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THE AFTER-EFFECTS OF REPETITIVE STIMULATION ON THE ISOMETRIC TWITCH CONTRACTION OF RAT FAST SKELETAL MUSCLE

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SUMMARY

1. The peak tension and time course of isometric twitch contractions of rat extensor digitorum longus muscle *in vitro* (35° C) have been measured at various stages of potentiation following repetitive stimulation at 20 c/s and 300 c/s.

2. Potentiation of the peak twitch tension increases with an increase in the number of repetitive stimuli up to a maximal level of about 1·9 times the control value. The relation between potentiation and numbers of stimuli is dependent on the frequency of stimulation.

3. Potentiation of peak twitch tension is maximal shortly after the end of repetitive stimulation and subsequently decays exponentially at a rate which is dependent on the number of stimuli in the train and the frequency of stimulation.

4. Short trains of stimuli bring about nearly maximal potentiation with little or no change in contraction time and a small decrease in half-relaxation time.

5. Long trains of stimuli increase the contraction time, the half-relaxation time and the twitch duration in addition to potentiating the peak tension. The changes in twitch time course are dependent on the number of repetitive stimuli and the frequency of stimulation.

6. The results are discussed in relation to possible mechanisms of post-tetanic potentiation and the degree of activation of mammalian and amphibian muscle fibres.

INTRODUCTION

Repetitive stimulation of mammalian fast skeletal muscle causes a transitory increase in the peak tension of the isometric twitch response (Lee, 1907; Brown & Euler, 1938; Euler & Swank, 1940; Bernhard, Euler & Skoglund, 1941; Standaert, 1964; Buller & Lewis, 1965). In the present work the influence of repetitive stimulation on peak tension and time

course of the isometric twitch of rat extensor digitorum longus muscles has been determined for various stages of potentiation. The abbreviation PTP is used throughout to refer to post-tetanic potentiation of peak twitch tension following a train of stimuli at 300 c/s and to post-train potentiation after stimulation at 20 c/s.

METHODS

The muscles used were extensor digitorum longus (EDL) muscles from hind limbs of 4-week old female rats (Wistar) which had an average body weight of 67·5 g ($\pm$ 2·95 g S.D.).

Dissection. EDL muscles were obtained from rats anaesthetized with pentabarbitone sodium (50 mg/kg body wt.). After cutting the blood vessels to EDL the muscle was transferred immediately to a bath of oxygenated Ringer solution at room temperature.

Tension recording. The muscle was held with its long axis vertical, with the proximal tendon tied directly to the frame below and the distal tendon tied directly to a short steel wire connexion which linked it with the tension transducer above the bath. The muscle was immersed in about 100 ml. of fluid (NaCl, 137 mM; KCl, 5 mM; $CaCl_2$, 2 mM; $MgCl_2$, 1 mM; NaH_2PO_4, 1 mM; $NaHCO_3$, 2 g/l.; glucose, 2 g/l.), which was bubbled continuously with a mixture of 95 % O_2 and 5 % CO_2, and replaced at the rate of a few millilitres per minute. The compliance of the transducer (Statham G1-80-350) plus the steel wire connexion was $4{\cdot}5 \times 10^{-5}$ cm/g and the natural frequency of vibration was 2 kc/s. The transducer was used in conjunction with a carrier amplifier (Tektronix Q) the output of which was displayed on an oscilloscope (Tektronix 565) and recorded photographically (Grass C4 camera). The oscilloscope amplifier (Tektronix 3A3 or 72) was used in the chopped mode; one of the channels carried the tension/time record and the other carried time marks from a pulse generator triggered by a time-mark generator (Tektronix 180A). All recordings were made with the muscle at the optimal length for twitch contractions at 20° C. The temperature of the fluid surrounding the muscle was maintained between 34·6 and 35·5° C in all experiments on PTP. The average temperature at the beginning of each series of measurements on PTP was 35·02° C ($\pm$ 0·2° C S.D., n = 35).

Stimulation. Neuromuscular transmission was blocked by adding tubocurarine chloride (2×10^{-5} g/ml.) to the Ringer fluid. The muscles were stimulated directly in a transverse electrical field applied to the bath fluid through 'massive' platinum electrodes (Gutmann & Sandow, 1965) set about 1 cm apart on either side of the muscle and extending beyond its ends. The stimulus amplitudes given below are the voltages applied to the electrodes.

Figure 1 shows records and graphs which describe the relation between mechanical response and stimulus strength for single stimuli applied to a curarized EDL muscle at 25° C. The curves relating stimulus duration and peak tension of the mechanical response are similar for all voltage gradients between 5 and 25 V but the threshold duration for the response is higher for low voltages. The curves show that increase in stimulus duration increases the peak tension up to a plateau followed by a decrease and then by a further increase in the maximum response. The plateau tension represents the maximum twitch response of the muscle to a single stimulus and this is only slightly greater for massive stimulation than for either indirect stimulation via the nerve *in situ* or direct stimulation at a point midway along the muscle fibres *in vitro*. The depression of the twitch response, shown for most voltage gradients with stimuli of several milliseconds duration, is not associated with a change in the time course of the twitch. This depression has been observed not only with massive stimulation but also with direct stimulation at a point and probably results from stimulus currents preventing the generation or conduction of the action potential in some muscle fibres. The large twitch-like responses to stimuli of long duration (10–20 msec) show an inflexion in the tension/time record shortly after the end of the stimulus (Fig. 1A, arrow) and there is a marked increase in both the contraction time and the half-relaxation time. It

