Effects of Previous Activity on the Energetics of Activation in Frog Skeletal Muscle

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ABSTRACT   Effects of previous activity on the ability of frog skeletal muscle at 0°C to liberate energy associated with contractile activation, i.e., activation heat (AH), have been examined. Earlier work suggests that activation heat amplitude (as measured from muscles stretched to lengths where active force development is nearly abolished) is related to the amount of Ca^{2+} released upon stimulation. After a twitch, greater than 2 s is required before a second stimulus (AH_t) can liberate the same activation heat as a first stimulus (AH_∞), i.e., $(AH_t)/(AH_\infty)$ = $1 - 0.83\ e^{-1.40t}$, where t is time in seconds. Caffeine introduces a time delay in the recovery of the ability to generate activation heat after a twitch. After a tetanus, the activation heat is depressed to a greater extent at any time than after a twitch. The activation heat elicited by a stimulus 1 s after a tetanus is depressed progressively with respect to tetanus duration up to 3 s. For tetani of 3, 40, and 80 s duration the posttetanus activation heat is comparably depressed. The time-course of the recovery of the ability of the muscle to produce activation heat after a tetanus can be described as: $(AH_t)/(AH_\infty)$ = $1 - 0.80\ e^{-0.95t} - 0.20\ e^{-0.02t}$. Greater than 90 s is required before the posttetanus activation heat is equal to the pretetanus value. The faster phase of recovery is similar to recovery after the twitch and the slower phase may be associated with the return of calcium to the terminal cisternae from uptake sites in the longitudinal sarcoplasmic reticulum.

INTRODUCTION

It has been shown (Homsher et al., 1972; Smith, 1972) that the energy liberated (measured as heat plus work produced) during an isometric contraction at long muscle lengths where myofilament overlap, and thus twitch force is vanishingly small, appears to be proportional to ATP split by the muscle. These results have been interpreted as providing a measure of the energy required to reaccumulate Ca^{2+} released during contraction. This energy liberation has been termed the activation heat (AH). Thus, the amplitude of the activation heat might be expected to be related to the amount of Ca^{2+} released with stimulation. Homsher and Kean (1978) and Smith (1972) have shown that at 0°C an interval of greater than 2 s is required before a second stimulus can liberate as much activation heat as an initial stimulus. These

results suggest that the amount of Ca^{2+} released by a second stimulus given within 2 s after a first stimulus would be decreased by a variable amount dependent on the stimulus interval. This activation heat repriming is temperature dependent with a Q_{10} of about 3 (Homsher and Kean, 1978). Also Winegrad (1970) and Connolly et al. (1971) have suggested that the amount of Ca^{2+} released in response to a twitch after a tetanus is transiently decreased. This presumably occurs because during the period after a tetanus more Ca^{2+} is at the "relaxation sites" of the longitudinal sarcoplasmic reticulum (SR) and less at the "release sites" of the terminal cisternae of the SR. This decrease in Ca^{2+} release associated with a twitch after a tetanus has been verified (Blinks et al., 1978; Rudel, 1978) by observing a decrease in the amplitude of the light emitted by the Ca^{2+}-sensitive photoprotein aequorin. One might predict that after a tetanus the activation heat would also be transiently diminished in amplitude. This report examines in detail the time-course of the repriming of the energetics of muscle activation at 0°C. The results are discussed in terms of Winegrad's hypothesis (1968 and 1970) of separate sarcoplasmic release and reaccumulation sites for activator Ca^{2+}.

METHODS

General

Rana pipiens of both sexes were obtained from the Mogul Corporation (Oshkosh, Wis.) and kept unfed in tanks with continuously running cold water (~5°C) at room temperature. Experiments were performed during the months of November to June. Generally, frogs were killed by decapitation on the day before an experiment, and the dorsal heads of a pair of semitendinosus muscles were dissected and incubated overnight in oxygenated Ringer's at approx. 1°C. On the day of the experiment, the muscle pair, still attached to the pelvic bone was mounted on a thermopile and aerated in 95% O_2, 5% CO_2 at 0°C in a Ringer's solution containing (millimolar): 95.0, NaCl; 20, $NaHCO_3$; 2.5, KCl; 1.0 $MgCl_2$; 1.0 $CaCl_2$; and 11, D-glucose. The muscle length at which isometric twitch force was maximum, l_0, ranged from 1.5 to 2.4 cm, and the blotted weight of the muscle pairs (m) ranged from 0.048 to 0.109 g. The blotted weight of the muscle pairs averaged 92 ± 0.3% (mean ± standard error of the mean) of the drained weight. The peak twitch force per cross-sectional area ($P_0 l_0/m$) averaged 178 ± 5 mN/mm^2 ($n = 24$), the accompanying energy liberation, E/m, averaged 9.48 ± 0.23 mJ/g, and the twitch to tetanus ratio at l_0 averaged 0.78 ± 0.01. All experiments were performed at 0°C.

