Effects of Twitch Train on the Tetanic Contractility of the Frog Skeletal Muscle

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Abstract The effects of twitch trains on the contractility of succeeding tetani were investigated in the frog toe muscle. The changes in isometric tension and its first derivative were analyzed. Tetani of 1 sec duration were induced every 5 min and trains of twitches (less than 250) with 0.1 to 3 Hz were interposed between two successive tetani. A twitch train which clearly shows an ascending staircase exerted at least three different effects on the following tetani. These were a rapidly decaying potentiation ($P_1$), a slowly decaying potentiation ($P_2$), and an inhibitory effect ($I$) which was regarded as fatigue. These after-effects were modified by various interventions. Increasing of the twitch frequency at a constant number of stimuli augmented both the potentiating and the inhibiting effects. When the bath temperature was lowered to 4°C, the potentiation was masked by a marked inhibition. At a higher temperature (28°C) the potentiating effect was facilitated. Prolonged perfusion of low concentration (0.5 mM) of caffeine mimicked the effects of low temperature. Effects of twitch train on the contracture induced by 5 mM caffeine were examined and it was found that the repetitive twitches had no effect on the following caffeine contracture. Although the exact mechanism for these after-effects was not clear, it was assumed that the intracellular calcium turnover may play some role in the observed phenomena.

Key Words: twitch train, tetanic contractility, staircase phenomenon, fatigue, excitation-contraction coupling.

It is known that the twitch force of the skeletal muscle can be modified by various physiological interventions such as the staircase effect or the phenomenon of post-tetanic potentiation. These two phenomena are of special interest because both are induced by repetitive stimuli. Applying the isometric quick-release method Ritchie and Wilkie (1955) found that the twitch potentiation caused either by staircase or post-tetanic effect was due to prolongation of the duration of the active state. The staircase phenomenon of the skeletal muscle, though less
obvious as compared with myocardial tissues (Hajdu, 1953; Niedergerke, 1956; Koch-Weser and Blinks, 1963), was further analyzed by Mashima et al. (1962) who first found that the positive staircase by increasing of the stimulation frequency could be induced without accompanying any noticeable change in the action potential measured intracellularly. Thus, these authors concluded that the phenomenon could be attributed to the events related to the excitation-contraction coupling. Post-tetanic potentiation, a twitch augmentation caused by tetanic stimuli, can be regarded as an after-effect of repetitive stimuli with far higher frequencies. The phenomenon is neither the result of recruitment of muscle fibers since it is not influenced by the conditions of stimulation, namely indirect via nerve or direct (Brown and von Euler, 1938), nor could it be due to increased stiffness of the series-elastic component (Close, 1972).

Recently Krarup (1981a) investigated these two phenomena on the mammalian skeletal muscle in detail and found that there exist several factors regulating them. He observed that in the staircase one potentiating parameter and one inhibiting parameter (fatigue) are operative, while the post-tetanic potentiation can be regarded as a sum of two potentiating effects. This author also pointed out that there is an additional negative effect which recovers rapidly with time after the cessation of repetitive stimuli, and suggested the possibility of a partial failure of propagation of excitation in the transverse tubular system.

The present investigation was aimed to clarify the effect of twitch train with successive stimuli on the following tetanic contractility since it has frequently been observed by the author that the tetanic tension was not potentiated, but rather depressed when tetani was induced immediately after the twitch potentiation, i.e. at the peak of positive staircase. If the same potentiating factor is developing during the staircase as the tetanic stimulation, then the tetanus should exclusively be augmented for some time after the cessation of the foregoing repetitive stimuli. It will be shown that in the frog toe muscle there are at least two potentiating factors with different decaying time constants and an inhibiting factor which plays the most dominant role.

