Graded and All-or-None Electrogenesis in Arthropod Muscle

I. *The effects of alkali-earth cations on the neuromuscular system of Romalea microptera*

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ABSTRACT Graded electrically excited responsiveness of *Romalea* muscle fibers is converted to all-or-none activity by Ba^{++} , Sr^{++} , or Ca^{++} , the two former being much the more effective in this action. The change occurs with as little as 7 to 10 per cent of Na^+ substituted by Ba^{++} . The spikes now produced have overshoots and may be extremely prolonged, lasting many seconds. During the spike the membrane resistance is lower than in the resting fiber, but the resting resistance and time constant are considerably increased by the alkali-earth ions. The excitability is also increased, spikes arising neurogenically from spontaneous repetitive discharges in the axon as well as myogenically from spontaneous activity in the muscle fibers. Repetitive responses frequently occur on intracellular stimulation with a brief pulse. The data indicate that the alkali-earth ions exert a complex of effects on the different action components of electrically excitable membrane. They may be described in terms of the ionic theory as follows: The resting K^+ conductance is diminished. The sodium inactivation process is also diminished, and sodium activation may be increased. Together these changes can act to convert graded responsiveness to the all-or-none variety. The alkali-earth ions can also to some degree carry inward positive charge during activity, since spikes are produced when Na^+ is fully replaced with the divalent ions.

INTRODUCTION

While all-or-none activity is characteristic of many varieties of electrically excitable tissue, muscle fibers of some invertebrates normally produce responses that are graded in amplitude depending upon the strength of the

depolarizing stimuli (1, 2, 4). Intracellular recordings from single muscle fibers of the grasshopper, *Romalea microptera* (4), have shown that the properties of the electrically excitable activity are similar to those of all-or-none decrementlessly propagated responses, except for the small size of the maximal potentials and the decremental propagation which is associated with graded responsiveness. Both modes of response are characterized by vanishingly brief latency to increasing strength of intracellularly applied depolarizing stimuli, by refractoriness, by sensitivity to anodal blockage, and by inactivation due to depolarization. Thus, the electrogenic processes of electrically excitable membranes that produce graded responses and of those which respond with all-or-none spikes appear to be basically similar.

Graded responses of crayfish muscle fibers are converted to large, all-or-none spikes if Ba^{++} or Sr^{++} is substituted for Na^+ (7). In the present paper it will be shown that the graded responses of *Romalea* muscle fibers are similarly altered and some aspects of the electrophysiological changes will be described. A second paper (26) details the essentially similar effects in muscle fibers of the lobster, *Homarus americanus*, and analyzes the nature of the changes that are produced not only by the alkali-earth but also by onium ions in the gradedly responsive muscle fibers of *Romalea* and *Homarus*. This analysis, though made possible by the particular circumstances of a study on gradedly responsive tissue, has wide applicability. Diverse phenomena appearing in many excitable tissues can be explained on the common basis of a four-factor ionic hypothesis (12) modifying, by inclusion of the process of K inactivation (10) the three-factor theory (14) that was constructed out of data for the squid giant axon.

METHODS

The general methods employed in the present work were similar to those used in the previous study of *Romalea* neuromuscular activity (4). The experiments were performed in decapitate preparations, on extensor muscles of the metathoracic legs, at room temperatures ranging from 22° to 26°C. The saline used to bathe the preparation was either that described by Hoyle (16) or modifications made by substituting sucrose, choline, or the divalent cations Ba^{++} , Ca^{++} , or Sr^{++} for equivalent amounts of Na^+ . The substitutions ranged from 5 to 100 per cent. The exoskeleton of the leg formed a convenient bath; as little as 0.3 ml. solution was adequate to change the fluid surrounding the preparation, and 10 ml. of saline were sufficient as a washing solution.

A four-trace oscillograph was available for simultaneous recordings of several functions, and up to four microelectrodes were sometimes employed to stimulate and record from the same muscle fiber. Intracellularly applied stimulating currents were monitored as the voltage drop across an 100 K ohm series resistance. In some experiments action potentials were recorded from the crural nerve which supplies the muscle

with its "fast" axon. Stimulating and amplifying equipment were standard for the laboratory.