Mechanical and Myothermal Measurements

Force measurements were made with capacitance transducers having a resonant frequency of 3 kHz and a compliance of 0.02 $\mu m/mN$. Energy liberation during contraction was measured as heat plus work produced. Because all contractions were isometric and because the internal work done by the muscle is quantitatively returned to the muscle as heat, energy liberation was measured by monitoring muscle heat production. Heat production was measured by employing two thermopiles consisting of constantan-silver plated constantan thermocouples (Ricchiuti and Mommaerts, 1965; Rall, 1979). The characteristics of the thermopiles, P2 and P3, have been described (Rall, 1979). The majority of the experiments were done with P3. The output of the thermopile was amplified by an Astrodata 120-nV amplifier. The

output of each thermopile in microvolts was divided by its sensitivity, multiplied by the estimated muscle heat capacity, and divided by the muscle blotted weight to obtain the energy liberation in millijoules per gram (Hill and Woledge, 1962). For purposes of calculating the heat capacity, the dried weight was assumed to be 20% of the blotted weight. Myothermal records were corrected electronically for an exponential heat loss. When stimulus heat was measured, muscles were incubated in 10 mM procaine Ringer's for 30 min to obliterate muscle action potentials and subsequent force development upon stimulation. Details of the control of temperature in the muscle chamber have been described (Rall, 1979).

Experimental Protocol

One muscle of a pair was mounted on each face of a thermopile and the tendons of the muscles were attached via thread to a piece of aluminum tubing connected to the force transducer. The thermopile and muscle were immersed in a Ringer's-filled glass chamber and aerated. The system was submerged in the 40L thermostatic bath at 0°C for an approximate 45 min equilibration period. During an experimental series of ~20–35 min, the Ringer's was drained from the chamber and the muscles were stimulated at 90-s intervals unless stated otherwise. After the muscles were reequilibrated in Ringer's for 20 to 30 min, another series began. During the first series optimum stimulus parameters and l_0 were determined. Maximal isometric twitch responses were obtained when the muscles were stimulated with square-wave pulses of 3 ms duration and 15-V amplitude. During a tetanus the muscles were stimulated at 10 Hz. An estimate of the activation heat was made during the second series. The muscles were first stimulated twice at l_0 and then stretched until the twitch force was reduced to ~3% (average 3.2 ± 0.3% at a muscle length of 1.42 ± 0.01 l_0) of the twitch force at l_0. Under these conditions the activation heat averaged 3.01 ± 0.13 mJ/g and was 32% of the energy liberated in a twitch at l_0. Succeeding series depended on whether the dual pulse or posttetanic twitch protocol was employed.

For determinations of the effects of a second stimulus on the activation heat production after a single stimulus, a dual pulse protocol was followed. The muscles were stimulated twice at l_0 then stretched to the predetermined length where twitch force was 3% of its value at l_0. Control twitches (two) were measured at 90-s intervals; then a series (usually four or five) of contractions were elicited by dual pulses with variable time intervals. The pairs of pulses were given at 90-s intervals. After the dual-pulse series, the activation heat produced from a single stimulus was measured again. Then the muscles were returned to l_0 and stimulated twice more. The muscles were reequilibrated in Ringer's for a time comparable to the experimental run (~20–30 min). Activation heat production before and after the dual pulse series was averaged and the effects of a second stimulus were determined by subtracting the activation heat production from the dual-pulse responses and normalizing by the control activation heat. The results are expressed as activation heat at time t divided by activation heat at time ∞ (or 90 s), i.e., $(AH_t)/(AH_\infty)$.

The activation heat produced by a single stimulus after a tetanus was determined in the following way. Muscles were first stimulated at l_0 and then stretched to the predetermined length where twitch force was 3% of its value at l_0. The activation heat was determined as the average of two twitches separated by 90 s. The muscles were tetanized 90 s later for varying durations from 1 to 80 s. A posttetanic stimulus was then applied at variable times after the last stimulus during the tetanus, i.e., from 0.2 to 300 s after a tetanus. The measurement of the activation heat after a tetanus provided a problem. Inasmuch as the energy liberation during a muscle contraction is an integral with respect to time of those events that liberate energy, the energy

liberation increases monotonically during a tetanus. The energy liberated by a stimulus after a tetanus will be less than 4 mJ/g and may be produced on a signal which could be as great as 250 mJ/g (80-s tetanus). The problem is to measure accurately this small amount of energy on a large, moving signal. The signal may be moving because of recovery heat production after the tetanus. Data during these experiments were recorded on a strip chart recorder and a digital oscilloscope (Nicolet Explorer III, Nicolet Instrument Corp., Madison, Wis.) arranged in parallel. For a twitch after a tetanus, the oscilloscope was triggered by the pulse delivered at a variable time after the tetanus. Another tetanus was recorded with a posttetanic pulse that triggered the oscilloscope but did not stimulate the muscle—this tetanus was used as a base line. With a digital oscilloscope it is possible to obtain a variable amount of pretrigger information. During these experiments 0.32 s of the base line before the posttetanic stimulus was recorded. Using the oscilloscope, posttetanic signals were superimposed, subtracted, expanded, and photographed or recorded on a X-Y plotter. Determination of the force production after a tetanus was done in an analogous manner. During the first 3-min period after a tetanus, only one stimulus was given at a variable time in order to eliminate any effects of the test stimulus on a succeeding stimulus. Thus, to determine the activation heat after a tetanus, it was necessary to tetanize the muscle twice, once followed by a posttetanic twitch and once followed by a base-line control. The two tetani were given at 15-min intervals for shorter contractions (40 s and less) and at 30–40-min intervals for 80-s tetani. For the 80-s tetani the muscles were reequilibrated in Ringer's during the rest period.