**METHODS**

Small bundles excised from the toe muscle (ext. dig. long. II) of the bullfrogs (Rana catesbeiana) were used in the majority of experiments. In some experiments whole muscles (ext. dig. long. II) obtained from Rana japonica were also used. The muscle preparation was mounted in a Lucite chamber containing 0.4 ml of bathing solution. One tendinous end of the muscle was pinned to the silicon rubber base of the chamber and the other end was connected to the sensitive arm of a strain gauge (Fuji-Keisoku, TDS-101). A pair of silver electrodes was placed interposing the muscle, through which an electrical current pulse delivered from the electronic stimulator (Nihon-Kohden, SEN-7103) was applied. The isometric
tension \((P)\) was measured and recorded on the ink-writing oscillograph simultaneously with its first derivative \((dP/dt)\) using an electronic differentiating circuit (time constant: 66 msec). The muscle was perfused with Ringer's solution containing \(10^{-4}\) g/ml \(d\)-tubocurarine with a rate of about 4.5 ml/min. The Ringer's solution had the following composition (mM): NaCl 111.2, KCl 3.0, CaCl\(_2\) 2.0, glucose 5.5, HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) 4.0 and pH of the solution was adjusted to 7.3 by titrating with \(1\) N NaOH. The temperature of the bathing solution was kept either to 20°C for the standard condition or lowered to 3.5°C or elevated to 38.5°C, according to the experimental purpose, by using a circulator pump device.

Principally the muscle was continuously stimulated with a tetanic pulse of 50 Hz for 1 sec (i.e. 50 stimuli) every 5 min. The twitch pulse was applied if necessary but was avoided as much as possible, since it became clear that twitch contraction exerts some depressive effects on the muscle contractility as shown below.

**RESULTS**

1. **Twitch staircase**

When the muscle bundle preparation was regularly stimulated with short single pulses of 0.5 msec duration, various steady tension levels were attained according to the stimulation frequency. Below 1/300 Hz (i.e. stimulus interval: 5 min) the peak tension was nearly constant. Figure 1A shows that a slight staircase was produced when the stimulation rate was increased from 1/300 Hz (a) to 0.1 Hz (b) and that a more marked staircase was induced at 0.5 Hz (c) or 1 Hz (d). The increase in the peak tension was accompanied by a parallel increase in both the rate of tension rise and fall. The potentiated twitch tension was never sustained at the maximum steady level, instead, it declined gradually (not shown). This indicates that the stage of fatigue was reached as Mashima et al. (1962) and Grabowski et al. (1972) have observed.

Figure 1B, from another muscle, shows the dependency of the staircase on the stimulation frequency. The basal frequency was fixed at 0.1 Hz in all examples. Furthermore, the number of stimuli during staircase was also fixed to 240 shocks in order to avoid an undesirable participation of fatigue, because this parameter clearly depends on the number of stimuli. The records a, b, and c show the effects of 1, 2, and 3 Hz, respectively. It can clearly be seen that the slope of staircase is augmented with increasing the frequency. So far as fatigue is still not operative, at least apparently, it would be reasonable to assume that the staircase phenomenon is produced by some intrinsic potentiating mechanism which lasts for several minutes after it. The decay phase of peak tension after the cessation of repetitive stimuli seems to consist of two components, a very rapid decrease and a slow one. The rapid decrease is, however, not necessarily accompanied by a parallel decrease in \((dP/dt)_{\text{max}}\).
2. Tetanus and fatigue

Figure 2 illustrates the contractile responses to various stimulation frequencies. After the twitch tension had reached a steady level with 0.1 Hz, trains of various frequencies were given for 2 sec with an interval of about 1 min. In the records the stimulation frequency was: a, 1/300; b, 0.1; c, 0.5; and d, 1 Hz. The recording speed was increased, if necessary, in order to show the changes in dP/dt more precisely (dots). In panel B the frequency was increased from the basal rate of 0.1 to 1 Hz (a), 2 Hz (b), and 3 Hz (c) under fixed number of stimuli (240). The records shown in panels A and B were obtained from different frogs.

Next, the dependency of the tetanic contraction on the interval of application of tetanic stimuli was examined. This type of experiment was aimed to clarity.
Fig. 2. Contractile responses induced by successive stimuli with various frequencies for 1 sec. These were produced with an interval of about 1 min and shown with faster recording speed (note the time calibration). The basal frequency of twitch was 0.1 Hz and shown with slower recording speed at the beginning and end of the record. Frequencies are: a, 1; b, 2; c, 5; d, 10; e, 20; and f, 50 Hz. The recording gain for both traces in f was lowered to 1/2 (horizontal bar).

