

# Action Potential Parameters Affecting Excitation-Contraction Coupling

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**ABSTRACT** In quantifying type B potentiation effects, given earlier merely qualitatively, it is found that  $Zn^{2+}$ , 1–50  $\mu M$ , causes increases in action potential duration, twitch tension, and twitch contraction period time, which are all directly proportional to the log of the concentration. Hence, the duration of the action potential, i.e. the magnitude of its mechanically effective period, is a causal factor quantitatively determining the degree of mechanical activation in the isometric twitch. In higher concentrations of  $Zn^{2+}$  up to 1000  $\mu M$ , the spike duration and the contraction time continue to increase but the twitch tension is disproportionately smaller, evidently because the high zinc (500–1000  $\mu M$ ) raises the mechanical threshold of excitation-contraction (E-C) coupling and reduces the intrinsic strength of the contractile system. Eserine (1.5 mM) and also high  $Zn^{2+}$  not only cause type B potentiation effects, but also slow the rise of the spike, thus causing retardation of the very onset of tension production, which is even greater for high  $Zn^{2+}$  because of the raised mechanical threshold. This retardation is then succeeded by the faster tension output characteristic of type B potentiation resulting from spike prolongation. Thus, the changes in the consecutive, rising and falling phases of the action potential explicitly register their separate effects in the respective very earliest and directly following periods of twitch output; i.e., each phase of the action potential produces its own mechanical "transform." These transforms, and other effects, suggest that the release of activator  $Ca^{2+}$  from the sarcoplasmic reticulum during E-C coupling can be graded in both the rate and the total amount of the release.

## INTRODUCTION

In a previous report (Sandow et al., 1965) we showed in broad outline, i.e. essentially qualitatively, that the effectiveness of excitation-contraction (E-C) coupling in activating the twitch of skeletal muscle is enhanced by lowering the level of the mechanical threshold, as caused typically by type A twitch potentiators, and by increasing the duration of the spike of the

action potential, as caused by type B potentiators. More recently we presented further evidence regarding the threshold under raised as well as lowered states (Taylor et al., 1969), which broadened the factual basis for the indicated role of the threshold in E-C coupling. In the present study we extend our consideration of the function of the action potential in the coupling process, by attempting to quantify the effects of prolonging the spike and by delineating the mechanical consequences of altering the rate of rise of the spike.

#### METHODS

We have experimented on the sartorius and toe muscles of the frog, *Rana pipiens*, by means of techniques that were in general essentially the same as those described earlier (Taylor et al., 1969). For certain special tests new procedures were used. For example, for simultaneous recording of electrical and mechanical responses of massively stimulated muscles, each toe or sartorius muscle, mounted as usual for mechanical recording, was oriented horizontally in the muscle chamber, which had the Pt plate electrodes mounted in its side walls, so that the plane of the muscle's broadest dimension was parallel to that of the stimulating electrodes. Action potentials were then recorded only from fibers on the uppermost edge. The shock artefact was minimized by altering the relative positions of the recording and indifferent electrodes until a null point was found for a subthreshold shock. Then, the recording electrode was advanced a few millimicrons to impale a fiber and the stimulus was restored to slightly supermaximal strength. For the special tests of the effects of varying the concentration of  $Zn^{2+}$ , the muscle in any particular experiment was equilibrated in oxygenated Ringer in turn at each succeeding  $Zn^{2+}$  concentration before making test recordings: first for 1 hr at 0.1  $\mu M$ , and then for 0.5 hr in the solutions containing 0.5, 1.0, 5.0, 10, 50, 100, and 1000  $\mu M$   $Zn^{2+}$  (added as  $ZnCl_2$ ). Control experiments showed that muscle deterioration possibly resulting from the long duration of an experiment (up to about 6 hr) could not account for the essential progressive effects we observed (see also Cooke and Grinnell, 1964). Furthermore, the spike prolongation and twitch potentiation that we attribute to the action of  $Zn^{2+}$  in concentrations up to 100  $\mu M$  were completely reversed after adding 0.1 M Ca-ethylenediaminetetraacetate (EDTA) to the test media (see Sandow and Isaacson, 1966). It should be noted, however, that greater concentrations of  $Zn^{2+}$ , such as 1000  $\mu M$ , may cause deleterious effects, especially after prolonged action (Sandow and Isaacson, 1960).

#### RESULTS

##### *Spike and Twitch with Massive Stimulation: Effects of $Zn^{2+}$ Concentration*

In our previous work (Sandow et al., 1965; Sandow and Isaacson, 1966), we recorded the mechanical and electrical responses in separate series of muscles. Moreover, the action potentials in the one series were recorded as propagated changes following focal stimulation and the mechanical changes in the other series as unpropagated responses to massive stimulation. We

thus questioned whether such procedures limited the dependability of attempts to quantify the correlation between the electrical and mechanical responses in zinc-treated muscles. We, therefore, studied the effects of the concentration of zinc on the simultaneously recorded twitch and the action potential of massively stimulated toe muscles.

