bacille Calmette-Guerin, whereas mononuclear cells from the spleens and lymph nodes of the same animals were inactive (*11*).

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Muscular Contraction as Regulated by the Action Potential

Abstract. *Lowering the mechanical threshold and, independently, prolonging the duration of the action potential cause an increased rate of tension development, as well as potentiation of the twitch, of frog skeletal muscle. The alterations of the two different features of function of the action potential modify excitation-contraction coupling in essentially identical fashion (probably by increasing the liberation of free Ca^{2+} into the myoplasm), and this results in early intensification as well as later prolongation of the active state.*

Diverse potentiators of skeletal muscle contraction cause their common mechanical effects—prolongation of the active state and thus potentiation of the twitch—by producing independent alterations in two distinct functions of the action potential in excitation-contraction coupling (*1*). Caffeine and the lyotropic anions (which we will call type A potentiators) lower the mechanical threshold (see also *2*) but produce no significant change in the shape of the action potential itself. Conversely, certain heavy metal ions, for example, Zn^{2+} and UO_2^{2+} (type B potentiators), greatly decelerate the repolarization phase of the action potential but have no effect on the mechanical threshold. These results are consistent with the generally proposed, though loosely defined, view that the duration of the active state of the contractile mechanism, and thus the output of the twitch, are determined by some function of the level of the mechanical threshold and the length of time during which the action potential maintains depolarization of the membrane beyond this threshold (*1–3*). Our earlier results were limited, however, in their capacity to elucidate especially the early events of excitation-contraction coupling, because they referred to mechanical features of the twitch that occurred long after excitation was over and that did not show any variation in relation to the different electrical effects of the two types of potentiators. We have extended this work and can now show (i) that the potentiators increase the rate of rise of tension during the earliest indications of contraction; (ii) that such increases differ significantly with the type of potentiator in regard to timing and yet are basically similar; and (iii) that these results in general provide new information about the link in excitation-contraction coupling of both normal and potentiated contractions.

We used excised frog sartorius muscles at 23° to 25°C, equilibrated to oxygenated Ringer solution containing 0.002 percent *d*-tubocurarine hydrochloride and adjusted to *p*H 7.0 with tris buffer. The muscles were set up at an initial tension of 2 g for isometric responses massively stimulated by slightly supermaximal square-wave shocks of 0.3 msec duration. Tensions were recorded by an RCA 5734 transducer tube, and ordinary and differentiating amplifiers and multichannel cathode-ray oscillography gave us traces as a function of time, t, of both the direct tension output, P, and also the first time derivative of the tension, dP/dt (that is, the rate of tension change).

Nitrate, in addition to producing characteristic potentiation (Fig. 1, top), accelerates development of tension. Thus, in the curves for P, the developed tension of the activated muscle 3.5 msec after the start of the shock is 1.0 g for treated muscle but only 0.72 g for untreated muscle. Such effects are more strikingly indicated by the dP/dt curves, especially when the curves are compared (Fig. 1, bottom). The curves show that at each instant the nitrate-treated muscle develops tension at a faster rate than does the normal muscle and that this difference becomes evident as early as 2 msec after the start of the shock—that is, about midway in development of the latency relaxation (*4*). This effect of the nitrate is also found when nitrate-treated and normal muscle are compared at common values of P. The effects of 1 mM caffeine are in all respects identical with those of nitrate.

As shown in Fig. 2, Zn^{2+} in 0.05 mM concentration causes changes generally like those of NO_3^- and caffeine: the usual potentiation and prolongation of the twitch, and increase in dP/dt. But an important difference is that the increase in rate of tension development begins about 3.3 msec after application of the stimulus—that is, about 1.3 msec later than that caused by NO_3^- or caffeine. Other heavy metal potentiators, such as UO_2^{2+} (1 μM) and Cd^{2+} (0.2 mM), act like Zn^{2+} (*5*).

Our most general result is that all potentiators tested cause an increase in dP/dt which at room temperature begins no later than 3.5 msec after stimulation. Similar increases in speed of shortening in isotonic contractions are also caused by potentiators (*6*). Thus we conclude that the present increases in dP/dt during the earliest part of isometric contractions result from comparable increases in velocity of shortening of the contractile component, which in turn effect greater rates of tension development in the series elastic component and thus in the muscle as a whole. Since the velocity of

shortening of the contractile component is a measure of the intensity of the active state (*7*), our results indicate that the potentiators not only prolong the active state (*8*) but, as measured by the shortening speed parameter, also intensify the process during the earliest phase of the twitch, which causes the active state to develop. This finding is contrary to earlier work (*9*); it has implications especially in respect to active state mechanisms.

Our main interest in this report, however, is in certain indications of electromechanical coupling that are revealed by correlating our new results with the known electrical changes produced by the potentiators (*1*). The time of onset of the increase in dP/dt is about 1.3 msec earlier under the type A action than it is under type B; the difference in the timing of the corresponding electrical changes is similar. Type A potentiators, by lowering the mechanical threshold, will begin to exert their action at a moment during the rise of the spike when the membrane potential reaches the new threshold; but the type B agents, by slowing the repolarization phase of the action potential, will have the onset of their action delayed until some moment during the fall of the spike.