RESULTS

The changes produced by the alkali-earth divalent cations will be detailed for Ba^{++} , which was the ion used most extensively in the present experiments and which was the most effective at any given concentration. Sr^{++} produced the

FIGURE 1. Conversion of graded responses to all-or-none by Ba^{++} . A_1 - A_3 , responses of muscle fiber before, and B_1 - B_2 after bathing preparation in Ringer's solution with 34 m.eq. Na^+ substituted by Ba^{++} . Upper trace in each set is the zero reference potential and also shows the current when intracellular stimulation was used. A_1 , A_2 , graded, oscillatory responses produced by weak and maximal stimulation of the muscle fiber by intracellular depolarization. A_3 , spike-like response evoked by stimulating the fast axon. The notch high on the rising phase represents the point of onset of the electrically excitable response from the postsynaptic potential (p.s.p.). B_1 , after treatment with Ba^{++} the p.s.p. became larger, as did the electrically excitable response which it evoked. B_2 , the latter now was an all-or-none response. The intracellularly applied current to evoke this spike was much weaker than the stimuli in A_1 or A_2 . Nevertheless, the depolarization which it produced was large, indicating the increased membrane resistance. The spike developed at a lower value of depolarization than was required for the maximal graded response of A_2 .

same effects at almost the same concentrations. Ca^{++} was used in only a few experiments. At high concentrations it gave results similar to those produced by dilute Ba^{++} or Sr^{++} . The effects of the latter two ions were completely reversible only in the lower range of concentrations.

Depolarization of the normal muscle fibers by intracellularly applied currents resulted in graded responses with a maximum amplitude measured from peak to trough rarely exceeding 30 mv. (Fig. 1 A_1 , A_2). The depolarizing, ex-

citatory postsynaptic potential (p.s.p.) might be twice as large (A_3). The resting potential averaged 50 to 60 mv.

Replacement of 7 to 10 per cent of the bath Na^+ by Ba^{++} resulted in marked changes in the responses of the muscle fibers. Both the p.s.p. and the electrically excitable response evoked by it grew in height (Fig. 1 B_1). Some depolarization of the membrane occurred. All-or-none spikes were now produced (B_2) in response to depolarizations by much weaker intracellularly

FIGURE 2. Increased effective resistance after treatment of muscle fibers with Ringer's solution in which 17 m.eq. Ba^{++} /liter was substituted for an equal quantity of Na^+ . Prior to this the rectification factor of the fiber as measured by the ratios of effective resistance was 5. Not shown in this experiment is the abolition of rectification which always occurred after treating the fibers with Ba^{++} . The effective resistances derived from the slopes of the current-voltage curves are indicated.

applied currents than were required to elicit even a small graded response (A_1) of the untreated fiber. The duration of the spike, at low Ba^{++} concentration was only slightly longer than that of the maximal graded response (A_2).

These low concentrations of Ba^{++} caused a marked increase in the time constant of the membrane and a given polarizing current now produced larger changes in the membrane potential, which denoted an increase in effective resting resistance (Fig. 2).¹ The increase, which in different experiments

¹ The membrane resistance is proportional to the square of the effective resistances in an ideal cable conductor (15, 19). The values approach direct proportionality as the system approaches an isopotential conductor. In this series of papers only the numerical values of effective resistance are used. First, serious deviations from ideal cable conditions occur in crayfish (7) and lobster (26) muscle

ranged from 50 to 300 per cent, was not proportional to the concentration of applied alkali-earth ion. The length constant, however, increased with the increased membrane resistance (15, 19). Values as great as 1 cm. were obtained in Ba^{++} -treated muscle fibers, whereas in normal fibers they ranged between 1.6 and 2.0 mm., as had also been found previously (4).

While Ba^{++} tended to depolarize the muscle fiber, the effect was marked only in high concentrations of this ion (Fig. 3). Resting potentials below 40 mv.