Fig. 1, which is a typical record of this series of tests, shows that  $50 \mu\text{M}$   $\text{Zn}^{2+}$  greatly potentiates the twitch and increases the duration of both the contraction and relaxation periods; and the action potential, though massively generated and not involving propagation as in our past records, shows characteristic effects, i.e. no significant changes in either time to peak or magnitude of the spike, but a great slowing of the falling phase so that at

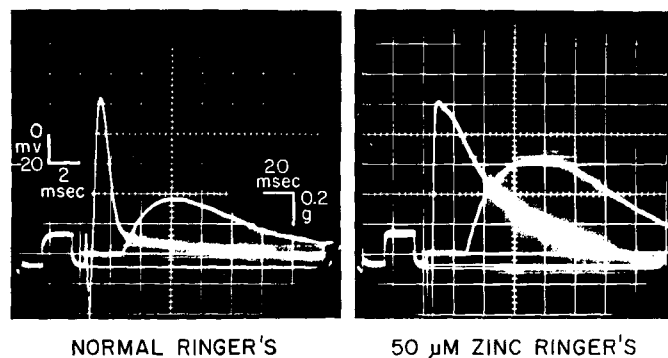


FIGURE 1. Action potential and twitch, simultaneously recorded from massively stimulated toe muscle. Calibrations at the *left* are for the action potential, those at the *right*, for the twitch. The pulse just preceding the action potential signals the 20 mV and 2 msec calibration for the action potential. Note that the position of the shock on the mechanical record is displaced to the right of that for the electrical records. Temperature,  $23^{\circ}\text{C}$ .

the  $-25 \text{ mV}$  level of the membrane potential the duration of the zinc-treated spike is about three times that of the normal. The time to peak of our massively produced action potentials was 1 msec, or slightly less, irrespective of the position of the recording electrode along the length of a given fiber. This demonstrates the absence of any measurable conduction time and indicates that the massively generated action potential was a pure “membrane potential” (Hodgkin and Huxley, 1952)—i.e., an unpropagated action potential.

The effects of increasing the concentration of  $\text{Zn}^{2+}$  are presented in Fig. 2. Spike duration, twitch tension, and twitch contraction period time show threshold increases at a zinc concentration of about  $1 \mu\text{M}$ . As the concentration is increased to somewhat less than  $50 \mu\text{M}$ , they exhibit generally parallel, progressively increasing changes, roughly proportional to the logarithm of the concentration. For concentrations greater than  $50 \mu\text{M}$ , the spike duration

continues to increase greatly, the contraction time increases only slightly, and the twitch tension falls so that at 1000  $\mu\text{M}$ , despite a threefold increase in spike duration, there is virtually no potentiation of the twitch at all.<sup>1</sup> Mashima and Washio (1964) made studies similar to ours (although they present no data on contraction time), and, in so far as comparison is possible, their and our results are essentially the same. Furthermore, Edman et al. (1966) have demonstrated that over the limited range of concentration from 2.5 to 10  $\mu\text{M}$ ,  $\text{Zn}^{2+}$  prolongs the active state of contraction, as measured at

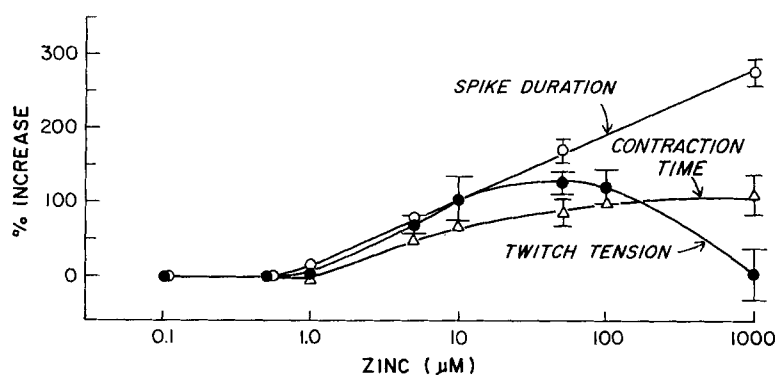


FIGURE 2. Effects of  $\text{Zn}^{2+}$  on the spike duration, as measured at the  $-25$  mv membrane potential (D25), peak twitch tension, and twitch contraction period time. The electrical and mechanical responses were obtained simultaneously from massively stimulated toe muscles. Temperature,  $25^{\circ}$ – $26^{\circ}\text{C}$ . The vertical bars indicate SEM values. To clarify plotting the data, the SEM bars for the increase in contraction times caused by 5 and 100  $\mu\text{M}$  zinc have been omitted; their values are, respectively,  $\pm 10$  and  $\pm 25\%$ ; where other such bars are omitted, their values are smaller than the height of the triangles indicating the associated means.

