.

stars) is consistent with this view. The large overshoot obtained in the Ba salines may be due to a large concentration gradient of the ion across the membrane and/or to an inactivation of K permeability by Ba.

Effect of Loading the Fibers with Rb

Preparations were soaked in media which contained 5 or 20 mM Rb substituted for an equal amount of Na. The conducted action potential recorded in normal saline after soaking for 48 hr in the 20 mM Rb saline did not overshoot, probably because of the reduced resting potential (59 mv); but its duration was much prolonged (Fig. 16 A_1). On stimulating repetitively both the amplitude and duration of the action potential tended to diminish, but the falling phase started at an approximately constant level of the membrane

potential (Fig. 16 A_2). In fibers which had been soaked in the 5 mM Rb saline for 42 hr the first experimentally induced action potential in normal saline had a large overshoot, 62 mv in the response of Fig. 16 B_1. After a rest of several minutes the overshoot was reduced to 47 mv (Fig. 16 B_2). The resting potential of this fiber was 69 mv. Rb depresses permeability to K in other excitable tissues (3, 36). If this is also the case in the crayfish abdominal muscle fibers, it would be expected to diminish the delayed increase in the con-

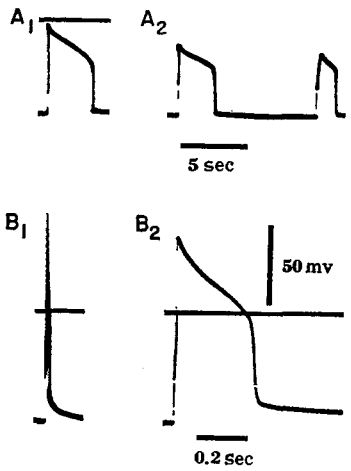

FIGURE 16. Conducted action potentials recorded in normal saline after soaking in saline containing 20 mM (*A*) or 5 mM (*B*) Rb. Upper line registers external potential. 5 sec calibration in *A* also applies to B_1. Explanation in text.

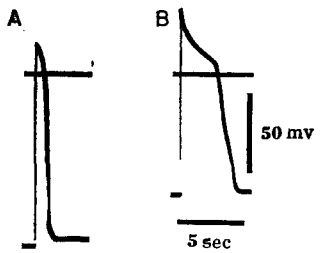

FIGURE 17. Conducted action potential recorded in procaine-containing K-free saline on soaking for 7 (*A*) or 24 (*B*) hr. Upper line registers external potential.

ductance, thereby causing prolongation of the action potential (7; but see reference 3). The observed increase in the overshoot implies that either Rb affects the Ca mechanism directly or, more likely, that Rb augments depolarizing electrogenesis by depressing the permeability to K.

Effect of K-Free Saline

The muscles were soaked in K-free procaine-containing saline with frequent renewal, in the expectation that the treatment would reduce internal K concentration (10; but see reference 22). After soaking for 7 hr, without renewal for the last half-hour, the resting potential was high (108 mv) and the overshoot of the first elicited action potential was 19 mv (Fig. 17 *A*). After 24 hr

soaking of another muscle, although the resting potential was reduced to 77 mv in the K-free saline, the first action potential had an overshoot of 39 mv and its duration was much prolonged (Fig. 17 *B*). These findings are consistent with the view that delayed K activation is involved in the repolarizing electrogenesis of the action potential and that K flux has an effect on depolarizing electrogenesis (6, 7, 23, 37).

DISCUSSION

The results indicate that the prolonged action potential in procaine-treated crayfish abdominal muscle fibers results from a time sequence of two rather widely separated phases of increased membrane permeability. The first increase is mainly for Ca, and is responsible for the depolarizing electrogenesis as indicated by the effects of altered external Ca. The increase in membrane conductance is fairly large initially, as shown by membrane current measurements. For some time after the peak of the action potential the conductance is still considerably elevated. The high conductance early in the plateau phase may be due to a remnant of the increased Ca permeability, as indicated by the effect of low Mn. The lack of effect of tetrodotoxin on the action potential is consistent with the role here ascribed to Ca, and with the selectivity of the drug in blocking the Na-activating mechanism (33, 35, 36, 38).

A delayed increase in the membrane permeability, shown by the increased membrane conductance and also by the undershoot potential, precedes the termination of the plateau phase. This differs from observations on other prolonged action potentials (54, 58, 59). The difference may possibly be related to the absence of depolarizing inactivation in the abdominal muscle fibers and/or to the different ions involved (see below). In the present conductance measurements, the electrogenic effects due to changes in permeabilities were eliminated. In this connection it may be noted that short pulses of stimulating current do not affect the membrane resistance appreciably before the start of the spikes (27) but long pulses do affect threshold currents (52).

The ion associated with the delayed increase in permeability, which is thus responsible for the repolarizing electrogenesis that terminates the action potential, is probably K as suggested by the effects of various ions (Cl, Ba, Rb, and K). Since the muscle fibers are permeable to Cl to some extent, the Cl permeability may contribute passively to the repolarization. However, the contribution of Cl permeability becomes significant if the K permeability is greatly reduced, which is the situation presumed to obtain with the Ba salines (see below). In this case, Cl permeability may be the main factor in membrane repolarization.

The action potential in standard saline may be terminated when the remaining Ca permeability is overcome by the delayed increase in K permeability (2, 19). Thus an earlier termination of the action potential, caused by repeti-

tive stimulation or by applied inward currents, indicates an earlier dominance of K permeability. The observed voltage-dependence for the start of the falling phase suggests that the repolarizing electrogenesis has a threshold potential, as does the depolarizing electrogenesis. Therefore the convergence of later potentials induced by weak currents (Fig. 5 *B*) implies that currents which started earlier failed to bring the membrane potential to a critical level earlier.

Since it is a voltage-determined process, K activation may start earlier if the action potential amplitude is increased as it is in the presence of high Ca. This interaction accounts satisfactorily for the shortening of the action potentials which is observed, along with increased amplitude, in the presence of high Ca. The faster repolarization of the plateau phase observed in the higher Ca salines may reflect the early contribution of K permeability.

Depolarizing K inactivation (18, 19) which may play a role in prolongation of the action potentials in cardiac fibers (18, 39) and in TEA-treated squid axons (5) was not observed in the abdominal muscle fibers. Therefore the prolongation may be ascribable to a slowing of the repolarization process. Although the procaine treatment may depress K permeability, thus causing the conversion of graded responses into all-or-none action potentials, the retrogression observed upon long term exposure to high Ca suggests that Ca is also a factor in the treatment.

As in other crustacean muscles (15, 24) Ba may substitute for the role of Ca as a charge carrier in the depolarizing electrogenesis. Moreover, Ba and other agents that depress K permeability (e.g., Rb) greatly prolong (15, 24, 58, 59) and augment the depolarizing electrogenesis. The delayed increase in K permeability seen in the Ca salines must therefore be presumed to be almost completely suppressed in the Ba salines, thus permitting the depolarizing electrogenesis to exert long lasting effects. Its maintenance is further prolonged with higher Ba. Similar prolongation when K flux is reduced or abolished due to decrease of internal K is observed for Na spikes (2, 6, 11, 37, 55) and for Ca spikes (23).

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