Fig. 3. Effects of interval of tetanus generation on the tetanic contractility. Constant tetanic stimuli with 50 Hz for 1 sec (i.e. 50 stimuli) were applied every 5 min. Tetanic contraction induced with this interval was considered to be a rested state response. The records shown in panels A and B are continuous. Intervals are: a, 10; b, 30; c, 60; and d, 10 sec. Note that a successive application of 5 tetani (250 stimuli) at a and d had no inhibitory effects on the following tetani.
how the fatigue develops during tetanic stimuli. Trains of 50 Hz for 1 sec were applied every 5 min as basal tetanic stimuli. This interval was adopted because it resulted in well-developed tetani for many hours without showing any sign of fatigue. In Fig. 3 a steady tetanic tension was gradually attained between a and b. At a, following a regular tetanus, four other tetani were successively induced, which resulted in an increase in both tetanic tension and its rate of rise, \((dP/dt)_{\text{max}}\). This effect was weakened after the steady tetanic tension had been attained (d). The records b and c show the changes where the interval between tetani was 30 and 60 sec, respectively. At all intervals tested it is seen that the maximum tetanic tension showed a slight dual change, a decreasing phase, and an increasing one, whereas \((dP/dt)_{\text{max}}\) showed only an increasing phase which could be termed as “tetanus staircase.” This contrasts with the results by NYSTRÖM (1968) that there was no difference between the first and the second tetani in the cat leg muscle.

In the experiment shown in Fig. 4 the relationship between number of stimuli
and the tetanic contractility was examined on another muscle in special respect of the interval of tetani, when the interval was further decreased. This muscle did not show any decreasing phase during the tetanic train in both peak tension and \((dP/dt)_{\text{max}}\) within the intervals tested (1.5 to 10 sec). Here, changes were expressed as % value relative to the first tetanus. The data clearly show that fatigue does not take place before 250 stimuli, provided that tetani were induced intermittently. In contrast, fatigue appeared already at the 150th stimulus, when a sustained (7 sec) tetanus was produced (filled circles).

3. Twitch and tetanic fatigue

Although tetanus *per se* scarcely produces a fatigue effect, it is decreased when twitch contraction is simultaneously present. Figure 5A illustrates the effects of trains of twitch with two different frequencies, 0.1 and 1 Hz, on the tetanic contraction. In panel A tetani of 50 Hz and 1 sec were produced every 5 min. From a to d, 25 twitch contractions with 0.1 Hz were interposed between each tetani. This procedure depressed the tetanic tension only slightly. When the frequency

![Diagram](image)

**Fig. 5.** Effects of twitch trains on the tetanic contractility. In panel A tetani of 50 Hz for 1 sec were regularly produced every 5 min. Trains with 25 twitches of 0.1 Hz were interposed between two tetani as indicated with a to d. The frequency of twitch was further increased to 1 Hz (250 stimuli) at e to g. Note a marked inhibition of the tetanic contraction when the twitch train is showing fatigue (g). Panel B is continuous to panel A and shows the recovery during which tetanus staircase was examined by applying five successive tetani with an interval of 10 sec.
of twitch was increased to 1 Hz with 250 stimuli, the tetanic tension was markedly depressed as seen in e, f, and g. It is of interest that in e train of twitch produced a positive staircase, nevertheless, this resulted in a depression of the following tetanus. The inhibition of tetanus was restored very gradually within the following 60 min, during which “tetanus staircase” was induced every 10 min (Panel B, continuous record of A). It is seen that the staircase is more marked when tetanus is inhibited and vice versa. These findings would suggest that the factors for tetanus inhibition and potentiation are of the separate origin and that the tetanus can be hardly suffered by fatigue.

4. Effects of twitch on following tetani

As shown previously, the twitch staircase does not necessarily result in an increase in the following tetanus. In example shown in Fig. 6 tetani of 50 Hz and 1 sec were produced every 5 min. Between the 4th and 5th tetani a train of twitch, 12 stimuli with 0.1 Hz (a) and 120 stimuli with 1 Hz (b), was applied. The post-tetanic potentiation almost disappeared during twitch train with 0.1 Hz and a marked positive staircase now developed on increasing the stimulation frequency to 1 Hz. This procedure does not seem to affect the immediately following tetanus, at least apparently. However, the tetani after it were inhibited and gradually returned to the original level, which consequently indicates the existence of rapidly decaying potentiation ($P_1$) in the first tetanus after the twitch train. By contrast, $(dP/dt)_{\text{max}}$ shows a long-lasting potentiation ($P_2$) together with $P_1$. It is of interest that the change in the rate of tension fall showed a mirror image pattern of the tension change. Thus, the inhibitory effect ($I$) seems to exist simultaneously with

\[ \begin{align*}
\text{a} & \quad \text{b} \\
\end{align*} \]