50% of maximal activity, in essentially direct proportion to the associated increase in spike duration, as measured at either the 0 or the  $-25$  mv level of membrane potential. Our earlier work (Sandow et al., 1965) has shown that  $\text{Zn}^{2+}$  in concentrations up to at least 100  $\mu\text{M}$  has no effect on the mechanical threshold. Thus the various aforementioned results clearly suggest that

<sup>1</sup> There was a seasonal variation in the absolute effect of a given concentration of zinc. The data of Fig. 2 were obtained from sartorius muscles sampled in the month of April. In November, the entire relationship between spike duration of sartorius fibers and  $\text{Zn}^{2+}$  concentration was shifted about one  $\text{pZn}^{2+}$  unit to the left (Taylor and Isaacson, 1965). Furthermore, special tests concerned only with twitch potentiation showed that 1000  $\mu\text{M}$   $\text{Zn}^{2+}$  did not cause as drastic a decline in twitch potentiation of summer toe muscles as it did of winter muscles, unless it acted for a very long time (Taylor, 1966). Thus, although this was not systematically investigated, winter muscles appeared to be generally more sensitive to the effects of zinc. Such a seasonal difference may account for certain essentially only quantitative disagreements between our current results (Fig. 1 and 2) and those previously reported (Sandow and Isaacson, 1960; Mashima and Washio, 1964; Taylor and Isaacson, 1965).

the capacity of  $Zn^{2+}$  in concentrations up to about  $50 \mu M$  to produce increases in the various features of mechanical activation is simply due to the parallel increases it causes in spike duration. This relation does not hold for the effects of the higher concentrations of zinc on the twitch tension, for this decreases despite the increases in the spike duration. It was this sort of dissociation that, in part, led Mashima and Washio (1964) to question whether the zinc-induced spike prolongation was the essential factor producing twitch potentiation. Their doubt may be resolved, however, by noting, first, that the contraction time increases, though slightly, even for the greatest  $Zn^{2+}$  concentrations, and this is important for it indicates that the duration of the active state continues to increase as the spike is prolonged. Thus, the spike, in proportion to its duration, is still tending to determine the degree of mechanical activation. As for the accompanying decrease in twitch output, we presume that this is caused by two mechanically depressing effects of high concentrations of zinc, the increase in mechanical threshold (Sandow et al., 1965; Taylor et al., 1969) and the decrease in maximal contractility, as demonstrated in both tetanus (Sandow and Isaacson, 1960) and K contracture (Sandow et al., 1965), and as might be expected if the zinc penetrated into the muscle fibers and acted deleteriously on the myofibrils (Edman, 1958; Hotta and Kojima, 1964).

Although not studied by us in detail, we have found that, in addition to  $Zn^{2+}$ , optimal concentrations of nickel ( $0.1 \text{ mM}$ ) (see also Fischman and Swan, 1967), cadmium ( $0.5 \text{ mM}$ ), and uranyl ( $0.5 \mu M$ ) ions increase spike duration by an average of 55, 61, and 108%, respectively, and this is evidently the means by which they all cause roughly proportionate increases in twitch tension output (Sandow and Isaacson, 1966). We have also found that imidazole ( $2 \text{ mM}$ ) and eserine ( $1.5 \text{ mM}$ ) prolong the spike by an average of 124 and 59%, respectively, thus providing again the characteristic electrical basis for the twitch potentiation these substances can produce (Sandow et al., 1964).

#### *Kinetics of Development of the Effects of $Zn^{2+}$*

To test further whether or not there is a causal relationship between spike duration and twitch output, it is of interest to know if these two parameters increase in parallel with the time of action of zinc. Mashima and Washio (1964) claim, though they do not give full details of either their procedure or results, that the increase in spike duration of fibers exposed to  $50 \mu M Zn^{2+}$  occurred "instantaneously" (pp. 544 and 546 of their paper), but that the associated twitch potentiation developed with a lag. Thus they conclude that these results also indicate that the potentiating effect of  $Zn^{2+}$  cannot be explained solely by its capacity to prolong the spike.

Our results, however, disagree with those of Mashima and Washio (1964). Fig. 3 shows that  $50 \mu\text{M Zn}^{2+}$  increases the spike duration not “instantaneously” but rather slowly. It took about 10 min for the full effect to develop and the half-time of development was 1.3 min. Furthermore, these results run fairly parallel with the rather slow development of the twitch potentiation, as indicated in Fig. 3 by the data (Sandow and Isaacson, 1966) showing the development of twitch potentiation produced by  $50 \mu\text{M Zn}^{2+}$  in the frog toe muscle. It is also noteworthy that twitch potentiation by  $\text{Zn}^{2+}$  re-

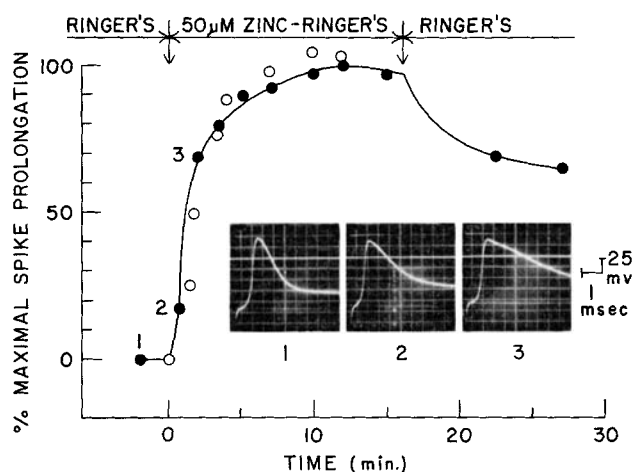


FIGURE 3. Gradual development of prolongation of the action potential of a surface fiber of a sartorius muscle exposed at time zero to  $50 \mu\text{M Zn}^{2+}$  in Ringer solution (solid circles). The three numbered *insets* show the action potential records corresponding, respectively, to the numbered data points plotted in the graph. Note partial reversal of the spike prolongation after restoration of the normal Ringer medium. The added open circles plot the gradual development of twitch potentiation of a massively stimulated toe muscle treated with  $50 \mu\text{M Zn}^{2+}$  as given in Fig. 3 of Sandow and Isaacson (1966). Temperature,  $24^\circ\text{C}$ .

