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TEMPERATURE DEPENDENCE OF ENHANCEMENT AND DIMINUTION OF TENSION EVOKED BY STAIRCASE AND BY TETANUS IN RAT MUSCLE

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SUMMARY

1. The effect of temperature (20–37·5 °C) on the potentiation of twitch tension was examined during and after the staircase (250 stimuli, 5/sec) and after the tetanus (188 stimuli, 125/sec) in the extensor digitorum longus muscle of adult Lewis rats.

2. During the staircase at 20 °C the twitch tension decreased (negative staircase) by 10–20 %. At 25–30 °C the staircase was initially negative and later positive. At 37·5 °C the staircase was positive throughout the train. Both at the end of the staircase and 2 sec after the tetanus the potentiation increased linearly with increasing temperature.

3. After the staircase and the tetanus at 20–30 °C the twitch tension increased initially rapidly and later after the staircase at a slower rate. Maximal potentiation at 20 °C was attained 3 min after the staircase ($+30 \pm 3$ %, $n = 10$, S.E. of mean) and 1 min after the tetanus ($+16 \pm 1$ %, $n = 10$, S.E. of mean). At 37·5 °C the potentiation decayed rapidly after the staircase and the tetanus.

4. During the staircase the time course of the twitch was shortened twice as much at 20 as at 37·5 °C. At the end of the staircase and 2 sec after the tetanus the contraction time was the more prolonged the greater the potentiation. At maximal potentiation the contraction time was prolonged three times as much at 20 °C ($+19 \pm 3$ %, $n = 10$, S.E. of mean) as at 37·5 °C ($P < 0{\cdot}005$). The half-relaxation time at the end of the staircase was prolonged 10 times more at 20 than at 37·5 °C ($P < 0{\cdot}02$).

5. When extrapolated to time zero after the staircase and the tetanus the potentiation at 20 °C was still marked (20–50 %). The rate of decay of potentiation (time constant, 20 °C, $561{\cdot}2 \pm 37{\cdot}4$ sec, $n = 20$, S.E. of mean) increased with increasing temperature ($Q_{10} = 2{\cdot}6$). The event of potentiation with a fast rate of decay, present after the tetanus but not after the staircase at 37·5 °C, was abolished below 30 °C.

6. The increase in twitch tension after the staircase and the tetanus at 20–30 °C was taken to indicate the recovery of events that diminished the twitch, occurring simultaneously with potentiation.

7. (i) One process of diminution, present after the staircase but not after the

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tetanus, increased on cooling and was assumed to be due to fatigue. The rate of recovery of the process (time constant, 20 °C, 79·6 ± 7·4 sec, $n = 10$, S.E. of mean) increased with increasing temperature ($Q_{10} = 1·9$). The half-relaxation time of the last twitch in the staircase was the more prolonged the greater the process. (ii) Another process causing diminution was present after the staircase and the tetanus at 20–30 °C. It recovered at 20 °C with a time constant of 14·9 ± 2·2 sec ($n = 10$, S.E. of mean). This process, possibly responsible for the initially negative staircase, was not thought to be due to fatigue. It may reflect a diminished depolarization of the transverse tubules by repetitive stimuli.

INTRODUCTION

The potentiation of twitch tension during a staircase and after a tetanus (post-tetanic potentiation) is diminished by cooling of the muscle (Walker, 1951; Close & Hoh, 1968; Hanson, 1974; Hoh, 1974). Thus, Close & Hoh (1968) and Hoh (1974) found potentiation in the extensor digitorum longus muscle of rat 10 sec after a tetanus to be absent at 20 °C. In the present study potentiation in the extensor digitorum longus muscle of rat after a staircase was still marked at 20 °C and some potentiation persisted after the tetanus at this temperature. These findings made it of interest to re-evaluate the underlying mechanism for the effect of low temperature on potentiation, using the model described previously (Krarup, 1981*a*).

METHODS

The recording of electrical and mechanical responses evoked by stimuli to the sciatic nerve or directly to the extensor digitorum longus muscle of rat has been described (Krarup, 1981*a*).

Temperature. The temperature of Liley's solution in the muscle chamber was adjusted to 19–38 °C by changing the temperature in a surrounding jacket. The solution in the chamber was exchanged at 1–2 ml./min *via* thin-walled tubes in the jacket. The temperature was monitored continuously by a thermocouple in the muscle chamber.