5 mN

\[ \begin{align*}
\text{5 mN/s} & \\
\end{align*} \]

5 min  5 sec

**Fig. 6.** After-effects of a twitch train consisted of 12 contractions with 0.1 Hz (a) and 120 contractions with 1 Hz (b). Fifty Hz-1 sec tetani were evoked every 5 min in order to establish the basal condition. The last twitch was elicited exactly 15 sec before the following tetanus. During the twitch train the recording gain was increased twice (horizontal bar). In this example, three kinds of the after-effect of twitch train on the following tetani are recognizable. The existence of $P_1$ is clearly seen in the change in the first tetanus to the second one; the latter consequently indicates an inhibitory effect ($I$). The long-term potentiation ($P_2$) is evident from the changes in $(dP/dt)_{\text{max}}$ together with the short-term $P_1$.  

*Japanese Journal of Physiology*
the potentiating parameters, $P_1$ and $P_2$. Although the mode of appearance of the after-effects of twitch train was not consistent and varied from muscle to muscle, the example shown in Fig. 6 is a typical one, because it clearly shows all three components of after-effect, an immediate potentiation ($P_1$), a long-lasting potentiation ($P_2$), and an inhibitory effect ($I$) which decreases slowly. Indeed, a marked inhibition in the first tetanus was frequently observed after twitch train (Figs. 10 and 12). An obvious potentiation was occasionally observed also in the peak tension (Fig. 11).

5. Effects of twitch frequency on the following tetani

Figure 7 shows the effects of twitch trains with various frequencies under fixed number of beats on the following series of tetani. At a, b, and c twitch train with

Fig. 7. Effects of twitch frequency on the staircase phenomenon and the following tetanic contractility. The twitch frequency was 1 Hz for a, 2 Hz for b, and 3 Hz for c. The number of twitch was fixed to 120 in all cases. The basal interval of tetani was 5 min as in other figures. The record of panel A is continuous to panel B.
1 Hz (120 stimuli), 2 Hz (120 stimuli), and 3 Hz (120 stimuli) were respectively given and stopped 15 sec before the following tetanus. As shown previously (Fig. 1B), the twitch staircase was increased with the frequency. The immediate potentiation seen in the first tetanus after twitch train was clearly increased with the frequency, i.e. the grade of staircase. Interestingly, the inhibition seen in the second tetanus was strengthened as the staircase was augmented. It could thus be concluded that the potentiating effect and the inhibitory one are independent of each other but are operative simultaneously.
While higher frequencies of twitch seemed to stimulate all of these three effects ($P_1$, $P_2$, and $I$) on the following tetani, increasing of number of twitch decreased the potentiating effect and augmented the inhibitory one. This is shown in Fig. 8 in which the records of panels A, B, and C were obtained from the same muscle preparation. A shows the control response and a train of 120 stimuli with 1 Hz was given at a. In B the number of twitch was increased to 240 without changing the number of tetani.

Fig. 9. Influence of low temperature on the after-effects of twitch train. Twitch trains consisted of $0.1 \text{ Hz} \times 12$ plus $1 \text{ Hz} \times 120$ were applied between two tetani as shown by a downward arrow in each panels. Panel A shows the record at $20^\circ C$, the standard condition. Panels B and C show the effects of 12 and $7^\circ C$, respectively. Note that the twitch/tetanus ratio increased with lowering of the bath temperature.
the frequency (b). The inhibitory effect on the peak tetanic force was now augmented and the potentiating effect seen in \((dP/dt)_{\text{max}}\) was slightly decreased. Panel C shows the effect of a twitch train of 240 stimuli with 2 Hz (c). The inhibitory effect on the tetanic tension was strengthened as compared with the control response (A), whereas a marked potentiation is seen in spite of the former change.