verses slowly when the treated muscle is replaced in pure Ringer solution and that the reversal can be greatly hastened by means of Ca-EDTA (Sandow and Isaacson, 1966). We have studied reversal of the spike prolongation under the same conditions and have found that this occurs with essentially the same kinetics as reversal of twitch potentiation, as indicated for simple partial reversal in Ringer solution in the graph of Fig. 3. Hence, our results indicate that in response to either application or removal of  $50 \mu\text{M Zn}^{2+}$ , both twitch potentiation and spike prolongation vary kinetically in essentially parallel fashion. This conclusion is obviously in accord with the similar one based on the equilibrium effects produced by increasing concentrations of zinc.

*Rate of Rise of Spike*

As shown in Fig. 4, 1.54 mM eserine slows the rise as well as the fall of the spike. It produces other electrical alterations, and its effects, in general, are given in Table I. Of special pertinence, however, is the decrease in speed of spike development; thus, the maximal value of  $dV/dt$ , where  $V$  is voltage and  $t$  is time, is about 45% of the normal, or, as indicated by the typical behavior presented in Fig. 4, eserine increases the total time for rise of the spike from 0.6 msec for the normal to 1.1 msec. The spike duration is also affected: at the  $-25$  mv level it is about 45% greater than the normal. In another series of tests involving massively stimulated toe muscles, the spike duration increased from  $0.85 \pm 0.06$  to  $1.68 \pm 0.07$  msec, i.e., by about 98% of the normal. Thus, eserine not only slows the rise of the spike, but it also prolongs the spike as do our type B potentiators.

Fig. 5 presents the corresponding effects of 1.5 mM eserine on the twitch of a massively stimulated sartorius muscle: (a) potentiation by about 100% of the peak twitch tension, (b) increases in duration of both contraction and relaxation periods, and (c) increase in rate of tension development which begins to appear only at about 4.7 msec after stimulation and then gets larger during the following 5 msec of the twitch. These changes are all typical mechanical effects of a type B potentiator, and they are clearly attributable to an increase in duration of the active state of the twitch, which, in turn, is caused by the prolongation of the spike. However, the mechanical effects of eserine also differ from those of a typical type B potentiator. For, during the interval from 2 to about 5 msec after the stimulus, when the type B potentiators typically produce no effects at all (Sandow and Preiser, 1964; Sandow et al., 1965), eserine causes a number of generally depressive effects which include: (a) an increase in the latent period, (b) a decrease in the depth of the latency relaxation, and (c) a general slowing of the development of tension. Since depressive effects of this sort may result from raising the level of the mechanical threshold (Taylor et al., 1969), we have determined whether 1.54 mM eserine has any such influence on the tension *versus* potential relationship of frog toe muscles, as given by the  $K^+$ -contracture technique described previously (Taylor et al., 1969). Our results show that eserine, in fact, shifts the tension/ $K^+$ -concentration curve to the right, i.e., as if it slightly raises the mechanical threshold. But additional tests proved that the eserine decreased the capacity of  $K^+$  to cause depolarization, this being consistent with the decrease it causes in resting potential (see Table II) and with it being a "stabilizing" compound (Shanes, 1958) and a depressor of  $K^+$  permeability (Varga and Horowicz, 1963). When this was taken into account by plotting a tension/potential curve, it clearly appeared that the drug caused no

change either in the mechanical threshold or in the whole curve relating contracture tension to membrane potential. We therefore conclude that eserine does not shift the mechanical threshold controlling the role of the action po-

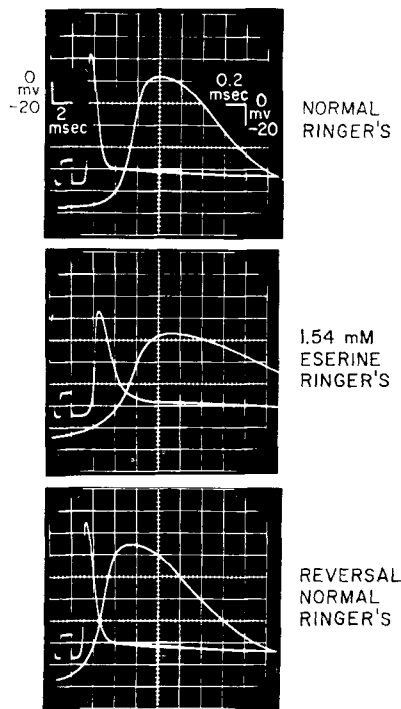


FIGURE 4

FIGURE 4. Effects of 1.54 mM eserine on the action potential of a sartorius fiber. Note especially the slowing of the rate of rise of the potential and the increase in its duration. Temperature, 22°C.