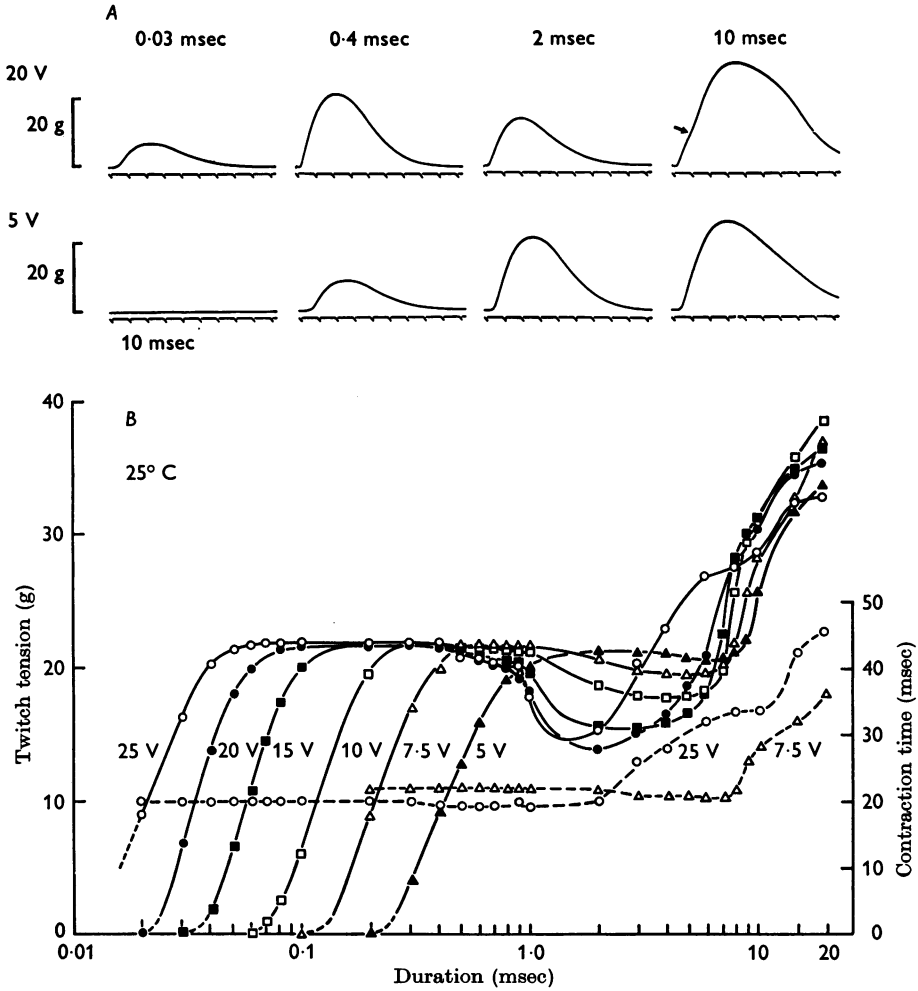

Fig. 1. The records in *A* are for isometric contractions of an EDL muscle in response to 'massive' direct stimulation *in vitro*. The amplitude of the stimulus was 20 V for the upper series and 5 V for the lower series. The stimulus durations shown above the records are for both series. The arrow over the large twitch-like response indicates an inflexion which occurs shortly after the end of a 10 msec stimulus. The records in *A* form part of the series in *B* which show the relations between peak tension (continuous curves) and stimulus duration (abscissa, log scale) for stimulus amplitudes of 25 V ○, 20 V ●, 15 V ■, 10 V □, 7·5 V △, and 5 V ▲. The contraction times for twitch responses to 25 V and 7·5 V are shown by the interrupted curves. The amplitude of the stimulus is given as the voltage applied to platinum electrodes placed 1 cm apart. Muscle weight = 22 mg. Average fibre length = 9·5 mm. Temperature: 24·6° C to 25·3° C.

seems likely that in these large responses some of the muscle fibres are excited twice, once during the stimulus and again at the end of the stimulus.

Figure 2 shows that both the peak twitch tension, indicated by the plateau values, and the threshold duration for excitation are increased by a decrease in temperature in the range 20–35° C.

The stimulus used throughout this work was 15 V for 0·2 msec or 0·3 msec. The heating effect of the current during one stimulus has been estimated to raise the temperature of the fluid around the muscle by less than 4×10^{-4}° C.

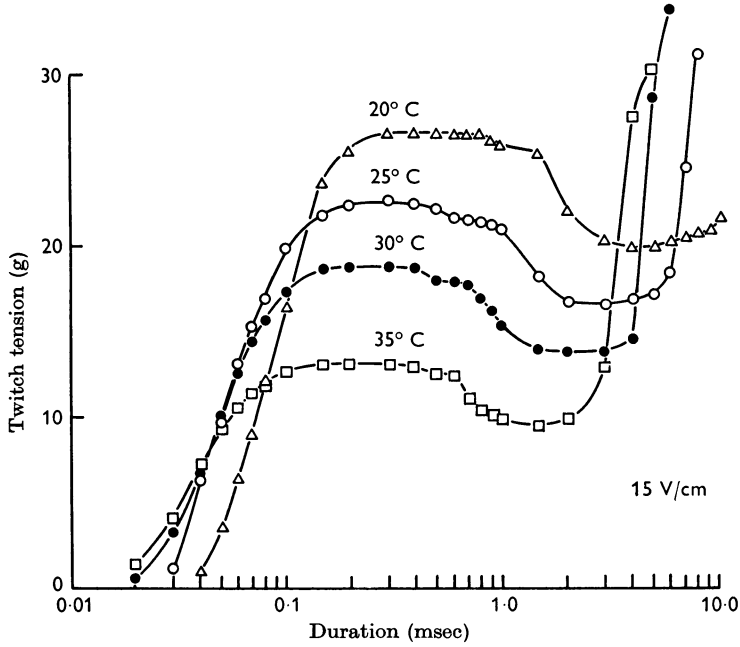

Fig. 2. The relations between peak tension (ordinate) of isometric contractions and duration (abscissa, log scale) of direct 'massive' stimuli (15 V) at different temperatures. Muscle weight = 25 mg. Average muscle fibre length = 10·5 mm.