The significant time difference between these two moments cannot be determined exactly because of our uncertainty about the turning-off of excitation-contraction coupling as a function of the degree of repolarization of the membrane during the falling phase of the action potential. Slowing the course of this phase, however, should certainly begin to augment excitation-contraction coupling when it causes the membrane potential to be maintained in a range above the mechanical threshold (−50 mv) for a longer duration than the norm, which is about 1.5 msec at room temperature. Hence we infer that this is a measure of the delay in onset of the electrical change produced by the type B potentiators that is significant in excitation-contraction coupling, relative to that produced by the type A potentiators. This is reasonably close to the period of 1.3 msec separating the instants of onset of the corresponding changes in dP/dt, and we therefore take this as evidence that the early increase in dP/dt due to the type A potentiators is a consequence of their ability to lower the mechanical threshold, and that the later increase in dP/dt caused by the type B potentiators is attributable to their capacity to prolong the action potential.

Our results show that the action potential is not a simple fixed trigger in the initiation of excitation-contraction coupling (see also *10*), but the effectiveness of its action in producing

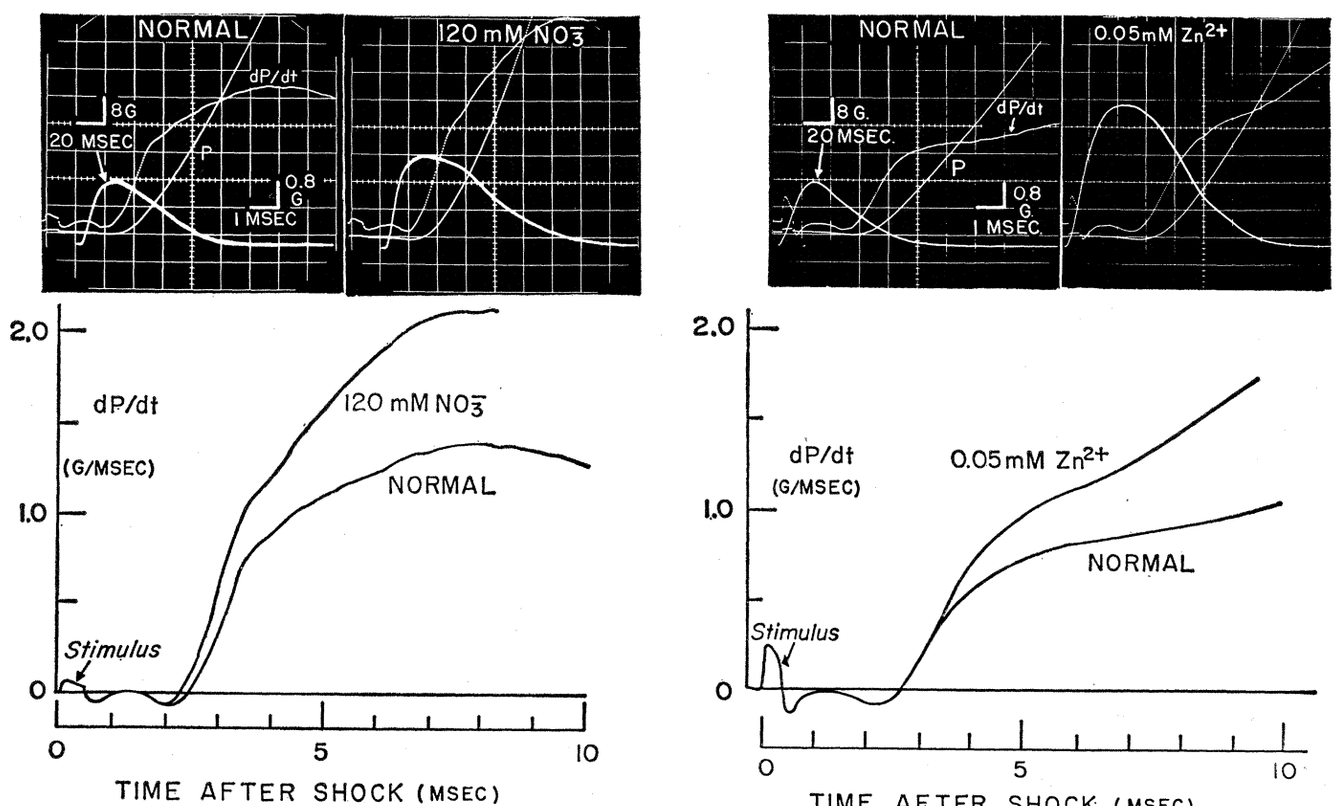

Fig. 1 (left). Increase in rate of tension development and associated potentiation of the massively stimulated maximal isometric twitch of frog sartorius muscle at 24°C, 30 minutes after exchange of NO_3^- for Cl^- in Ringer solution. Each record at the top depicts the entire twitch traced out on one beam of the oscilloscope and both the direct (P) and differentiated (dP/dt) traces of the very early part of the twitch on the two channels of the electronically switched second beam. Note the different tension and time calibrations. The twitch records demonstrate general potentiation effects. The dP/dt curves include first the shock artifact, and then the derivative of the latency relaxation followed by that of the early increase in tension. In the diagram below the records, the normal and NO_3^- dP/dt curves (given separately above) are directly compared. Note that the increase in dP/dt induced by nitrate onsets during the latency relaxation. Fig. 2 (right). Increase in rate of tension development after 30-minute action of 0.05 mM Zn^{2+} added as $ZnCl_2$ to Ringer solution. Details as in Fig. 1. Note the delayed onset of the increase in dP/dt.