FIGURE 3. Lowering of threshold for responses with increasing concentrations of Ba^{++} . Each point is the average of four experiments. Both critical firing level and resting potential were reduced by adding Ba^{++} , the former to a much greater degree. Since the depolarization might itself cause a lowering of the threshold for eliciting a response, the depolarization was subtracted from the reduction in firing level to give the adjusted critical firing level.

were then frequently seen. The critical firing level for eliciting the first observable response in the fibers, normally about 25 mv. depolarization (*cf.* Fig. 1A₁), decreased even more markedly than did the resting potential. This decrease was approximately linear with Ba^{++} concentration (Fig. 3). Overshooting spikes were very frequent, even in severely depolarized cells. Spike amplitudes were then commonly above 70 mv. and occasionally as high as 100 mv.

fibers. The membrane resistance therefore lies somewhere between the values of effective resistance and its square. Second, the significance of membrane resistance as a parameter in electrogenic activity is not at all clear. Membrane resistances calculated for various excitable cells range over about four orders of magnitude, and vary widely even among cells of the same type (8).

Accompanying these changes were marked prolongations in the duration of the action potential that will be described below.

Repetitive Activity The substitution of as little as 10 per cent of the Na^+ by Ba^{++} resulted in strong, long lasting "spontaneous" contractions of the muscle with marked asynchrony among the fibers; the normally quiescent muscle was transformed into a squirming, worm-like mass. These changes were

FIGURE 4. Repetitive firing in Ba^{++} . A, neurogenic repetition. The lowest of the three traces registers the potentials of the nerve. A single shock to the crural nerve produced repetitive firing in the nerve, each spike being followed by a response of the muscle. A notch which occurred on the rising phase of the muscle spike indicates when the electrically excitable responses are initiated by the p. s. p. B, myogenic repetition. Repetitive firing without notching (B_1) was blocked by hyperpolarization (B_2). Even stronger hyperpolarization (B_3) failed to reveal a p. s. p.

manifested by spontaneous repetitive firing of muscle fibers, often with definite rhythm.

The source of this firing was both neural and muscular in origin. A single stimulus to the crural nerve produced a rhythmic, repetitive firing of the muscle fiber, each response being preceded by a discharge in the nerve (Fig. 4A). A notch on the rising phase of the muscle potential denotes the initiation of a spike from a p.s.p. Repetitive activity initiated "spontaneously" in the muscle fiber itself also occurred (B_1). These spikes were devoid of a notch, and were blocked by hyperpolarizing the muscle fiber (B_2). Very strong hyperpolarization (B_3) failed to disclose p.s.p.'s which, if present, would have been augmented by this procedure (4).

FIGURE 5. Repetitive firing in Ba^{++} . A, single shock to the crural nerve produced three neural spikes (*lowest trace*), each followed by responses in the muscle (*middle trace*). The muscle spikes exhibit refractoriness. B, two successive records superimposed. A 10 msec. intracellularly applied depolarizing current produced a long spike. Between sweeps, spontaneous depolarization occurred. The same applied current now produced a repetitive train of spikes following an evoked response.

Early after application of high concentrations of Ba^{++} both the nerve and the muscle fibers exhibited increased irritability, manifested by repetitive firing in response to a single, brief stimulus (Fig. 5A). At this frequency of repetition (*ca.* 60/sec.) the muscle fiber was in its relative refractory period and the responses after the first were small. When the repetitive responses followed a prolonged initial spike they were also briefer (B), but a tendency to increased duration was again seen in the later responses of the repetitive train. The frequency of repetition, and even more, the duration of the burst varied irregularly from one stimulation to another and repetitive activity was absent at times.