Procedure for determining PTP. The optimal length for twitch contractions was determined for each muscle at room temperature. The temperature of the bath fluid was raised subsequently and maintained at 35 °C during measurements on PTP. In every series of measurements control twitches were elicited at 0·05 c/s followed by the response to a train of stimuli at either 20 c/s or 300 c/s timed to end 30 sec after the last control twitch, followed by the post-train potentiated responses to single stimuli at 0·05 c/s beginning 10 sec after the end of repetitive stimulation. The influence of frequency of testing on the disappearance of PTP was not studied extensively but there seemed to be no difference between results obtained for testing at 0·1 c/s and 0·05 c/s. Usually only one or two series were obtained for each muscle before the peak tension of the control, pre-train, twitch had decreased to below 90 % of the original value (see below), but up to four series were obtained for small numbers of repetitive stimuli.

Condition of muscles in vitro. Isometric contractions have been recorded for several preparations in which each muscle was stimulated maximally first by indirect stimulation of the nerve *in situ*, then by direct stimulation with platinum wire electrodes placed midway along

the muscle fibres *in situ* and finally by direct stimulation through massive platinum electrodes *in vitro*; for each muscle the maximum isometric twitch tension was almost exactly the same for all methods of stimulation. Consequently all, or nearly all the muscle fibres of EDL remained excitable when the muscle was excised. The length of time these muscles remained fully excitable at 35 °C *in vitro* depended on the rate of stimulation and the activity of the muscle. For example, the twitch response of one muscle did not change during 6 hr of continuous stimulation once every 10 min and the maximum isometric tetanic tension of another muscle showed no decline over a period of 1 hr in response to stimulation at 300 c/s for 1 sec every 10 min. On the other hand there is usually a small reduction (*ca.* 5 %) in the peak twitch tension after the disappearance of PTP following a maximum tetanic contraction to stimulation at 300 c/s for 1 sec. All the results on PTP described below were obtained from those series of measurements in which the peak tension of the control, pre-train, twitch exceeded 90 % of the original peak tension of the initial control twitch.

Definitions. Optimal length (L_0) is the length of the muscle at which the peak twitch tension, in excess of initial tension, is maximal at 20 °C. The optimal length is the same for twitch and tetanic contractions at 20 °C.

Maximum isometric twitch tension (P_t) at 35 °C is the peak twitch tension in excess of initial tension at L_0.

Maximum isometric tetanic tension (P_0) at 35 °C is the maximum tension in excess of initial tension at L_0 during stimulation at 300 c/s.

Contraction time (T_c) at 35 °C is the time from onset of contraction to the peak of the isometric twitch at L_0.

Half-relaxation time ($T_{\frac{1}{2}R}$) at 35 °C is the time for decay of tension from the peak of the isometric twitch to one half of the peak tension at L_0.

Cross-sectional area of muscle in square centimetres was estimated by dividing the weight of the muscle in grams (M) by the average fibre length at L_0 in centimetres (L).

The degree of potentiation is the ratio of the peak tension of a post-train potentiated twitch (P_t*) to the peak tension of the pre-train twitch (P_t), i.e. P_t^*/P_t.

RESULTS

Some of the properties of EDL muscles *in vitro* are listed in Table 1. The results do not differ greatly from those described previously for juvenile rat EDL muscles *in situ* (Close, 1964). The values listed for the twitch are for responses before repetitive stimulation in the first series of measurements on PTP in each muscle. The ratios of values for pre-train twitches in second and subsequent series to the values obtained for the initial series were 0·95 ($\pm$ 0·03 S.D., n = 18) for maximum twitch tension, 0·986 ($\pm$ 0·034 S.D., n = 18) for contraction times and 0·96 ($\pm$ 0·06 S.D., n = 18) for half-relaxation times. These small changes in the twitch were not due to differences in temperature; the average temperature was 35·07° C ($\pm$ 0·2° C, S.D., n = 17) for the initial series and 34·97° C ($\pm$ 0·2° C, n = 18) for subsequent series.

An example of PTP of the twitch contraction of EDL is shown in Fig. 3; the records are for the control pre-train twitch contraction (A) and the potentiated response (B) recorded 10 sec after the end of a train of 300 stimuli at 300 c/s. The time course of the two contractions is almost

the same except that relaxation is a little more rapid in the potentiated twitch.

Figure 4 *A* and *B* show a few examples of the time course of decline of potentiation following repetitive stimulation at 20 c/s and 300 c/s and

TABLE 1. Properties of EDL muscles from 4-week old rats. The values are the mean values ± the standard deviation for a number (n) of muscles. All measurements were made at 35° C.

Muscle weight (mg)	26·2 ± 2·4, n = 15
Muscle fibre length (mm)	9·5 ± 0·4, n = 16
P_t(g)	15·25 ± 2·45, n = 17
P_t/P_0	0·21 ± 0·03, n = 17
P_0(g)	73 ± 8·2, n = 17
$P_0.L/M$ (kg/cm²)	2·57 ± 0·286, n = 15
T_c (msec)	9·8 ± 0·7, n = 17
T_{tR} (msec)	9·4 ± 0·6, n = 17

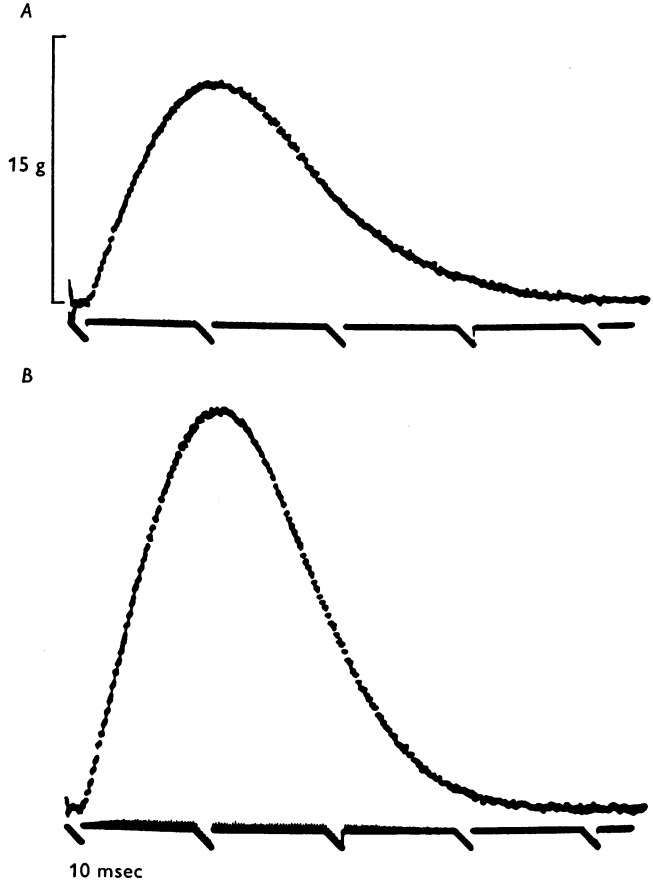

Fig. 3. Isometric responses of an EDL muscle to a single stimulus, *A* before and *B* 10 sec after a train of 300 stimuli at 300 c/s. Temperature: 35° C.