6. Effects of temperature

Variety of appearance in the pattern of tetani after high-frequency twitches suggests the possibility of a participation of some metabolic factors. Therefore, the temperature of the muscle bath was either lowered or elevated by using the thermo-regulating pump without changing the flow rate of the perfusing solution. Figure 9 illustrates the effects of twitch train (0.1 Hz \(\times\) 12 plus 1 Hz \(\times\) 120) on the following tetani which were measured at three different temperatures (20, 12, and 7°C) on the same muscle. At 20°C (A) all three components of the after-effect of twitch train are recognizable. At 12°C (B) both the twitch tension and tetanic tension were considerably increased and the twitch/tetanus ratio was markedly increased as compared with A. Moreover, the short-term potentiation was de-

![Image](image_url)

Fig. 10. Influence of high temperature on the after-effects of twitch train. Effects of elevating the bath temperature up to 28°C were examined with the similar design of experimental procedure as in Fig. 9. Panel A shows the data at 20°C and panel B shows those at 28°C. The long horizontal bar in panel B indicates that the recording gain was increased twice. In contrast with Fig. 9, the twitch/tetanus ratio was decreased at high temperature. Note the appearance of the after-potentiation in \((dP/dt)_{\text{max}}\) at 28°C.
creased, whereas the inhibitory effect (I) was augmented. The long-term potentiation seen in the dP/dt record in A can hardly be seen, although the ascending staircase is still present. These changes were further strengthened at 7°C (C). The twitch/tetanus ratio is now more than one third and the ascending staircase almost disappeared. Thus, lowering the temperature resulted in an inhibition of the staircase simultaneously with a marked alteration in the after-effects of twitch train. Elevating the bath temperature brought about a reverse phenomenon. Figure 10 compares the results obtained at 20 and 28°C. Although this muscle showed a relatively large inhibition and seemed to lack the long-term potentiation at 20°C (A), elevating the temperature resulted in a decrease in the inhibitory effect together with an appearance of the potentiation phase (B).

The results described above are not exclusive for bullfrog's muscle. The data shown in Fig. 11 were obtained from the toe muscle preparation of *Rana japonica*. In this preparation the long-term after-potentiation was most dominant among three after-effects at 20°C (open circles). When the muscle was cooled down to 4°C and equilibrated for about 45 min at this temperature before application of
twitch train, the tetanic contractility was considerably depressed. Under this condition the muscle failed to show any ascending staircase. In addition, as shown in the figure, the long-term potentiation completely disappeared and, instead, a marked inhibition and a very slow recovery were observed (filled circles). It should be noted that the short-term potentiation is still discernible even at such a low temperature (compare the first tetanus after twitch train with the second one).

7. Effects of caffeine

Figure 12 shows the effects of low concentration (0.5 mM) of caffeine on the staircase phenomenon and its after-effects. In the record A a twitch train with 1 Hz, which caused a slight ascending staircase, resulted in a marked inhibition together with a long-term potentiation in the following tetani. The rapidly decaying potentiation was not clear in this example. When 0.5 mM caffeine was applied, both the peak tetanic tension and (dP/dt)\text{max} were increased. At the same time the twitch/tetanus ratio was increased to about one third within 30 min after the start of caffeine perfusion. The twitch staircase seen in the control was completely suppressed and the inhibition in the following tetani was strengthened,

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Fig. 12. Influence of low concentration (0.5 mM) of caffeine on the after-effects of twitch train. Panel A shows a control response while panel B shows the response in the presence of 0.5 mM caffeine. Ringer’s solution containing caffeine was perfused for 30 min before the application of twitch train and throughout the record in panel B. Data shown in panels A and B were obtained from the same muscle preparation.

*Japanese Journal of Physiology*
though the long-term potentiation can still be identified in the tension record. Thus, the effects of low concentration of caffeine on the twitch/tetanus ratio, twitch staircase and some after-effects resemble those of low temperature.