RESULTS

Activation Heat Repriming after a Twitch

Fig. 1 shows an example of the effect of a second stimulus given at variable times after a first stimulus on the activation heat production. In this example the stimulus intervals were 0.05, 0.2, 0.3, 0.6, and 1.5 s. Fig. 1 A shows the force and heat traces produced by the dual-pulse stimulation pattern superimposed on that obtained from a single pulse. The heat and force of the single pulse was subtracted from the dual-pulse experiments to determine the effect of the second stimulus and this result is shown in part B. This figure clearly shows how the activation heat reprimes with time. Greater than 1.5 s at 0°C is required for a second stimulus to produce the same activation heat as a first stimulus. The average results from seven experiments are shown in Fig. 2 (●). In this figure the ratio of the activation heat at some time, t, (AH_t) to the control activation heat (AH_∞) is plotted vs. time. The equation shown in Fig. 2 is the result given from a least squares regression fit for a single exponential applied to all points except the 0.01 s point (solid line). To plot the figure the values of (AH_t)/(AH_∞) from seven experiments (52 observations) were averaged at time t and the exponential was then fit to the averages. One derives essentially the same relation if an exponential is fit to each experiment separately and the values are then averaged.

It is clear that the data during the first 0.05 s rise faster than predicted by the calculated single exponential recovery. This early recovery of the activation heat cannot be attributed to stimulus heat production because the heat produced by a second stimulus at a 0.01 s interval is approximately equal to the stimulus heat produced in a procainized muscle. The value of the stimulus

heat was only 3% of the activation heat. Thus stimulus heat has not been subtracted from any of these results. Likewise the early recovery cannot be attributed to differences in force development because the force produced by the second stimulus is less than 3% of the force produced at l_0 (see Fig. 1 B). In that the AH is 30% of the energy liberated at l_0 and since force development is linearly related to energy liberation, the calculated energy liberation associated with a force production that is less than 3% of that produced at l_0 is less than 7% of the AH. The activation heat does not appear to reprime as a first-order process. Nonetheless, 83% of the AH can be attributed to a single exponential process which reprimes with a rate constant of 1.4 s^{-1} (half-time
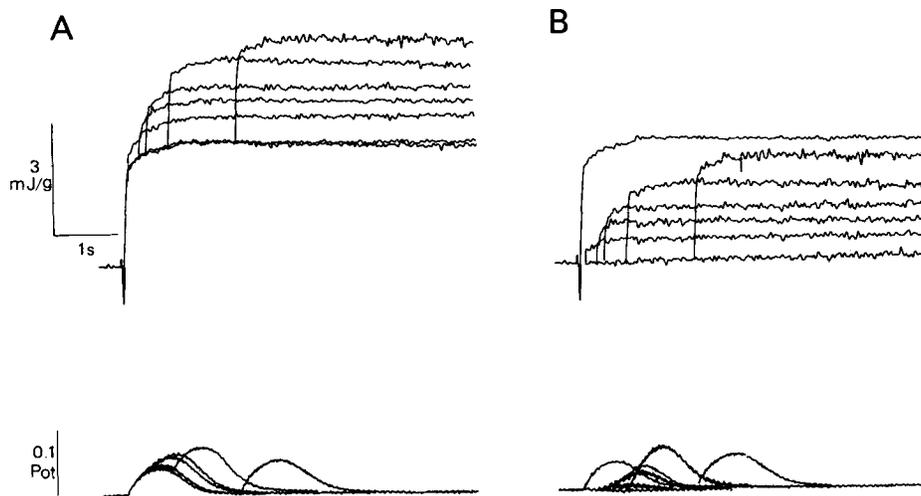

FIGURE 1. Effects on energy liberation and force development of a second stimulus given at variable times after a first stimulus at 1.38 l_0. (A) Results of two pulses given at 0.05-, 0.2-, 0.3-, 0.6-, and 1.5-s intervals plus a single stimulus response 90 s before and after the dual pulse series. Twitch force is ~ 4% of the force produced at l_0. (B) Energy liberation due to a single stimulus before dual-pulse series, energy liberation due to a single stimulus before minus energy liberation after dual-pulse series, dual-pulse results minus energy liberation due to a single stimulus before dual-pulse series. Same for force.

of 0.49 s) at 0°C. In this context in preliminary experiments ($n = 2$), at l_0 the peak force was increased by 9% and the accompanying energy liberation by 18% when a second pulse was delivered 0.05 s after the first. Also, it is apparent that >2 s is required before a second stimulus will liberate the same activation heat as a first stimulus at 0°C.