Effect on the Axon Prolonged application of Ba^{++} depressed the p.s.p. (Fig. 6A) and eventually eliminated the activity of the nerve fiber, but the

muscle continued to be spontaneously active (*B*) and to respond to direct stimuli. The spike amplitude and form remained unchanged. Thus, the failure of alternate p.s.p.'s to evoke a spike (Fig. 6*A*) is ascribable to their reduced size. During the course of block of neural excitability the p.s.p. evoked in the muscle developed after a longer latency, indicating that conduction in the axon was slowed. However, diminution of the p.s.p. occurred independently of the changes in the irritability of the axon (Fig. 7). The response of the directly excited muscle fiber (*A*) was followed by a repetitive discharge of the

FIGURE 6. Neuromuscular blockade in Ba^{++} . *A*, stimuli to the crural nerve produced small p.s.p.'s, but only every other p.s.p. was capable of producing a spike. *B*, About 1 min. later complete neuromuscular blockade occurred and was followed by spontaneous myogenic firing. The spikes were unaltered in appearance except for the absence of a prefatory p.s.p.

axon. Each of these impulses evoked a p.s.p. in the muscle fiber, but these potentials were too small, even when summated, to reexcite the relatively refractory muscle fiber. Later in the same experiment, when the nerve became inexcitable, a strong, direct stimulus evoked a sustained train of spikes whose amplitude was the same as before neural excitability was abolished (*B*).

Non-Uniformities in the Membrane The all-or-none action potentials seen after the addition of Ba^{++} ions suggested that decrementless propagation might be present. This was tested by insertion of three microelectrodes into a fiber, one for direct stimulation, the others for recording. In general, the Ba^{++} action potentials appeared to be propagated. However, it was obvious that there were marked irregularities in propagation, indicating spatial non-uniformities in the Ba^{++} -treated membrane.

Fig. 8 shows two examples of simultaneously recorded propagated repetitive activity following a brief depolarization in the presence of Ba^{++} . Inspection of

the records reveals that there are some important deviations from the usual conditions of decrementless propagation. There are reversals of order of precedence and differences in the duration of the action potentials recorded at the different loci. Small membrane potential changes can be seen and

FIGURE 7. Repetitive activity induced by depolarization of a muscle fiber with intracellularly applied current. *Upper trace*, applied current. *Middle trace*, intracellular records from the muscle fiber. *Lower trace*, external record from crural nerve. *A*, response of muscle fiber followed by repetitive propagated all-or-none activity in axon which caused p.s.p.'s that were too small to re-excite the muscle fiber. *B*, after the nerve failed to respond to activity of the muscle fiber the latter still responded repetitively to a direct stimulus.

FIGURE 8. Two examples of repetitive firing of a muscle fiber produced by brief depolarizations and simultaneously recorded at two sites. Further description in text.

appear to have been caused by electrotonic spread from an active region into refractory membrane at another point. However, this phenomenon was irregular. The changes in latency reflect variations in the refractoriness and critical firing level of the membrane, variations which were distributed both spatially along the membrane and at different times at given loci in the membrane.

It would appear likely from these findings that spikes would arise at a site

some distance from the stimulating electrode and sometimes at several sites independently; the spikes from these ectopic foci then would propagate into areas which remained gradedly responsive. These effects did occur and were particularly marked in preparations which were treated with low concentrations of Ba^{++} . Fig. 9 illustrates records from three different fibers. The membrane potentials were simultaneously recorded at two sites, "near" and "far"

FIGURE 9. Non-uniformities in responses of Ba^{++} -treated muscle fibers. Three examples of responses to depolarization recorded simultaneously at two sites in muscle fibers treated with 25 to 50 m.eq. Ba^{++} /liter. The recording site nearest the current electrode is the upper of the two voltage traces and registered a larger amount of the electrotonically spread stimulating pulse. The current trace is on top in *A*, and on the bottom in *B* and *C*. Further description in text.

from an intracellular stimulating electrode. In *A*, the stimulating electrode was between the two recording electrodes. The far electrode (*lower trace*) recorded a spike while the potential change at the nearer electrode was below threshold for this region. The spike therefore arose in only one part of this fiber. In *B*, the response at the near electrode was graded and was decrementally propagated to the far electrode. The far site eventually responded with an all-or-none spike. Probably, a region even more distant from the stimulating electrode than that of the far electrode had reached its firing level and discharged with incomplete propagation toward the stimulating electrode. In *C*, firing initially appeared to originate almost equidistant from the two recording

electrodes, but the response on break of the depolarizing current had a point of origin clearly nearer to the far electrode.