different numbers of stimuli; the degree of potentiation is shown as the ratio of the peak tension of the post-train twitch to the peak tension of the pre-train twitch. The decay of PTP is approximately exponential though in some instances (e.g. Fig. 4 *B*), particularly following several hundred stimuli at 300 c/s, the experimentally determined values for potentiation

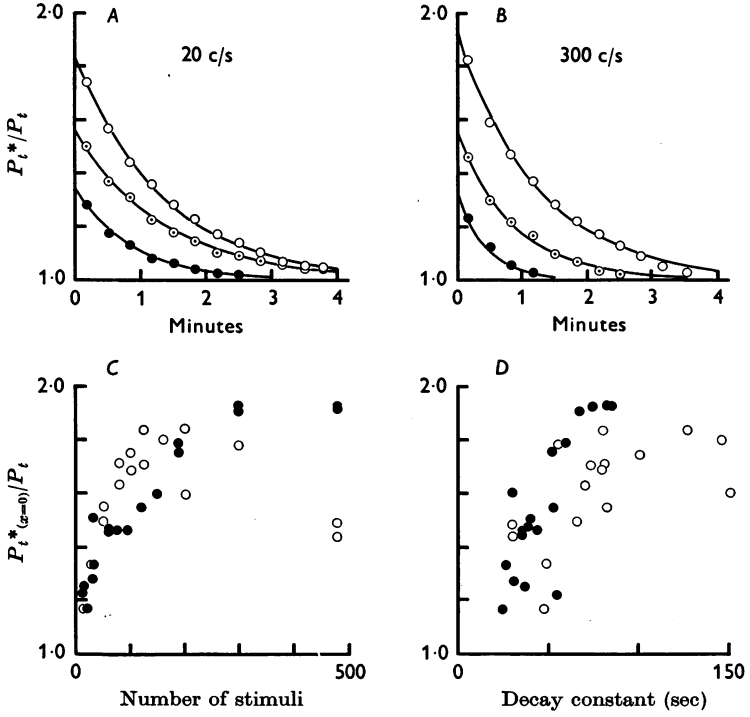

Fig. 4. The time course of decay of PTP after 126 (○), 50 (⊙) and 26 (●) stimuli at 20 c/s (*A*) and 300 (○), 120 (⊙) and 30 (●) stimuli at 300 c/s (*B*). In *A* and *B* the degree of potentiation P_t^*/P_t of post-train twitches (ordinates) is plotted against the time after the end of repetitive stimulation (abscissae). *C* shows the relation between maximum degree of potentiation at the end of repetitive stimulation $P_t^*{}_{(x=0)}/P_t$ estimated as described in the text, and the number of repetitive stimuli at 20 c/s (○) and 300 c/s (●). *D* shows the relation between the estimated maximum potentiation at the end of repetitive stimulation and the time constant (τ in seconds) for decline of PTP after stimulation at 20 c/s (○) and 300 c/s (●). The results shown in *C* and *D* are for seventeen EDL muscles the properties of which are summarized in Table 1. Temperature: 35 °C.

fall below the fitted curves in the region where potentiation is less than 1·1 P_t due to decline in P_t after the disappearance of PTP (see above). It has been assumed that potentiation is maximal soon after the end of repetitive stimulation at 35° C and a value for maximum potentiation has been estimated for each series of measurements by extrapolating the

exponential decay curve to zero time. The degree of potentiation of the twitch at any time later than 10 sec after the end of repetitive stimulation is given by

$$\frac{P_t^{*}{}_{(x)}}{P_t} = \left(\frac{P_t^{*}{}_{(x=0)}}{P_t} - 1\right) e^{-x/\tau} + 1,$$

where P_t is the peak tension of the control twitch, $P_t^{*}{}_{(x=0)}$ is the estimated peak tension of the potentiated twitch at zero time, $P_t^{*}{}_{(x)}$ is the peak tension in the potentiated twitch at any time x sec after the end of repetitive stimulation and τ is the time constant in seconds for the decline of PTP.

Figure 4 *C* shows the relation between the estimated maximum degree of potentiation ($P_t^{*}{}_{(x=0)}/P_t$) and the number of stimuli (S) for thirty-five series of measurements following repetitive stimulation at either 20 c/s (○) or 300 c/s (●). The general form of the relation is described approximately by

$$\frac{P_t^{*}{}_{(x=0)}}{P_t} = 0{\cdot}93(1 - e^{-0.012S}) + 1$$

for all points except those for 480 stimuli at 20 c/s. Repetitive stimulation at 20 c/s is a little more effective in potentiating the twitch than stimulation at 300 c/s for trains of less than 150 to 200 stimuli and more accurate descriptions of the relation between maximum potentiation and numbers of stimuli are given by

$$\frac{P_t^{*}{}_{(x=0)}}{P_t} = 0{\cdot}069\sqrt{S} + 1$$

for 20 c/s and 10 to 150 stimuli and

$$\frac{P_t^{*}{}_{(x=0)}}{P_t} = 0{\cdot}055\sqrt{S} + 1$$

for 300 c/s and 10 to 300 stimuli. For larger numbers of stimuli the maximum degree of potentiation decreased following stimulation at 20 c/s whereas it continued to increase for stimulation at 300 c/s up to a maximum of about 1·93 for trains of 300–500 stimuli.