Next, the effects of twitch train on caffeine contracture were examined. In Fig. 13, caffeine in 5 mM was perfused for 2 min after the steady tetanic contractility had been established. This concentration of caffeine induced a fairly large contracture which roughly corresponded to the control tetanic tension (a). Reaplication of caffeine 5 min after the first contracture induced, however, far smaller contracture (b) indicating either a decrease in the capacity of activator calcium to be released or some desensitization of the intracellular sites for which caffeine acts. The twitch train of 0.1 Hz × 12 plus 1 Hz × 120, which was applied between the third (c) and the fourth (d) contractures, seems to hardly affect the height of the latter one, although the ability to produce contracture per se gradually declined with time. Figure 13B further shows that the tetanic contractility could be returned to the original level.

DISCUSSION

The present results revealed that twitch trains with high frequency (above
H. KAWATA

1 Hz) leave at least three different effects on the following tetani. These were a short-term potentiation ($P_1$) which decayed rapidly after cessation of twitch train, a long-term potentiation ($P_2$) which lasted for a sufficiently long time, say 30 min under the present experimental conditions, and an inhibitory effect which showed the most striking feature. Figure 14 shows a scheme for the phenomenon and summarizes the after-effects of twitch train on the tetanic contractility. All of these after-effects were caused when the twitch train was showing a distinct ascending staircase, but never when twitch train passed into a declining phase, namely the fatigue. Moreover, the effects were clearly dependent on both the frequency and number of twitch (Figs. 7 and 8). In the present study it was confirmed that both twitch and tetanus did not result in fatigue, provided that the number of stimuli does not exceed 250 (Figs. 1 and 4).

However, there was an essential difference in the after-effects of high-frequency stimuli between twitch and tetanus. The twitch train results in a complicated effect on the following tetani, while the tetanus leaves far lesser effect on them. Among the various effects of twitch train the inhibitory effect was the most dominant and was consistently observed. This would mean that the process of inhibition had already started with the onset of twitch train, regardless of the presence

Fig. 14. A scheme for the after-effects of twitch train. The change in either peak tetanic tension or $(dP/dt)_{\text{max}}$ was resolved into three different effects, namely a rapidly decaying potentiation ($P_1$), a slowly decaying potentiation ($P_2$), and an inhibition ($I$), and their hypothetical temporal changes were plotted against time. The timing of successive stimuli for twitch generation is indicated with the downward arrow.
or absence of any sign of fatigue during the staircase phenomenon itself. When
the ascending staircase reached its peak or passed into the declining phase, that
is a virtual fatigue, a marked after-inhibition was usually observed and a considera-
bly long time was required for the recovery. Recently, KRARUP (1981a) investi-
gated the after-effects of staircase and tetanus on the twitch contractions of rat’s
extensor digitorum longus muscles and found that high-frequency twitch or tetanus
could induce different types of after-effect. The twitch train with positive staircase
causd a potentiating effect simultaneously with an inhibitory effect and both
effects decayed exponentially with time. On the contrary, the decay of post-tetanic
effect consisted of two potentiating parameters with different time constants. Al-
though the experimental procedures considerably differed between the above and
the present studies, the results seem to coincide strikingly. Both investigations
clearly revealed that the high-frequency twitch evokes a diminution of contrac-
tility together with a potentiation, while tetanus fails to leave any inhibitory effect
(fatigue). Although the results shown in Fig. 3 suggest that there are both positive
and negative after-effects when 50 Hz tetani were induced with various intervals,
the negative effect almost disappeared when the total number of stimuli was con-
fined within 250 and the interval between tetani was decreased below 10 sec (Fig. 4).
Moreover, such a successive train of five tetani (250 stimuli) did not leave any in-
hibitory effect on the following tetani in the steady state (Fig. 3).

The difference in the time course of the phenomena between Krarup and the
present author could be attributed to that in either the experimental animal or
temperature or both. The inhibitory effect of rat’s EDL muscle decays relatively
rapidly, whereas that of frog’s muscle has a considerably large time constant.
The present results revealed that the potentiating effect can more easily be induced
at higher temperature but masked by the marked inhibitory effect at lower temper-
ature, suggesting that both effects are in some way linked to the metabolism regu-
lating the contractility. While the long-term potentiation was almost completely
abolished at low temperature, the short-term potentiation seemed to still exist at
such low temperature as 4°C. KRARUP (1981b) also found that effects are tem-
perature dependent.