Complete Replacement of Na^+ with Sucrose or Ba^{++} When all the Na^+ was replaced with sucrose there was no longer evidence of an electrically excitable response. The loss of responsiveness was not associated with changes in either resting resistance or potential. When the sucrose solution was replaced by isotonic Ba^{++} chloride, a depolarizing stimulus now produced all-or-none spikes but never longer than 100 msec.

Prolonged Responses Replacement of 50 per cent or more of Na^+ by Ba^{++} invariably resulted in a markedly prolonged spike. Responses lasting 10 sec. or more were then frequent and a spike of 32 sec. occurred in one preparation.

FIGURE 10. Refractoriness of Ba^{++} -treated muscle fiber. *A*, prolonged spike exhibited marked refractoriness manifested chiefly by diminished duration of the testing response. *B*, a train of brief depolarizations produced progressive loss of amplitude and later of duration in the evoked spikes.

Like the similarly prolonged responses of crustacean muscle fibers (7, 23–26), these spikes had a slowly declining falling phase and in appearance (Figs. 10A and 11A) they resembled potentials of cardiac muscle fibers (21). However, as will be shown below (Fig. 12), the underlying phenomena must have been different.

The relatively refractory period after the prolonged spikes was very long, particularly so after the longest spikes (Fig. 10A). Evidence of relative refractoriness often persisted for 1 min. after a conditioning response. The prolonged refractoriness was usually manifested by diminution of duration, while appreciable amplitude diminution was not seen until the testing stimulus was applied at relatively brief intervals after the conditioning response. Decrease in amplitude and duration was also observed in the course of repetitively elicited briefer spikes produced in lower concentrations of Ba^{++} (Fig. 10B). Because of the long refractoriness of the very prolonged responses quantitative data were more conveniently obtained for responses which lasted only 50 to 100 msec. This was made feasible by the finding that the properties of the prolonged responses had no obvious relation to the concentration of the alkali-earth ions.

Resistance Effective resistance during the spike was measured by applying relatively brief hyperpolarizing or depolarizing pulses (Fig. 11). During the peak of the spike (*B*) there was much less change in resistance than is found in squid axons (5), or cardiac muscle fibers (21). Furthermore, unlike

FIGURE 11. Membrane resistance during prolonged responses. *A*, a train of brief cathodal pulses demonstrates the diminished membrane resistance throughout the entire plateau of a spike. *B*, anodal pulses of constant amplitude produced smaller voltage changes at the peak of the spike than subsequently in the plateau. Voltage and current calibrations same for both experiments, but time scales are different.

FIGURE 12. Average relative impedances calculated from fourteen fibers with spikes of 50 to 150 msec. duration by methods shown in Fig. 11. Effective resistance at peak of spikes is taken as 100.

the case in cardiac fibers, the effective resistance was lower than resting values throughout the prolonged spike. The relative values, averaged for fourteen action potentials from as many experiments, are shown in Fig. 12. The resistance increased continuously from the lowest value at the peak and rose

most rapidly during the phase of rapid decline of the potential which was seen at the end of the spike. An appreciable post-spike conductance change was seen only rarely, although undershoots of the potential following the spike occurred occasionally. Delayed rectification (asymmetry in the outward current-membrane voltage relation) was approximately fivefold in the untreated muscle fiber (Fig. 2), but was never seen after addition of Ba^{++} , either at rest or during the spike plateau (*cf.* Fig. 14).

FIGURE 13. Abolition of prolonged spikes by hyperpolarizing pulses. Muscle fiber treated with Ba^{++} was stimulated by 20 msec. depolarizing pulses (upward deflection in current monitor trace). Hyperpolarizing pulses of 11 msec. duration (downward deflection on monitor trace) were applied at various intervals in relation to the stimulus. Record A serves as the control. B, C, increasing current strengths applied at end of spike. D-G, progressively larger anodal pulses during rising phase eventually abolished spike, but the stronger pulses evoked anodal break responses (H, I).