Figure 4 *D* shows that the time constant (τ) for decay of PTP increased with increase in the degree of maximum potentiation $P_t^{*}{}_{(x=0)}$. Furthermore for a given maximum degree of potentiation the rate of decline of PTP was usually less following stimulation at 20 c/s than following stimulation at 300 c/s. The relation between τ in seconds and the number of stimuli (S) is approximately linear and is given by

$$\tau = 0{\cdot}55\,S + 35$$

for 10–200 stimuli at 20 c/s and

$$\tau = 0{\cdot}12\,S + 32$$

for 10–500 stimuli at 300 c/s. The decreased degree of maximum potentiation following 480 stimuli at 20 c/s (Fig. 4 *C*) was accompanied by a decrease in τ to about 30 sec (Fig. 4 *D*).

Figure 5 shows the relations between the number of repetitive stimuli and both the degree of potentiation and the time course of isometric

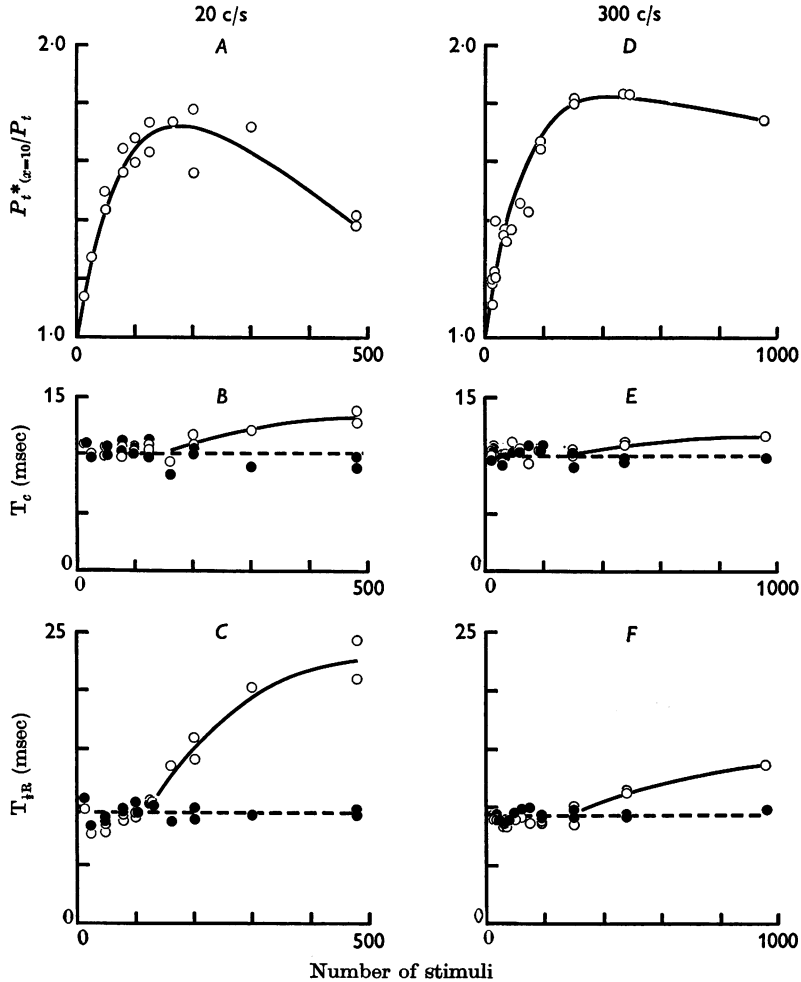

Fig. 5. The relations between number of repetitive stimuli (abscissae) and the degree of potentiation $P_t^*{}_{(x=10)}/P_t$ (*A*, *D*), contraction time (*B*, *E*), and half-relaxation time (*C*, *F*) on ordinates for twitches recorded 10 sec after the end of repetitive stimulation at 20 c/s (*A*, *B*, *C*) and 300 c/s (*D*, *E*, *F*). The contraction times and half-relaxation times of both the pre-train (●) and post-train (○) twitches have been plotted at the same position along the abscissa in *B*, *C*, *E* and *F* and the mean pre-train value is shown by the interrupted line. The results were obtained from seventeen EDL muscles described in Table 1.

twitches recorded 10 sec after the end of repetitive stimulation at 20 c/s and 300 c/s. The peak tension of the first post-train twitch in each series has been plotted as a multiple of the pre-train value (i.e. $P_t^*{}_{(x=10)}/P_t$); mean $P_t.L/M$ was 0·55 kg/cm² for muscles stimulated at 20 c/s (Fig. 5 *A*) and 0·58 kg/cm² for muscles stimulated at 300 c/s. T_c and $T_{\frac{1}{2}R}$ for both the pre-train twitch (filled circles) and the post-train twitch (unfilled circles) are plotted for comparison in Fig. 5 *B*, *C*, *E*, *F*, and in each of these graphs the average value for the pre-train twitch is indicated by the interrupted

TABLE 2. Contraction times and half-relaxation times before, and 10 sec after, the end of short trains of stimuli which caused PTP with little or no change in time course of post-train twitches. The values given are mean values ± the standard deviation for a number (n) of series. The probability values (P) were obtained from t-values calculated from the ratio of the mean difference between pre-train and post-train values of T_c and $T_{\frac{1}{2}R}$ and the standard error of this mean difference. The mean values have been plotted in Fig. 5