mechanical change can be modulated by varying either the mechanical threshold or the duration of the spike. It is of interest to consider such alterations in terms of the role of the action potential in excitation-contraction coupling, the role of releasing an agent into the myoplasm that activates contraction of the actomyosin sliding-filaments; this agent—the link that couples excitation to contraction—may be the calcium ion (*11*). The work of Bianchi and Shanes (see *12*) indicates that such a release of Ca^{2+} is increased in nitrate-potentiated twitches. But our results suggest that the action potential causes an increased release of Ca^{2+} whenever the mechanical threshold is lowered or the spike is prolonged. This inference is supported by consideration that the rate of contraction of the sliding-filament mechanism of contraction is inherently dependent on the rate of hydrolysis of adenosine triphosphate (*13*), and that this in turn is, within limits, proportional to the concentration of the free Ca^{2+} (*14*). It is thus possible that the differences we observed in dP/dt indicate corresponding differences in the level of the Ca^{2+} very early after its release in excitation-contraction coupling. It is conceivable that the higher this initial level, the longer the Ca^{2+} may continue at sufficiently high concentration to activate contraction and thus prolong the active state.

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Interferon-Like Viral Inhibitor in Rabbits after Intravenous Administration of Endotoxin

Abstract. *Intravenous injection into rabbits of endotoxin or killed cells of* Escherichia coli *induced, in 1 hour, a viral inhibitor detectable in serum. The inhibitor disappeared from the serum in 7 to 24 hours, and was only active after incubation with rabbit cell cultures. Like interferon, it did not preferentially inactivate virus directly, was ineffective in chick cells, was inactivated by trypsin, and was not sedimentable. Unlike interferon, the inhibitor was heat labile. Nucleic acid or nucleotides apparently play no role in its induction.*

While studying circulating interferon, which appears after an intravenous inoculation of certain viruses in rabbits, a rough correlation between the capacity of the virus to induce inteferon and its pyrogenicity was noted (*1*). This observation stimulated a reexamination of the hypothesis that bacterial endotoxins (pyrogens) may induce interferon or interferon-like viral inhibitors. Gledhill found in mice and a serum factor which "spared" viral infections appeared after inoculation with endotoxin (*2*). Whether this factor acts within the cell, directly on the virus, or by way of other host defense mechanisms is not known. Youngner and Stinebring showed that an intracellular bacterium, such as *Brucella abortus*, induced interferon in chickens, but endotoxin was not an effective inducer (*3*). However, they later reported that 2 hours after inoculation with 40 mg of endotoxin or other bacteria, an inhibitor appeared in the blood of chickens (*4*).

The lethal (LD_{50}) and pyrogenic doses of the endotoxin (*5*) used in these experiments were 468 and 0.00045 μg, respectively, in rabbits weighing 2.6 to 3.0 kg. The endotoxin was suspended in phosphate buffered saline (*p*H 7.2) and inoculated in ear veins of albino male rabbits (1 to 2 kg). Serums were titrated for viral inhibitory activity as follows. Serial twofold dilutions (0.3 ml) were overlaid on duplicate tube cultures of rabbit kidney cells and incubated at 37°C for 18 hours. The fluids were then decanted, and each tube was inoculated with 10^4 to 10^5 plaque-forming units (PFU) of vesicular stomatitis virus (VSV, Indiana) suspended in 1.0 ml of medium consisting of lactalbumin hydrolyzate (0.5 percent) in Hanks balanced salt solution and 6 percent calf serum. Observations of cytopathic effect were made at 24 and 48 hours. Inhibitory effect was expressed as the last dilution of rabbit serum which inhibited cytopathic effect in 50 percent of the cultures as calculated by the Reed-Muench method. Virus was titrated by plaque formation in chick embryo cell cultures (*6*).

While normal serums showed no inhibitory effect when tested as described, an inhibitory factor developed in all rabbits 1 to 7 hours after inoculation of 2 μg of endotoxin or more (Table 1). Inhibition appeared to be greatest about 2 hours after inoculation and disappeared in 24 hours. In separate experiments those serum dilutions which inhibited the cytopathic effect of VSV also inhibited virus replication.

Whereas 0.5 μg did not consistently elicit the inhibitory factor, doses equal to or greater than 2.0 μg did (Fig. 1). There was little increase in the inhibitory titer of serum obtained from rabbits receiving exponentially increasing doses of endotoxin.

Properties of this inhibitor were studied with a pool of rabbit serum (titer of 1 : 32) obtained 2 hours after inoculation of 100 μg of endotoxin. The inhibitory effect was largely eliminated by heating at 56°C for 30 min-