MASHIMA et al. (1962) analyzed the staircase and fatigue on the frog twitch
muscle and concluded that the staircase phenomenon of twitch is not dependent
of the action potential but is related to excitation-contraction coupling mechanism.
KRARUP (1981a) also reported that the staircase of rat’s EDL muscle was not ac-
accompanied by any critical changes in the action potential measured extracellularly.
Thus, both in amphibian and mammalian muscles, the staircase phenomenon,
so far as it does not undergo fatigue, could be considered to be independent of the
membrane excitation. Although the action potential was not measured in the
present investigation, the possibility of participation of changes in the action po-
tential for the observed phenomena, especially for the after-inhibition, may prob-
ably be low. The data shown in Fig. 15 may support this view. In the figure

Vol. 33, No. 3, 1983
it is seen that the pattern of changes in the tetanic response, especially in \( \text{dP/dt} \), after twitch train was fairly different from that when changing the tetanic frequency. Furthermore, throughout the present study, the number of twitch train was confined within 250, which is comparable to the tetanic stimuli that hardly cause any fatigue during as well as after them. If the after-inhibition were due to fatigue or omission of the action potential, this should be more pronounced after tetanus than after twitch train, since the action potentials during a tetanus tend to decline (see Fig. 9 of MILEDi et al., 1982).

The present observation that twitch train induced more readily fatigue than tetanus would suggest that the process of relaxation in the twitch generating cycle is somehow related to the after-inhibition. The classical observation by RITCHIE and WILKIE (1955) that twitch potentiation during the staircase or post-tetanus is accompanied by a prolongation of active state could be interpreted, in terms of the

*Japanese Journal of Physiology*
modern concept, as that the time required for release and sequestration of activator calcium is prolonged under these conditions. The present results on the caffeine contracture ruled out the possibility of any participation of the calcium releasing process at the level of sarcoplasmic reticulum in the after-effect of twitch train, since this drug is known to facilitate the release of calcium from the sarcoplasmic reticulum (Ogawa, 1970; Sakai and Yoshioka, 1973; Endo, 1977). Although caffeine contracture itself declined with time, when induced repeatedly, this by no means indicates any deterioration of the muscle, since the full-sized tetanic contractility could be restored by washout of the drug. However, Yoshioka et al. (1981) recently reported that caffeine concentration above 4 mM could induce an irreversible damage on the rapid-cooling contracture as well as the ultrastructure of sarcoplasmic reticulum of the frog sartorius and toe muscles.

On the other hand, it may be possible that the changes in the sequestrating process of activator calcium is somehow related to the after-inhibition. The inhibition could result from that of the energy-consuming process of the sequestration of calcium into the sarcoplasmic reticulum. The effect may not interfere with the progressing positive staircase per se, within a certain limit of number of stimuli, but could well influence the following tetani by decreasing the supply of activator calcium to the releasing sites. Applying calcium-sensitive bioluminescent aequorin, Blanks et al. (1978) measured the calcium transients in the single twitch muscle fiber of frog and observed a descending staircase of the peak-light intensity when repetitive stimulation was applied. This was interpreted as reflecting a gradual decline of sequestration of calcium by the sarcoplasmic reticulum. The calcium transients during repetitive stimulation and tetanus were also investigated by Milelli et al. (1982) in frog's twitch muscle by using arsenzao III. In agreement with Blanks et al. (1978) they observed a negative staircase of the amplitude of light response together with slowing of its decay (summation). In this respect, the recent observation by Homsher and Kean (1977) that the activation heat per stimulus declined with decrease in the stimulus interval would be interesting. The present finding that the effects of caffeine in low concentration were similar to those of low temperature would suggest the possibility of an increase in the sarcoplasmic calcium level which consequently affects the intracellular calcium turnover process.

The impairment of the contractile protein system after twitch train is unlikely because Grabowski et al. (1972) showed that maximum contracture tension could be produced even in the fatigued muscle, when muscle membrane is sufficiently depolarized by potassium solution which contained caffeine. The mechanism for the after-potentiation is not clear. The long-term after-potentiation could not be a mere reflection of the ascending staircase of twitch, because its decay time constant differed considerably from that of the recovery from the staircase (compare Fig. 11 with Fig. 1B). Rather, the short-term potentiation may reflect this. Although one could assume that the origins of the after-potentiation and after-inhibition should not be identical and that both may functionally and structurally be separated,
further experiments are required for identifying them.

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