Frequency of stimulation (c/s)	Number of stimuli	T_c (msec)		$T_{\frac{1}{2}R}$ (msec)	
		Pre-train twitch	Post-train twitch	Pre-train twitch	Post-train twitch
20	12 to 126	10·3 ± 0·664	10·24 ± 0·476	9·68 ± 0·731	9·15 ± 0·89
		$n = 10$, $P > 0·5$		$n = 10$, $P < 0·02$	
300	10 to 300	9·8 ± 0·441	10·06 ± 0·489	9·25 ± 0·45	8·85 ± 0·53
		$n = 14$, $P < 0·05$		$n = 14$, $P < 0·01$	

line. The graphs show that there is little or no change in the time course of the isometric twitch with increase in PTP up to nearly maximum levels of potentiation brought about by 126 stimuli at 20 c/s and 300 stimuli at 300 c/s. The mean values for T_c and $T_{\frac{1}{2}R}$ for pre-train twitches and post-train twitches after 12–126 stimuli at 20 c/s and 10–300 stimuli at 300 c/s are listed in Table 2. The probability values (P) given in Table 2 were obtained from t-values calculated from the ratio of the mean difference between pre-train and post-train values of T_c and $T_{\frac{1}{2}R}$ and the standard error of this mean difference. T_c is only slightly altered with increase in PTP up to the maximum level in both series. The small change in T_c following stimulation at 300 c/s is significant at the 5% level but the values show no obvious trend with increase in the number of stimuli up to about 300 stimuli. In contrast, $T_{\frac{1}{2}R}$ of the post-train twitch decreased progressively with increase in the number of stimuli in the train to a minimum of about 0·9 times the control value of $T_{\frac{1}{2}R}$ following 50–70 stimuli at 20 c/s and 150 stimuli at 300 c/s but increased gradually with further increase in the number of repetitive stimuli and was approximately the same as $T_{\frac{1}{2}R}$ of the pre-train twitch after 100–126 stimuli at 20 c/s and

300 stimuli at 300 c/s. Both T_c and $T_{\frac{1}{2}R}$ of the post-train twitch are increased markedly following trains in which the number of stimuli exceeds about 126 stimuli at 20 c/s and 300 stimuli at 300 c/s and these changes are much more pronounced following stimulation at the lower frequency.

Figures 6 and 7 show examples of the time course of change of degree of potentiation and of T_c and $T_{\frac{1}{2}R}$ during the decline of PTP. Figure 6 shows representative records of twitch contractions and graphs illustrating the effects of trains of 100 and 300 stimuli at 20 c/s on contractions of two muscles and Fig. 7 shows similar results obtained from two other muscles after trains of 300 and 960 stimuli at 300 c/s. The degree of potentiation and the time course of decay of PTP are similar in the four series (Fig. 6 *B*, 7 *B*). The short trains of stimuli, 100 at 20 c/s and 300 at 300 c/s, caused potentiation of twitch tension with little or no alteration of the time course of the twitch. Prolonged stimulation by 300 stimuli at 20 c/s and 960 stimuli at 300 c/s markedly increased both T_c and $T_{\frac{1}{2}R}$ in addition to potentiating the peak twitch tension (Figs. 6 *B*, 7 *B*). The increase in T_c and $T_{\frac{1}{2}R}$ after prolonged stimulation declines along a time course which depends on the number of stimuli in the train and the frequency of repetitive stimulation. For example, after 160 or 200 stimuli at 20 c/s T_c and $T_{\frac{1}{2}R}$ decreased to pre-train values at a time when the peak tension of the twitch was still 20–40 % potentiated and the twitch time course remained virtually unaltered thereafter (not shown in Fig. 6). The disappearance of the increase in T_c and $T_{\frac{1}{2}R}$ after 300 stimuli at 20 c/s followed a time course which was almost the same as that for the decline of PTP (Fig. 6). The effects of prolonged stimulation at 300 c/s were less marked and even after trains of 480 or 960 stimuli the increase in T_c and $T_{\frac{1}{2}R}$ disappeared before the end of PTP. More observations are required to define precisely the time course of disappearance of changes in twitch duration which occur following prolonged repetitive stimulation.

DISCUSSION

The results described above have revealed two distinct aspects of potentiation of the twitch of rat EDL following repetitive stimulation at 35° C. These are the increase in peak tension with little or no change in time course of the twitch and prolongation of both the contraction and relaxation phases. These effects have not been examined in detail for other muscles but the results available show that PTP of the twitch of mammalian fast skeletal muscle at 35° C is similar to that observed in frog twitch muscle at 20 °C not only in the degree of maximum potentiation but also in changes in time course of twitches during potentiation. For example, short trains of stimuli increase peak tension with little or no change in