Effects of Caffeine on Activation Heat Repriming after a Twitch

Fig. 3 shows a representative example of a muscle pair soaked for 60 min in 1 mM caffeine Ringer's. On the average ($n = 3$), the time to peak force was prolonged by 41 ± 10% (mean ± S.E. of mean), the one-half relaxation time

was increased by 49 ± 10%, the energy liberated at l_0 was increased by 27 ± 11%, and the activation heat was increased by 60 ± 14% whereas the peak twitch force decreased by 14 ± 7%. Each muscle served as its own control. In two experiments control values were obtained; then caffeine-treated values were obtained. In the third experiment the protocol was reversed. The parameters measured changed in the same way in each experiment. The decreased force may be due to the fact that the first and second series in caffeine usually displayed a definite prolongation of twitch force suggestive of a contracture phenomenon. Energy was continually liberated during this
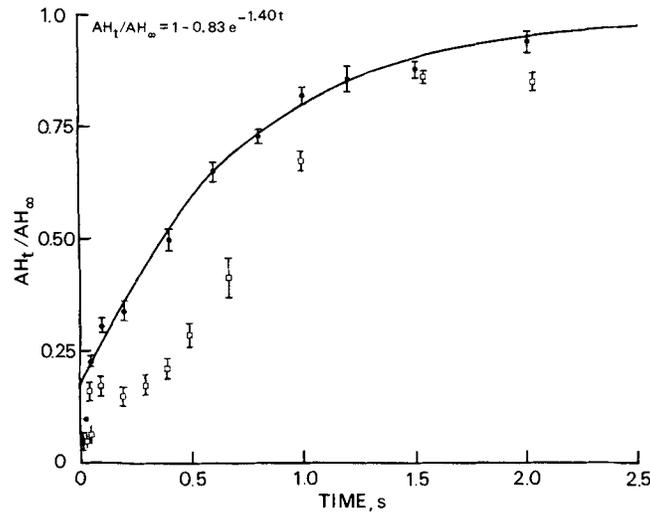

FIGURE 2. Repriming of the energy liberation at long muscle lengths in response to a stimulus after a twitch in normal and in 1 mM caffeine Ringer's. The energy liberation in response to a stimulus at some time t after a twitch, (AH_t), divided by the single stimulus energy liberation before a twitch, (AH_∞), is plotted vs. time. (●): Average results ± standard errors from posttwitch experiments ($n = 7$) in normal Ringer's. The line and equation are derived from a least-squares fit for a single exponential utilizing data from 0.05 to 2 s (●). The data at 0.01 s is approximately what would be expected for stimulus heat. (□) Results after muscles had rested for 60 min in 1 mM caffeine Ringer's. Note the definite plateau in caffeine Ringer's and its relationship to the intercept of the line drawn from the data in normal Ringer's.

period. After the muscle was stretched to a long length and returned to l_0 the twitch showed no evidence of contracture but was still prolonged as in Fig. 3. Experiments were done when the contracture was absent. The kinetics of the activation heat were slowed in caffeine Ringer's as shown in Fig. 3 C where the activation heat in normal and caffeine Ringer have been scaled to unity to unmask changes in kinetics. Fig. 2 shows a plot of $(AH_t)/(AH_\infty)$ vs. time for caffeine-treated muscles (□) along with the data from other untreated muscles already discussed. Control data from the caffeine experiments are not displayed in Fig. 2 because they were superimposable upon the untreated

muscle results. The caffeine results show a definite plateau from 0.05 to ~0.4 s and then the recovery accelerates. This plateau occurs at a value (0.18 ± 0.01, average of data points from 0.05 to 0.4 s in caffeine) near to the value of the intercept (0.17) determined from the exponential fit of the untreated data in Fig. 2. Caffeine appears to introduce a time delay in the exponential recovery of the ability to generate activation heat.

Activation Heat Repriming after a Tetanus

Fig. 4 shows an example of the effects of a stimulus delivered after a 3 s tetanus at intervals of 0.2, 2, 10, and 30 s. Contrary to the twitch data the
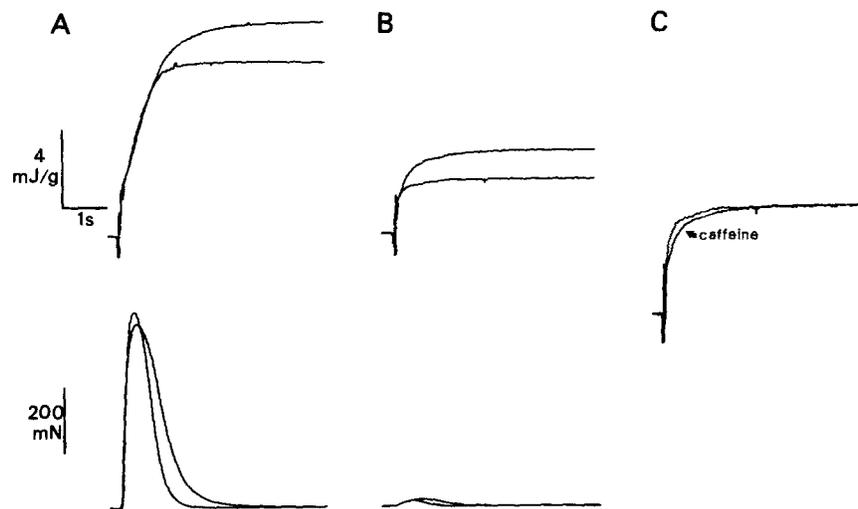

FIGURE 3.   Effects of 1 mM caffeine on energy liberation and force development at l_0 and 1.47 l_0. (A) Results at l_0: upper panel, energy; lower panel, force. In this example caffeine prolongs the time to peak force by 28% and the one-half relaxation time by 43% and increases the amount of energy liberated by 22% but decreases the peak force amplitude by 6%. (B) Results at 1.47 l_0 upper panel, energy; lower panel, force. Twitch force decreased by ~ 98% at 1.47 l_0. Caffeine increases energy liberation by 53% with force about the same but prolonged. (C) The energy liberation at 1.47 l_0 in normal and caffeine Ringer's has been scaled to expose the effects of caffeine on the activation heat kinetics. Caffeine slowed the evolution of the energy liberated at 1.47 l_0.