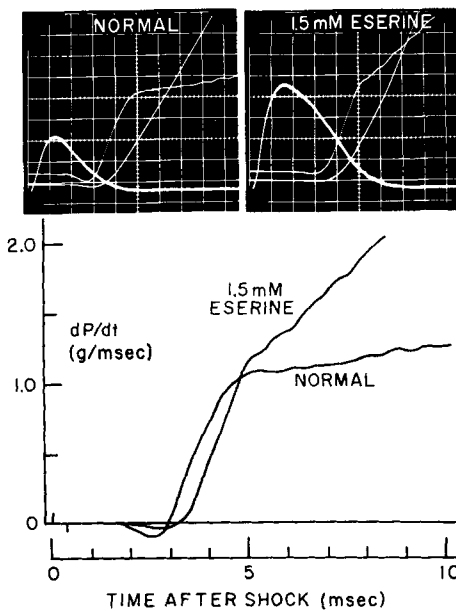


FIGURE 5

FIGURE 5. Effects of 1.54 mM eserine on the maximal twitch of a massively stimulated, curarized sartorius muscle at room temperature. The upper panels present the entire twitch (thick line) with tension calibration of 10 g/square and time of 20 msec/square. The thinner lines depict the tension output during the first 10 msec of the twitch (time calibration, 1 msec/square). The lower of these lines give the tension/time curve (tension calibration, 1 g/square), and the upper of these lines, the time derivative of the tension rise. The lower panel superimposes the derivative records of the upper panels to show directly, as described further in the text, the sequence of the two different rate changes produced by eserine on the early tension rise of the twitch. 20°C.

tential in E-C coupling. Hence, we attribute the eserine-induced depressive changes in the mechanical output described above to retardation in the rise of the spike.

Similar depressive effects are produced also by  $Zn^{2+}$  in relatively high concentrations, e.g., 250–1000  $\mu M$ . For, in addition to causing type B potentiating



effects, these higher  $Zn^{2+}$  concentrations slow the rise of the spike, as shown by Kobayashi (1962) and also by ourselves as indicated in Fig. 6 and Table II. Fig. 6 also indicates that  $500 \mu M Zn^{2+}$  causes corresponding depressive effects

TABLE I  
EFFECTS OF ESERINE ON ELECTRICAL PROPERTIES OF FROG SARTORIUS MUSCLE FIBERS

| Medium (pH 7.0)                 | Resting potential | Action potential |                      |                 |
|---------------------------------|-------------------|------------------|----------------------|-----------------|
|                                 |                   | Overshoot        | Maximum rate of rise | D25*            |
|                                 | <i>mv</i>         | <i>mv</i>        | <i>v/sec</i>         | <i>msec</i>     |
| Normal Ringer                   | $-93.0 \pm 0.5$   | $+27 \pm 1.2$    | $534 \pm 11$         | $0.78 \pm 0.01$ |
| 1.54 mM eserine Ringer (30 min) | $-87.9 \pm 1.3$   | $+9.4 \pm 0.7$   | $294 \pm 9$          | $1.24 \pm 0.02$ |

All the data give mean values and their standard error of the mean based in each case on data from several fibers in each of three different muscles.

\* D25 gives the duration of the spike at the  $-25$  mv level. Similar tests for D25 on massively stimulated toe muscles gave  $0.85 \pm 0.06$  msec for the normal fibers and  $1.68 \pm 0.07$  msec for the fibers treated with 1.54 mM eserine. Temperature,  $23^\circ C$ .

TABLE II  
EFFECTS OF  $Zn^{2+}$  ON ELECTRICAL PROPERTIES OF FROG SARTORIUS MUSCLE FIBERS

| Medium                      | Resting potential | Action potential |                      |
|-----------------------------|-------------------|------------------|----------------------|
|                             |                   | Overshoot        | Maximum rate of rise |
|                             | <i>mv</i>         | <i>mv</i>        | <i>v/sec</i>         |
| Normal Ringer               | $-90.2 \pm 0.7$   | $+29.5 \pm 3.4$  | $395 \pm 28$         |
|                             | (6)               | (6)              | (3)                  |
| 250 $\mu M Zn^{2+}$ Ringer  |                   |                  | $349 \pm 21$         |
|                             |                   |                  | (3)                  |
| 500 $\mu M Zn^{2+}$ Ringer  | $-92.6 \pm 1.0$   | $+27.1 \pm 4.3$  | $290 \pm 6$          |
|                             | (6)               | (6)              | (3)                  |
| 1000 $\mu M Zn^{2+}$ Ringer | $-89.8 \pm 1.0$   | $+17.5 \pm 7.9$  | $182 \pm 3$          |
|                             | (6)               | (6)              | (3)                  |

Each value is a mean and SEM based on data from several fibers in each of the number of muscles indicated in parentheses. Temperature,  $20^\circ$ - $23^\circ C$ .

on the very early phases of tension development, which are qualitatively the same as those caused by eserine.<sup>2</sup> We have shown (Sandow et al., 1965; Taylor et al., 1969), however, that 500-1000  $\mu M Zn^{2+}$  increases the mechanical