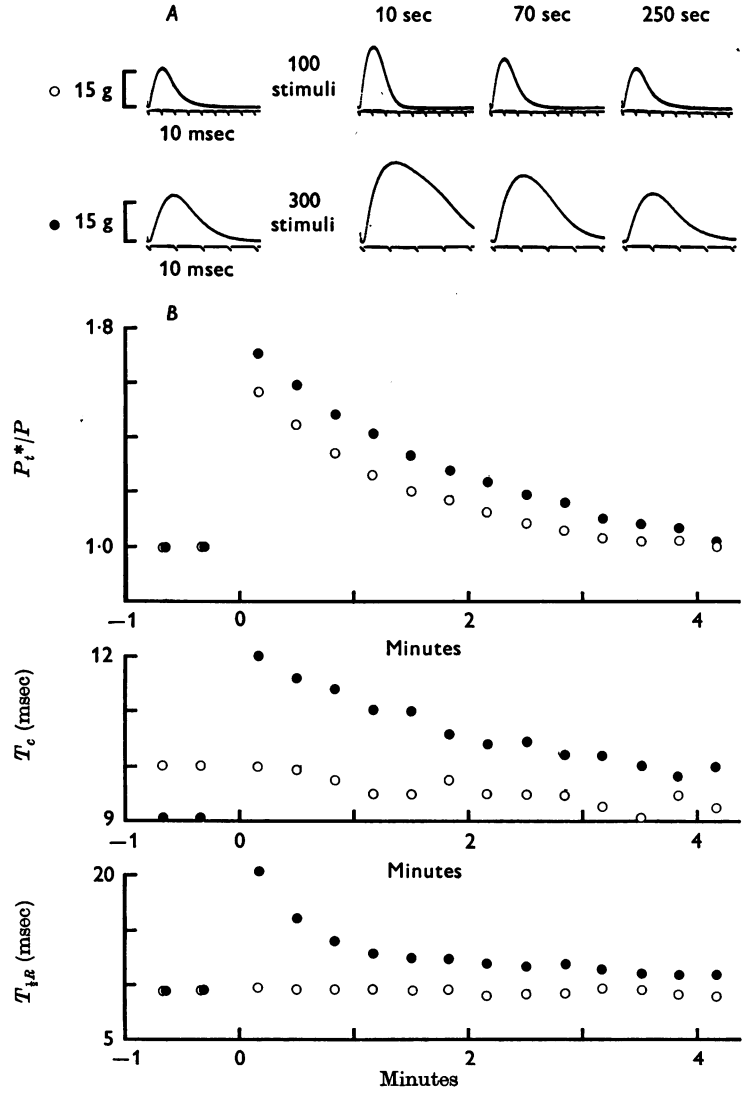

Fig. 6. The two series of records in *A* are, from left to right, a pre-train twitch and post-train twitches recorded 10, 70 and 250 sec after 100 stimuli at 20 c/s in one muscle (○) and 300 stimuli at 20 c/s in the other muscle (●). The records in *A* form part of the series in *B* which show the degree of potentiation P_t^*/P_t, the contraction time and half-relaxation time of post-train twitches at different times after 100 stimuli at 20 c/s (○) and 300 stimuli at 20 c/s (●). Values for pre-train twitches are shown between −1 and 0 on abscissae and P_t is expressed as 1·0. The time scale on the abscissa refers to time before and after the end of repetitive stimulation at 20 c/s. Muscle weights ○ = 30 mg. ● = 28 mg. Average muscle fibre lengths ○ = 9·5 mm, ● = 9·0 mm. Temperature: 35 °C.

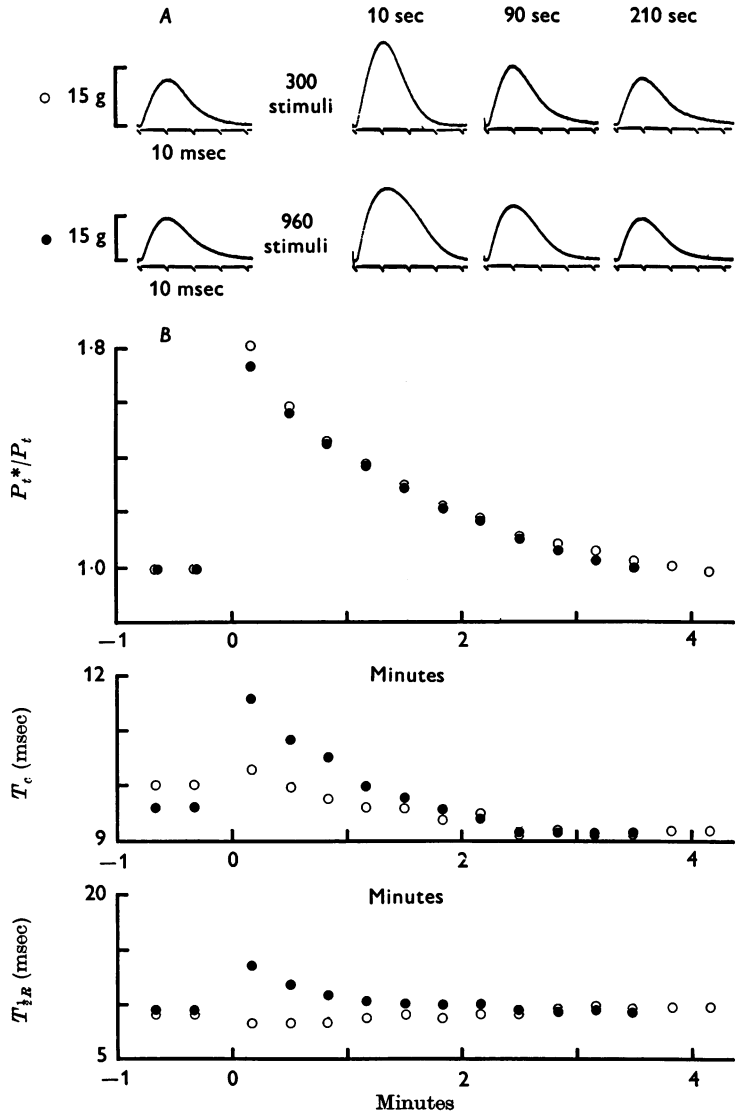

Fig. 7. Representative records (*A*) and graphs (*B*), similar to those in Fig. 6, showing the degree of potentiation, contraction time and half-relaxation time of post-train twitches after 300 stimuli at 300 c/s in one muscle (○) and 960 stimuli at 300 c/s in another muscle (●). Muscle weight ● = 27 mg. Average muscle fibre length ● = 10 mm. The weight and average fibre length of the other muscle (○) are not available. Temperature: 35 °C.

contraction time, depending on the number of stimuli in the train, the frequency and method of stimulation and the time after the end of the train of stimuli, in cat (Brown & Euler, 1938; Standaert, 1964), rat (above), human (Desmedt & Hainnaut, 1968) and frog muscles (Colomo & Rocchi, 1965) and this is usually accompanied by either no change or a small decrease in the half-relaxation time. Following prolonged stimulation there are marked increases in contraction time, half-relaxation time and total duration of the twitch in both rat (above) and frog (Ramsey & Street, fig. 7, 1941) muscles.

PTP has been observed in single fibres of frog muscles (Ramsey & Street, 1941; Colomo & Rocchi, 1965) and indirect evidence indicates the same for mammalian muscles. It has been shown above that all, or nearly all, muscle fibres must have been excited in the isolated EDL muscles. Consequently the increase in peak tension during maximum PTP of whole EDL muscle could not have resulted merely from an increase in the number of muscle fibres excited, with every muscle fibre responding with an all-or-nothing twitch. This is consistent with the observation of Brown & Euler (1938) and Bernhard *et al.* (1941) that the compound action potential of whole cat muscle is not increased during PTP. On the other hand the results obtained for whole rat muscle must be regarded as providing only average values for the whole population of muscle fibres with no indication of the range of P_t/P_0 and the degree of PTP of individual fibres. Ramsey & Street (1941) observed a considerable range of P_t/P_0 for frog single muscle fibres and found that the extent to which the twitch was potentiated after repetitive stimulation was inversely related to P_t/P_0 of the pre-train twitch (fibres for which P_t was about 0·1 P_0 showed a fivefold increase in peak twitch tension during PTP whereas those with P_t about 0·64 P_0 showed little or no increase in P_t) and that the twitch to tetanus ratio of maximally potentiated twitches never exceeded the maximum P_t/P_0 of 0·64 recorded for control twitches.