activation heat after a 3 s tetanus remains substantially depressed for >30 s. The posttetanic twitch force is increased. Fig. 5 illustrates an experiment to test the effect of tetani shorter than 3 s on the production of activation heat 1 s after the tetanus or twitch. Three separate series are shown in Fig. 5. Each series has a control activation heat (labeled pre) and a dual-pulse stimulation (labeled twitch, where the effects of the second stimulus are shown) and either a 1-, 2-, or 3-s tetanus where the effects of a single stimulus after the tetanus are shown. There is a small amount of variability in the activation heat from series to series which is mirrored by the variability in the posttwitch results. It

is apparent that the longer the muscle is stimulated (up to 3 s), the greater is the suppression of the activation heat in the next stimulation. Fig. 6 shows results in which different muscles were tetanized for 3, 40, or 80 s and then activated by a single stimulus 1 or 30 s after the tetanus. The finding is that for a twitch 1 s after a tetanus the depression of the activation heat is similar for tetani from 3 to 80 s in duration. The same conclusion is reached for the twitch 30 s after a tetanus. It also should be noted that the kinetics of the activation heat after a tetanus seem insensitive to tetanus duration at this resolution.

Inasmuch as a 3-s tetanus seemed to give the maximum effect on posttetanic activation heat production, the time-course of the activation heat repriming
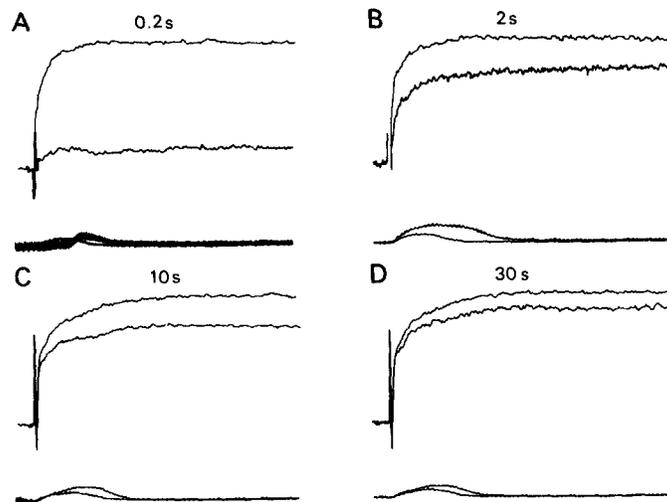

FIGURE 4.   Effects on energy liberation and force development of a stimulus given at variable times after a 3-s tetanus at 1.4 l_0. Results from three different muscle pairs. Responses to a single stimulus at 90-s intervals have all been scaled to the same size to show the effects of the tetanus on the subsequent twitch more clearly. Average control twitch force, 0.02 P_{0t}. $(AH_t)/(AH_\infty)$: 0.15 at 0.2 s, 0.74 at 2 s, 0.87 at 10 s, and 0.88 at 30 s. Posttetanic twitch force is greater than control values. Control energy liberation averaged 3.0 mJ/g. Records are 4.8 s in duration.

was derived from stimulation after 3-s tetani. That data along with 40- and 80-s data are plotted in Fig. 7 as $(AH_t)/(AH_\infty)$ vs. time. The graph and equation were constructed from 50 observations on 13 muscle pairs. The data has been fitted by a least-squares regression equation describing the sum of two exponentials. One gets essentially the same relation if the data are fit as individual points or as averages at each time interval. The graph shows that >90 s is required after a tetanus of 3 s duration or longer to produce an activation heat equivalent in magnitude to the activation heat produced 90 s before the tetanus. 3 min after the tetanus the activation heat is not statistically different from the value measured before the tetanus. The reprim-

ing of the activation heat after a tetanus apparently has two exponential components with rate constants of 0.95 s^{-1} (half-time of 0.73 s) and 0.02 s^{-1} (half-time of 34.5 s) at $0°C$. There is a substantial difference between the results for posttwitches (Fig. 2) and posttetani (Fig. 7). If the slower phase ($\tau = 0.02$ s^{-1}) in the posttetanic data is subtracted from the total, only the faster phase ($\tau = 0.95$) remains and its time-course is similar to the posttwitch results ($\tau = 1.4$).