<sup>2</sup> It should be noted that the zinc-induced changes in  $dP/dt$  presented in Fig. 6 are typical for the 500  $\mu M$  concentration. But the amount of twitch potentiation is extraordinarily high, and it is not clear how to account for this. Presumably, this is another, though rather extreme, example of the variability mentioned in footnote 1.

threshold, and this should cause certain mechanical effects along with those produced by slowing the rise and increasing the duration of the spike. Our

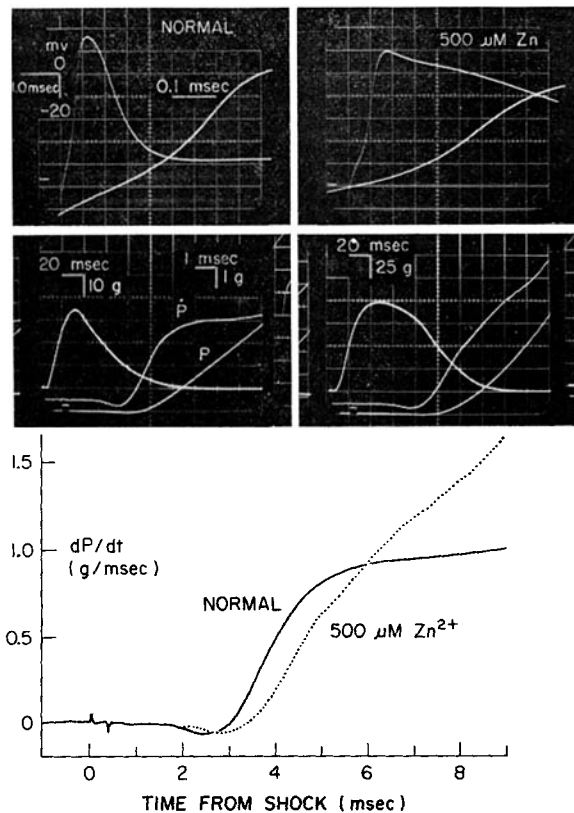


FIGURE 6. Effects of  $500 \mu\text{M Zn}^{2+}$  on the twitch and the action potential of the sartorius muscle. *Top* panels: action potential at room temperature, each simultaneously presented on a slow sweep ( $1.0 \text{ msec}/2 \text{ squares}$ ) to show the essential contour of the whole spike, and on a fast sweep ( $0.10 \text{ msec}/2 \text{ squares}$ ) to show only the rising phase of the spike. Note that the  $500 \mu\text{M Zn}^{2+}$  not only prolongs the spike (by greatly retarding repolarization) but it also retards the rising phase. *Middle* panels: twitch responses at  $20^\circ\text{C}$  with recording details like those of Fig. 5, except that calibrations are as shown. Note that the effects of the  $500 \mu\text{M Zn}^{2+}$  are (a) the characteristic type B potentiator changes of a great potentiation of the twitch and prolongation of the twitch time parameters, and, (b) as can be seen by especially comparing the derivative records in the *bottom* panel, a double effect on the rate of early tension output, i.e. an initial slowing of the rise of tension which, at about  $6.0 \text{ msec}$  after the shock, changes into a hastening of the output. See text for further details.

earlier work showed that the increase in threshold disadvantageously biases the entire mechanically effective period of the action potential and thus introduces a delay in onset of tension and also tends generally to reduce mechanical

activation (Taylor et al., 1969). The delay, though small (since it would be caused by the slightly longer time for the developing spike to depolarize sufficiently to reach the higher threshold), would presumably add to that produced by an actual slowing of the rise of the spike. We have not attempted to determine whether this extra delay can be quantitatively detected in our present results. However, a particular depressive effect of the increase in threshold is definitely in evidence, as shown in Fig. 6, in that the type B increase in  $dP/dt$  (where  $P$  is tension) above the normal, which results from prolonging the spike, does not appear until the abnormally long time of about 6.0 msec after the shock. This is to be compared with the uncomplicated type B increase in  $dP/dt$ , which occurs at the 4.5 msec point (Sandow et al., 1965). Comparison may also be made with the typical effects produced by eserine (see Fig. 5), which result from simply reducing the rate of rise and increasing the duration of the action potential, where it is seen that, after the initial delay, the treated  $dP/dt$  begins to exceed the normal one at 4.7 msec, i.e., much earlier than is the case when the corresponding event is additionally delayed by the raised mechanical threshold caused by high zinc. Thus, the increased mechanical threshold caused by the high zinc is evidently revealed in the mechanical output by the delayed development of the type B increase in  $dP/dt$ . However, we presume that, as in the case of the action of eserine, the slowing of the rise of the spike induced by high zinc acts additively with the increase in threshold to retard the very onset of tension development, although further experiments are needed to demonstrate this explicitly.