The question arises whether PTP results from a decrease in the compliance of series elastic elements within muscle fibres, an increase in the response of the contractile material itself, or both. The properties of the series elastic elements have not been determined during PTP but it is important to note that a decrease in series compliance would probably lead to either no change in T_c or a decrease in T_c, depending on the time course of decay of the active state, whereas in the results shown above T_c was usually increased in twitches recorded 10 sec after a tetanus. Furthermore, a decrease in compliance of the elastic elements would not cause prolongation of the twitch duration which occurs after prolonged stimulation. The effects of temperature on PTP are also interesting in this connexion. The peak tension of the maximally potentiated twitch of rat EDL

is about twice that of the normal twitch at 35° C and is independent of temperature from 35 to 20° C despite a twofold increase in the peak tension of the normal twitch as temperature is decreased over that range (Close & Hoh, 1968). The same appears to hold for cold-adapted frog sartorius muscle in the range 20–0° C (Hill, 1951; also unpublished observations) even though the properties of the series elastic elements in that muscle are only slightly altered by change in temperature (Jewell & Wilkie, 1958). So far as can be judged from these observations PTP does not result merely from changes in passive series elastic elements but it remains for measurements to be made to determine whether the properties of the elements are altered in any way by repetitive activity. On the other hand, all the observations listed above, as well as that of Ramsey & Street (1941) on the relation between PTP and P_t/P_0 of frog muscle fibres at 20 °C are entirely consistent with the view that many muscle fibres in mammalian fast muscles near body temperature and some frog twitch muscles at 20 °C are only partially activated, and that PTP at these temperatures leads to an increase in the response of the contractile material itself (Close, 1965; Colomo & Rocchi, 1965; Close & Hoh, 1968; Desmedt & Hainnaut, 1968). On this basis PTP with little or no change in twitch time course may be regarded as being due largely to an increase in the degree of activation of individual muscle fibres whereas prolonged stimulation, in which the number of stimuli exceeds that required for full potentiation, leads to an increase in the duration of the twitch presumably as a result of prolongation of the active state as described by Ritchie & Wilkie (1955) for frog muscle at 0°C.

As regards a change in the response of the contractile material it is possible that PTP results from an increase in the number of fully activated myofibrils contracting in individual muscle fibres. This hypothesis has the advantage that potentiation would result simply from addition of the same kind of response of elements contracting in parallel. It has been reported that even the central myofibrils of frog fibres are activated following a single stimulus at 20° C and undergo shortening (Gonzalez-Serratos, 1966) but in the absence of a mechanical record of the contraction which was photographed there remains the possibility that the fibre was one of the few which appear to be fully activated and show little or no PTP (Ramsey & Street, 1941). In any event the shortening of fibrils observed by Gonzalez-Serratos was free shortening with no external load and those results do not preclude the possibility that the response of individual fibrils may be increased during PTP, either by an increase in the number of active filaments or cross-bridges or by some other means.

Alternatively, all the cross-bridges of a fibre may contribute to the normal twitch contraction of rat EDL at 35° C and frog muscle at 20° C, but the amount of activator liberated following the action potential may

be submaximal and limit the average rate of cycling of each cross-bridge at any particular load. PTP may then result simply from an increase in the amount of activator liberated in all regions of the fibre following a single action potential, thus raising the activator concentration throughout the fibre, with a corresponding increase in the average rate of cycling of individual cross-bridges at a particular load and an increase in the number of bridges formed and tension developed at any given time in the twitch. According to this hypothesis the degree of activation or potentiation would increase with increased liberation of activator up to a maximum corresponding to the maximum rate of cycling of cross-bridges for each load and this would not necessarily alter either the intrinsic speed of shortening or the isometric twitch contraction time.

A possible explanation for the effects of prolonged stimulation increasing the twitch duration is that there is an increase in the amount of activator liberated, thereby exceeding the amount which saturates and fully activates the contractile material. As a consequence it would take some time for the concentration of activator to fall below the saturation level, as suggested by Hodgkin & Horowicz (1960*b*) for potassium contractures, and this may result in delayed decline of the active state, thereby increasing T_c, $T_{\frac{1}{2}R}$ and twitch duration, and the appearance of a hump on the record of relaxation (Fig. 6) normally seen in rat and frog muscles only at low temperature.

It has been suggested that PTP results from accumulation of a substance in some part of the muscle fibre (Lee, 1907; Brown & Euler, 1938; Martini, 1939; Giachetti, 1950; Zingoni, 1954). Brown & Euler (1938) pointed out the similarity in potentiation of the twitch of mammalian muscle by potassium ions and PTP but there is as yet no direct evidence that accumulation of this ion in, or about, the muscle fibre actually causes PTP. Diffusion of excess external potassium from frog single muscle fibres occurs within about 10 sec after a sudden decrease in external potassium ion concentration (Hodgkin & Horowicz, 1960*a*) whereas PTP in frog single muscle fibres persists much longer (Ramsey & Street, 1941; Colomo & Rocchi, 1965). Nevertheless, it is possible that potassium ions accumulate in a space such as the transverse tubules during repetitive excitation, thereby affecting excitation–contraction coupling and enhancing activation, and that diffusion of these ions out of the tubules is retarded following repetitive stimulation. There is also the possibility that the mechanical threshold (Hodgkin & Horowicz, 1960*b*) is lowered during PTP, as suggested by Desmedt & Hainnaut (1968), or that the muscle membrane is altered in some other way. Some changes in the action potential after repetitive stimulation disappear with a time course similar to that for PTP (Persson, 1963), but it is not known whether these electrical changes are causally

related to the potentiation of twitch tension or whether they are independent effects of repetitive stimulation.

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