Since the activation heat 1 or 30 s after a tetanus is independent of tetanus duration from 3 to 80 s, one might expect that the twitch force after a tetanus at l_0 would also be independent of tetanus duration in the same manner. Fig. 8 shows that this does not appear to be the case. At 1 s (Fig. 8 A) and 30 s (Fig. 8 B) after a tetanus at l_0 the twitch force decreases as the tetanus duration
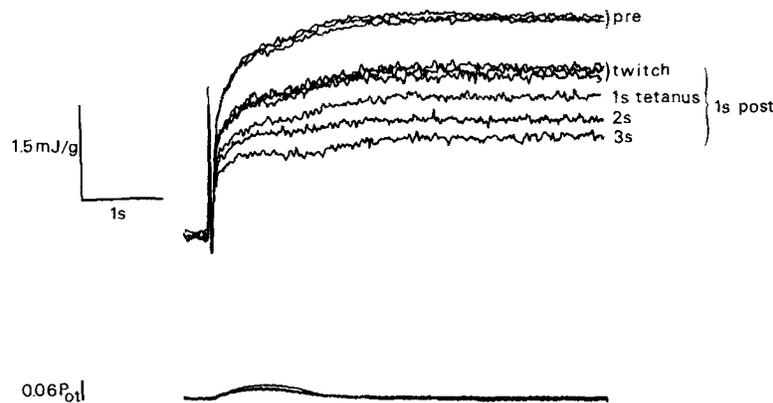

FIGURE 5.   Effects on energy liberation and force development of a stimulus given 1 s after a twitch or after a tetanus of 1–3 s duration at 1.5 l_0. Results from three separate series with the same muscle pair. Pre: single stimulus (90-s interval) results from each series. Twitch: results from each series for a second stimulus given 1 s after a twitch (effects of the first stimulus have been subtracted). 1-, 2-, 3-s tetanus: results of a stimulus given 1 s after a 1-, 2-, or 3-s tetanus. Note the progressive decrease in posttetanic energy liberation. $(AH_t)/(AH_\infty)$: 1 s posttwitch, 0.74, 0.77, 0.75; 1 s after a 1-s tetanus, 0.64; 1 s after a 2-s tetanus, 0.51; 1 s after a 3-s tetanus, 0.49.

increases. Thus, at a time when the activation heat produced is the same, the twitch force can be considerably different. This suggests that the tetanus duration can modify the influence of subsequent activation. Finally, if a stimulus is delivered at an interval of 2.5 s or longer after a 3-s tetanus, the resulting twitch force at l_0 is approximately equal to the pretetanus value but prolonged.

DISCUSSION

Homsher et al. (1972) proposed that activation heat represents the "thermal accompaniments of the liberation of calcium into the sarcoplasm, its movement to and from the myofibrillar binding sites, and its return to its storage

site by an ATP-dependent transport process in the sarcoplasmic reticulum." Currently the best estimate of the activation heat is derived from stretched muscle preparations (Homsher and Kean, 1978). Assuming that released Ca^{2+} is accumulated via an ATP-dependent transport process with a fixed stoichiometry, one would expect the amplitude of the activation heat to be related to the amount of Ca^{2+} released with stimulation. Thus a depressed activation heat after a twitch or tetanus suggests a depressed Ca^{2+} release with stimulation. This interpretation is consistent with the direct measurement of Ca^{2+} release provided by the Ca^{2+}-sensitive photoprotein aequorin. After a twitch
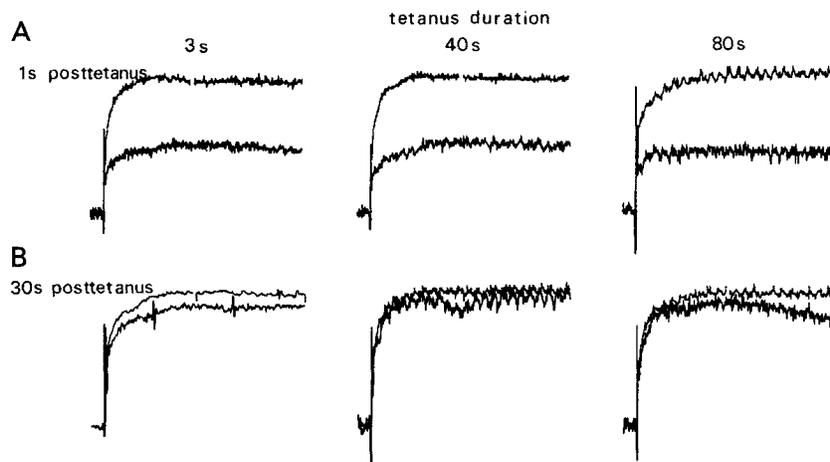

FIGURE 6. The effects of tetanus duration on the energy liberation in response to a stimulus 1 or 30 s after the tetanus at 1.44 l_0. Results from three different muscle pairs. Responses to a single stimulus at 90-s intervals have all been scaled to the same size to show more clearly the effects of tetanus duration on the subsequent twitch. Average control twitch force: 0.04 P_{0t}. $(AH_t)/(AH_\infty)$: (A) 1 s posttetanus: 0.50 for a 3-s tetanus, 0.53 for a 40-s tetanus, and 0.44 for an 80-s tetanus; (B) 30 s posttetanus: 0.90 for a 3-s tetanus, 0.91 for a 40-s tetanus, and 0.91 for an 80-s tetanus. Posttetanic twitch force is slightly greater than control values. Control energy liberation averaged 3.4 mJ/g. The single stimulus energy liberation for the 80-s tetani averaged 1.3% of the energy liberation during the tetanus. Each record is 4.8 s long.

or a tetanus the light emitted by aequorin due to a subsequent stimulus is diminished (Blinks et al., 1978; Rudel, 1978). This interpretation is also in agreement with Winegrad's (1970) prediction of a diminished Ca^{2+} release with a posttetanic stimulus.