Abolition Anodal pulses of increasing strength applied during the spike could terminate the action potential prematurely (Fig. 13). During the plateau the current necessary to cause termination was roughly proportional to the voltage of the spike at the time the pulse was delivered, but during the course of the plateau the current required appeared to decrease somewhat more rapidly than the height of the action potential. Stronger currents were required to terminate the response during its rising phase than later. Reexcitation frequently occurred at the end of a hyperpolarizing pulse much stronger than required to terminate the spike. When the spike was terminated during

the plateau the effective resistance rose immediately from its low value during the response to the resting value (Fig. 14). This was accompanied by a discontinuity in voltage approximating the height of the plateau at the time tested.

FIGURE 14. Return of resistance to higher resting value when a prolonged response was abolished. Filled circles, current-voltage relation of membrane at rest. Crosses, relation during plateau of prolonged spike, showing lower resistance than in resting membrane. Level of potential at plateau taken as origin. When the hyperpolarizing current depressed the plateau level by about 20 mv. the spike was abolished. The resistance as well as the membrane potential returned to their original values.

DISCUSSION

That insect muscle fibers react to the alkali-earth ions in the same way as do crustacean muscle fibers (7, 13, 26) is a matter of considerable interest, since the neuromuscular complexes in these two arthropod orders have different organizations (*cf.* reference 17). The pharmacology of neuromuscular transmission not only differs between insects and crustaceans (3, 11, 13, 27), but even within the group of decapods, crabs apparently having a pharmacologically different neuromuscular system from that of the lobster and crayfish (9). However, they all have in common the presence of an electrically excitable, gradedly responsive electrogenic mechanism (1, 2, 4, 8). It is tempting to re-

gard this common feature as a major factor in their similar reactions to divalent alkali-earth and/or onium ions. In the following paper this matter will be treated in further detail. For the present it will be sufficient to outline this postulated relation between graded responsiveness and reactivity to Ba^{++} , Sr^{++} , or Ca^{++} , expressing it in terms of the ionic hypothesis (14).

If either the K^+ conductance at rest were sufficiently high or if the increase triggered by activity occurred early enough, the regenerative response initiated by inward movement of Na^+ would produce only a highly damped action potential. In this connection, it is of interest that frog muscle possesses the safety factor of a lower threshold for regenerative Na^+ conductance than is that for activation of K^+ conductance (18). If high K^+ conductance were responsible for graded responsiveness, K^+ inactivation (10, 12) or merely passive blockage of potassium valves by the alkali-earth cations would lead to far larger action potentials.

That this may, indeed, be the case is indicated by both the increased resting resistance and the loss of rectification seen after application of the alkali-earth cations, since both of the affected phenomena are manifestations of K^+ conductance (14).

Prolongation of the response is probably also associated with low K^+ conductance. As in heart muscle (21) diminished K^+ conductance would repress repolarization. It is of interest that Ba^{++} blocks K^+ conductance much more than it does Na^+ conductance in certain tissues (6). In *Romalea* and also in lobster muscle fibers (26) Ba^{++} apparently decreases Na^+ inactivation, so that during the plateau of the prolonged spike the membrane conductance continues to be high relative to that of the resting membrane.

Fatt and Ginsborg (7), who first called attention to the conversion of graded responses to all-or-none potentials by the alkali-earth ions in crayfish muscle, assumed that Ca^{++} was the dominant carrier in the normal muscle fiber since the fiber remained responsive when Na^+ was entirely removed from the medium. They regarded Ba^{++} as a more efficient carrier of inward positive charge than Ca^{++} . In *Romalea* also, Ba^{++} and Sr^{++} can replace Na^+ , but only to a limited extent. The responses in media which still had at least 10 per cent of the normal Na^+ were larger and longer lasting than those obtained in Na^+ -free Ba^{++} solutions. Furthermore, actions of Ba^{++} or Sr^{++} were already manifested almost fully with relatively low concentrations of these ions. Among these actions, reported in the present paper, were the large increase in resting membrane resistance and the elimination of rectification. In the subsequent paper using a more favorable preparation, that of the gradedly responsive muscle fibers of lobster (26), other data will be presented to show that the major action of onium as well as of alkali-earth ions is to diminish or block K^+ conductance.

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