In further consideration of the role played by the rate of development of the spike, we have studied the mechanical effects of lowering the  $\text{Na}^+$  concentration of the Ringer medium. Such treatment is known to reduce the rate of rise of the spike of various nerve fibers roughly in proportion to the reduction of the  $\text{Na}^+$ , and similar behavior occurs in frog skeletal muscle (Nastuk and Hodgkin, 1950; Hodgkin, 1951). Our experiments, however, have dealt only with the mechanical changes. We have found that a stepwise  $\text{Na}^+$  decrease to 80, 60, and 40% of the normal (with sucrose replacement) causes a progressively greater depressive effect on the very early features of tension development which are basically similar to those produced by eserine and by relatively high zinc. These changes due to  $\text{Na}^+$  deficiency are especially interesting because they are not accompanied by any potentiation effects on the twitch, nor do they raise the mechanical threshold. There are various other effects of the  $\text{Na}^+$  deficiency on the twitch (Sandow et al., 1968), but these will be fully presented elsewhere. We stress here that, apart from any other effects on the twitch response, the depressive effects of the low  $\text{Na}^+$  on the early tension output of the twitch can be attributed to an associated slowing of the rise of the spike.

## DISCUSSION

Our results demonstrate (*a*) that the greater the duration of the action potential the greater, within certain limits, is the mechanical activation in the twitch, and (*b*) that slowing the rise of the spike causes a corresponding slowing in the onset of the twitch tension.

In our original indication of the first of these effects, our evidence was based on the influence of only a single (50  $\mu\text{M}$ ) concentration of zinc (Sandow et al., 1965). But in the present work we have studied the effects of zinc over a range of concentration from 1 to 1000  $\mu\text{M}$ , and our results, taken in conjunction with related findings of Mashima and Washio (1964) and Edman et al. (1966), show that the zinc ions, up to at least 50  $\mu\text{M}$  concentration, cause quantitatively parallel increases in spike duration and in mechanical activation as indicated by increases in both twitch tension output and contraction period time. These clear correlations are not so straightforwardly evident for the effects of higher concentrations of zinc. But our results and analysis indicate that the continued increases in spike duration are still tending to increase mechanical activation by prolonging the active state and that the failure of the twitch tension output to keep pace is evidently owing to the depressive effects of high zinc concentrations which tend to reduce the contractile output of the fibers. Thus these results are significant in showing not only that the duration of the spike is causative, in general, of proportional mechanical activation, but that the expression of such activation in overt tension output may be reduced or even negated by concurrent depressive effects of other mechanically significant factors such as the level of the mechanical threshold (see also Taylor et al., 1969) and the intrinsic contractility of the myofibrils.

It is important to determine the particular feature of the action potential which mediates its role in excitation-contraction coupling. The increase in spike duration, as produced by  $\text{Zn}^{2+}$  under all conditions of our work, is a consequence of slowing the repolarization phase of the action potential, and Kao and Stanfield (1970) have clearly shown that this occurs because  $\text{Zn}^{2+}$ , and  $\text{UO}_2^{2+}$  as well, slow the development and partially inhibit the increase in K conductance during delayed rectification. Although we have no direct evidence regarding our other type B agents such as eserine and imidazole, we presume that they similarly retard the spike's repolarization phase. It had been proposed that delayed rectification may be directly involved in activating the mechanical response (Freygang, 1965; for review, see Ebashi et al., 1969), but this is now disproved by various results (Adrian et al., 1969; Heistracher and Hunt, 1969; Stanfield, 1970; Kao and Stanfield, 1970; Lorkovic and Edwards, 1968). Hence, the potentiating effects of our type B agents do not directly result from the changes they may produce in delayed rectification.

It is clear that the low Na of our experiments reduced the rate of rise of the action potential by simply decreasing the availability of Na for influx; and

eserine and high zinc slowed the rise of the spike presumably by depressing Na activation.<sup>3</sup> In any case, these agents in common decrease the rate of depolarization during the rise of the spike. As will be discussed later, the rise of the spike individually produces certain explicit mechanical effects. Hence, the virtually complete absence of delayed rectification during this phase precludes an increase in K conductance as the cause of at least these particular mechanical changes. Furthermore, Beeler and Reuter (1970) have clearly shown that mechanical activation of depolarized ventricular myocardial fibers does not depend on sodium currents, and this suggests, although the details of electromechanical coupling in this preparation are rather different from E-C coupling in frog skeletal muscle, that Na activation is not directly involved in producing the effects we find in our work.

In view of the foregoing, we conclude that the mechanical changes we observe are, in general, not direct consequences of alterations in the Na and K activation systems underlying production of the action potential but of the changes in the rate of rise and general duration of the sequence of membrane potentials themselves that characterize our variously altered spikes. Furthermore, our results showing that massive stimulation of Zn-treated muscles produces essentially the same sort of twitch potentiation and spike prolongation as are evoked by focal stimulation prove that longitudinal currents of classical cable theory are not essential in the E-C coupling processes determining the changes in mechanical activation we have observed. This is consistent with the results of Watanabe (1958) and with the currently generally accepted views that essentially transverse, inward propagation along T tubules and consequent Ca release from the sarcoplasmic reticulum are basic links in coupling membrane depolarization to activation of contraction.