Activation Heat Repriming after a Twitch

At 0°C in frog semitendinosus muscle dual pulses must be separated by >2 s for both pulses to liberate the same activation heat. At 2 s the activation heat is still significantly depressed by 6 ± 2% (n = 3). Activation heat is not reprimed as a single process. 83% of the activation heat can be explained by

a single exponential with a rate constant of 1.4 s^{-1} (half-time of 0.49 s) (Fig. 2). The first 17% is reprimed in 0.05 s. This behavior may reflect a threshold for the activation heat repriming process near 0.05 s. Homsher and Kean (1978) showed that a stimulus 1 s after a twitch results in an ~20% reduction in the activation heat which can be compared to the 18% reduction in Fig. 2. Smith (1972) has shown that almost 2 s are required between dual pulses for full repriming of the activation heat. In considering mechanisms it should be noted that Homsher and Kean (1978) observed that the activation heat-repriming kinetics approximately scale at 0°C and 23°C with a 10-fold reduction in the time axis. This suggests a Q_{10} of about 3 for the repriming of the activation heat after a twitch. Also, it appears as if caffeine Ringer's delays

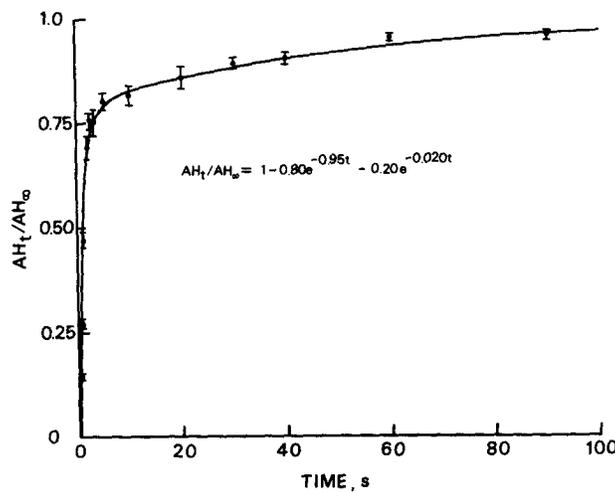$$AH_t/AH_\infty = 1 - 0.80e^{-0.95t} - 0.20e^{-0.020t}$$

FIGURE 7.   Repriming of the energy liberation at long muscle lengths in response to a stimulus after a tetanus of 3–80 s duration. The energy liberation in response to a stimulus at some time t after a tetanus, (AH$_t$), divided by the single stimulus energy liberation, (AH$_\infty$), is plotted vs. time. (●) averages of 50 observations from 13 muscle pairs for tetani of 3, 40, and 80s duration. The line and equation are derived from a least squares fit of a sum of two expoentials to the data. The rate constants differ by 48-fold.

the onset of the slower phase of repriming without altering the faster phase or threshold (Fig. 2).

Mechanisms to explain the time-course of activation heat repriming after a twitch reside in the series of steps associated with excitation-contraction coupling and relaxation. These steps include: (a) action potential, (b) conduction in transverse tubules, (c) charge movement, (d) possible potential changes associated with release of Ca^{2+} from the sarcoplasmic reticulum (SR), (e) Ca^{2+} release, (f) Ca^{2+} interaction with and debinding from troponin, (g) Ca^{2+} uptake by SR, and (h) Ca^{2+} transport back to release sites. At 4°C Oetliker and Schümperli (1979) and Suarez-Kurtz and Parker (1977) have shown that the birefringence signal, thought to be associated with a SR potential change

accompanying Ca^{2+} release, can fully reprime in 0.15–0.3 s after an initial stimulus. Unfortunately, there is disagreement as to whether the birefringence signal mirrors the repriming of the Ca^{2+} transient. In an experiment at 5°C the activation heat was still depressed by 54 and 35% at 0.15 and 0.3 s, respectively, after an initial stimulus.[1] Thus, steps a–d in excitation-contraction coupling do not seem likely to be the primary limitation to the ability of a second stimulus to produce activation heat.

It has been suggested that repriming of the activation heat after a twitch may reflect uptake of Ca^{2+} and its return to release sites (steps g and h above).[2] The time expected to be associated with the uptake of Ca^{2+} by the longitudinal SR and its return to the terminal cisternae of the SR is much longer than the observed repriming of the activation heat after a twitch (Winegrad, 1970 and
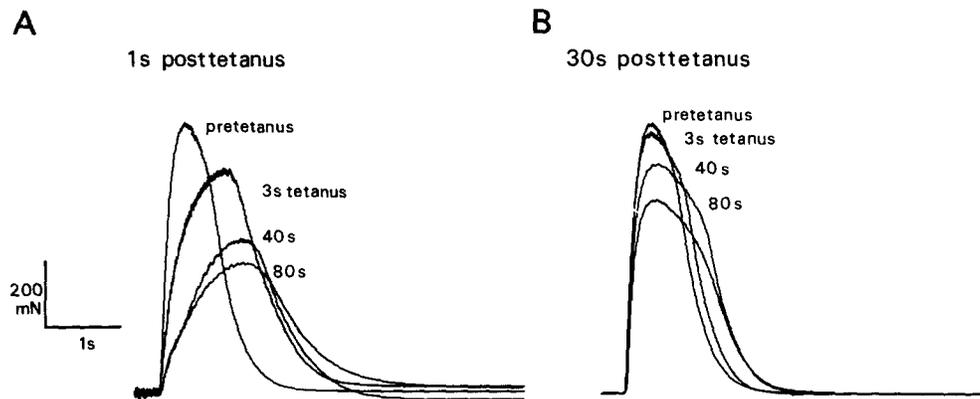

FIGURE 8. The effect of tetanus duration on the force development at l_0 in response to a stimulus 1 or 30 s after a tetanus. Force records 1 s (A) and 30 s (B) after a 3-, 40-, and 80-s tetanus plus the pretetanus control. The records 1 s posttetanus require the subtraction of base-lines from control tetanus. Ratios of posttetanus force to pretetanus force: 1 s—0.84 for a 3-s tetanus, 0.58 for a 40-s tetanus, 0.49 for an 80-s tetanus; 30 s—0.97 for a 3-s tetanus, 0.85 for a 40-s tetanus, and 0.71 for an 80 s tetanus.

see below). Possibly some Ca^{2+} is resequestered by that part of the SR from which it is released. The fragmented SR thought to be associated with the terminal cisternae possesses an ATP-driven Ca^{2+} pump (Meissner, 1975). The repriming kinetics of the activation heat after a twitch may then reflect the ability of this portion of the SR to accumulate Ca^{2+} and subsequently release it. Shifting of the repriming kinetics to longer times in caffeine Ringer (Fig. 2) and slowing of the kinetics of activation heat evolution in a single twitch (Fig. 3 C) would be consistent with such an interpretation. Along with potentiation of Ca^{2+} release (Sandow et al., 1964), caffeine is thought to inhibit the rate of

[1] Rall, J. A. Unpublished results.
[2] Homsher, E. Personal communication.

Ca^{2+} uptake by the SR (Weber and Herz, 1968) and thus may delay repriming of the activation heat.

Activation Heat Repriming after a Tetanus

After a tetanus of a 1 s or longer duration, activation heat in response to a posttetanic stimulus is more depressed than after a twitch (Fig. 5). This depression increases with tetanus duration up to 3 s. Longer tetani (up to 80 s) do not give a greater depression of the activation heat repriming kinetics than does a 3-s tetanus. Fig. 7 shows that after a tetanus more than 90 s is required for the complete repriming of the activation heat, and that the activation heat is reprimed in two exponential phases. The first phase constitutes 80% of the recovery with a half-time of 0.73 s and the second phase comprises 20% with a half-time of 34.5 s at 0°C. Winegrad (1968, 1970) proposed, from autoradiographic studies of intracellular Ca^{2+} movements, that Ca^{2+} is released from the terminal cisternae and reaccumulated by the longitudinal portion of the SR and only slowly returned to the release sites. Winegrad (1970) has made quantitative estimates of the half-times of the assumed exponential return of Ca^{2+} to release sites from uptake sites. Using this information the calculated half-time for this process at 0°C is ~34 s. This value is close to the half-time (34.5 s) of recovery of the slow phase of the activation heat after a tetanus. This agreement supports the suggestion that the depression of the activation heat from 3 to >90 s after a tetanus is due to part of the Ca^{2+} being located in nonrelease sites within the SR. The fast recovery after a tetanus has a half-time of 0.73 s, which is similar to the half-time of the exponential component of recovery after a twitch (0.49 s). The repriming of the activation heat after a twitch has a Q_{10} of about 3 (Homsher and Kean, 1978), whereas Winegrad (1970) suggests that the Q_{10} for the slow intrasarcoplasmic Ca^{2+} movement is 1.7. Thus one would predict that the fast recovery phase after a tetanus would be more temperature sensitive than the slow recovery phase.

Implications for Mechanical Activation

A twitch produced at l_0 after a tetanus is not potentiated at 0°C where the average twitch:tetanus ratio is 0.78. This is consistent with the observation made by Ramsey and Street (1941) that posttetanic twitch potentiation occurs only if the twitch to tetanus ratio is less than 0.64. 2.5 s or longer after a tetanus, the twitch is of normal amplitude but prolonged in duration (the prolongation occurs in decreasing magnitude for at least 90 s after the tetanus). Activation heat in response to a single stimulus is decreased by 20% 2.5 s after a tetanus despite a normal amplitude twitch. Connolly et al. (1971) and Blinks et al. (1978) suggest that this phenomenon can be explained by a decreased rate of Ca^{2+} uptake by the SR. No systematic changes were observed in the kinetics of the activation heat evolution after a tetanus. Either the kinetics of the activation heat do not closely follow the rate of Ca^{2+} uptake or another interpretation must be suggested. One possibility might be that there is a 20% excess in Ca^{2+} release in a fully reprimed twitch, and that only 80% of the Ca^{2+} release is needed to fully activate the muscle. Prolonged relaxation

may be due to an increase in sarcomere inhomogeneity after a tetanus and may not reflect the rate of Ca^{2+} uptake.

A twitch produced 1 or 30 s after a tetanus at l_0 decreases as the tetanus duration increases from 3 to 40 to 80 s (see Fig. 8). This is true despite the fact that the activation heat has reprimed to a comparable extent at each tetanus duration. Decreased force development may be due to other mechanisms than Ca^{2+} release, e.g., mechanisms which may be associated with the amount of energy liberated by a contracting muscle.

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