Our results, that eserine and low Na, and zinc in high concentration, slow the rise of the spike and evidently consequently depress the magnitude of the latency relaxation and slow the very onset of tension development, indicate that the action potential regulates the development of the mechanical response not only by its mechanically effective duration, as previously discussed, but also by its rate of rise. Similar mechanical alterations result from increasing the mechanical threshold (Taylor et al., 1969), and, when caused by a high con-

<sup>3</sup> Since eserine is a penetrating anticholinesterase, its various effects, in particular, naturally raise various interesting questions regarding the mechanism of its action, e.g.: (a) whether it acts in its cationic or uncharged form, (b) whether its action depends on penetration and thus whether it acts superficially or internally, and (c) whether its action involves anticholinesterase activity. We do not consider such questions here since our basic interest is to study the special, correlated electromechanical effects it, as well as our other agents, produce, as a means of determining the role of the action potential in E-C coupling. Nevertheless, in regard to mechanism, it is worth noting that we have found that Prostigmin, a nonpenetrating anticholinesterase, in even 3 mM concentration, does not cause twitch potentiation, nor does it alter the resting potential or the action potential's overshoot or D25 value. However, it slightly reduces the action potential's rate of rise (by about 17%), but we have not attempted to determine whether this produces any of the early depressive effects on tension output that we find are associated with the much larger reduction in this parameter caused by eserine.

centration of  $Zn^{2+}$ , they must add to those produced by the associated slowing of the rise of the spike, for this agent very greatly retards tension onset. (The reduction in depth of the latency relaxation that occurs under these various conditions is of special interest, but it will be discussed in detail elsewhere.)

The diverse conditions we have described which affect the role of the action potential in E-C coupling indicate that although the rising and falling phases of the spike combine to make up its mechanically effective period they nevertheless act individually to produce explicit "transforms" in the early tension output of the activated muscle. Thus, when the rise of the spike is slowed, as by eserine, this produces a corresponding slowing of the earliest signs of tension development, which begins at the end of the latent period (i.e., 2.2 msec after stimulation). Furthermore, lowering the mechanical threshold, and thus hastening the initiation of E-C coupling by the rising phase of the action potential, accelerates the earliest development of tension (Taylor et al., 1969); raising the mechanical threshold has consistent, opposite effects. But when the change impressed on the action potential is delayed, as when only the falling phase of the spike is affected by the type B agents which merely slow the spike's rate of fall (e.g.,  $50 \mu M Zn^{2+}$ ,  $0.5 \mu M UO_2^{2+}$ , 2 mM imidazole), then the mechanical consequence is correspondingly delayed, appearing first in the increase in  $dP/dt$  which does not begin until about 3.5 msec following stimulation. Especially interesting are the full effects of eserine and of high (e.g., 0.5 mM)  $Zn^{2+}$ , since each of these agents causes, in sequence, first a slowing of the rising phase of the spike (which in the case of the zinc, combines simultaneously with the similarly acting increase in the mechanical threshold) and then a prolongation of its falling phase, so that a corresponding sequence is produced in the early mechanical output, i.e., the initial slowing and the later hastening of the tension development.

Considering the timing of all the pertinent events presented here and in previous work (Sandow and Preiser, 1964; Sandow et al., 1965), the transform of the spike's rising phase is evidently made up of the tension output appearing from the end of the latent period, at about 2.2 msec following initiation of the action potential, until about the 3.5 msec point; the transform of the falling phase consists of the tension development at least beginning at about the 3.5 msec instant and extending until some time later which is not clearly defined by our present results. It is now particularly clear that, as previously mentioned, electromechanical coupling does not directly depend on K activation, for the spike's rising phase, although essentially free of delayed rectification, nevertheless produces explicit mechanical effects as evidenced in its own transform.

Further details regarding the transforms and their relation to various features of E-C coupling, active-state development, and the course of the entire twitch have appeared in preliminary form elsewhere (Sandow, 1971). We present in the following, however, some inferences regarding the means by

which the action potential produces its mechanical transforms by modulating the pulse of activator  $\text{Ca}^{2+}$  released from the sarcoplasmic reticulum (SR). Certain indications regarding the shape of this pulse under the particular conditions of our experiments may be obtained by: (a) attributing the slowed rise of initial tension output we have observed to a slowed release of the activator  $\text{Ca}^{2+}$  and thus a retarded buildup of activation of the myofibrils, and (b) ascribing the various augmented mechanical effects we observe in later phases of the twitch to release of a supernormal total amount of activator in the Ca pulse (see Sandow et al., 1965). We have shown that these alterations in the mechanical responses are consequences of particular changes in the function of the action potential. Thus, our results indicate that as the action potential courses through its mechanically effective period, it exerts a continuous control on the mechanisms causing release of  $\text{Ca}^{2+}$  from the SR so that the shape of the action potential is, in essential outline, impressed on the waveform of the Ca pulse. On this basis, it is noteworthy that the longer the duration of the action potential, the more prolonged must be the Ca pulse, i.e., the greater is the release of  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum. Thus our various results suggest that the release of activator  $\text{Ca}^{2+}$  is not a fixed process but, depending on the shape and duration of the action potential, it is graded in both the rate of release and the total amount of the activator that is produced. The implications of this inference for elucidating the mechanisms causing release of  $\text{Ca}^{2+}$  from the SR during the twitch, in relation, e.g., to the possibility of a regenerative release of Ca (Endo et al., 1970; Ford and Podolsky, 1970), are under consideration and will be presented in another report.

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