Procedure. Twitch potentiation was examined during runs of increasing and decreasing temperature. The results were similar and therefore pooled. At 37–38 °C in a run with decreasing temperature and at 20 °C in a run with increasing temperature, the following data were collected. (i) The amplitude, duration, and latency of the muscle action potential and the tension, latency, contraction time, and half-relaxation time of the isometric twitch (pre-staircase and pretetanic twitches). (ii) The potentiation of twitch tension during a staircase (250 stimuli, 5/sec). (iii) The poststaircase potentiation obtained by comparing the pre-staircase twitch with twitches evoked 15, 30, 45, 60, 120, 180, 240, 300, 360, 600, 900, and 1200 sec after the staircase. (iv) The post-tetanic potentiation (188 stimuli, 125/sec) by comparing the pretetanic twitch evoked 0·5 min before the tetanus with twitches evoked 2, 5, 10, 30, 60, 120, 180, 240, 300, 360, 600, 900, and 1200 sec after the tetanus. Up to 10 sec after the tetanus, the stimuli were delivered automatically by the programmed stimulator. (v) The temperature was then decreased to 30 °C or increased to 25 °C within 30 min. A further 10 min were allowed to assure equilibration of the temperature between the muscle and the surrounding fluid. The potentiation evoked by a staircase and by a tetanus was examined at 20 °C (19·6–20·2 °C), 25 °C (24·6–25·3 °C), 30 °C (29·7–30·3 °C), and 37·5 °C (37·4–37·7 °C) as described in (ii) and (iii). Single responses were recorded at each full degree C. Responses were compared that were evoked by stimuli to the nerve and directly to the muscle. In addition, at the final temperature (20 or 37·5 °C) responses evoked by stimuli to the nerve were compared with responses of the curarized muscle (cf. Krarup, 1981*a*). The responses evoked by direct stimuli did not differ in the curarized and non-curarized muscle.

RESULTS

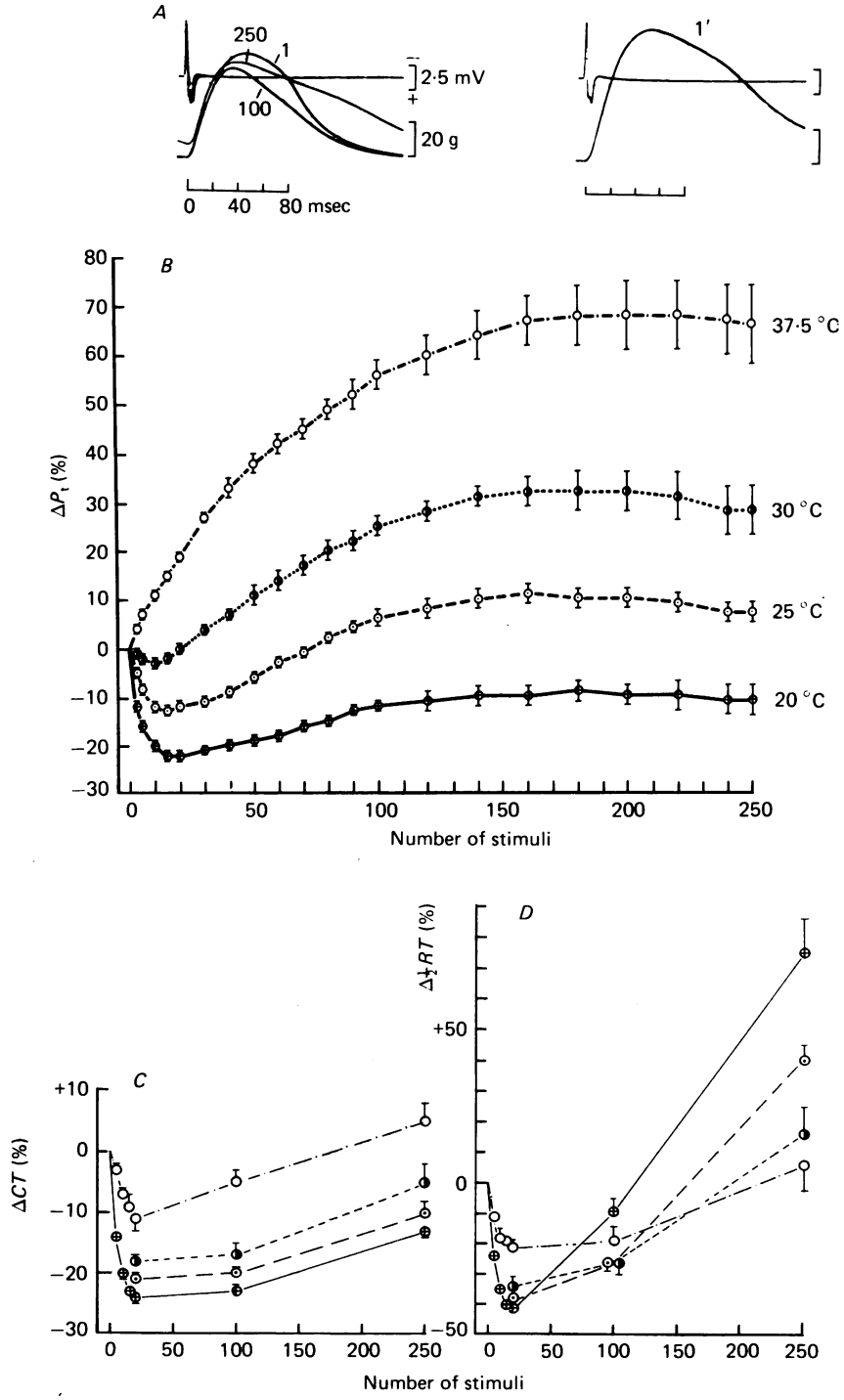
The effects of changes in temperature on the twitch and on the tetanus were similar to those reported by others (Truong, Wall & Walker, 1964; Close & Hoh, 1968; Hoh, 1974; Ranatunga, 1977 *a*); the twitch tension increased almost linearly by 40 % when the temperature was decreased from 37·5 to 20 °C. At 20 °C the twitch tension was 650·3 ± 27·0 g/cm² ($n = 11$, S.E. of mean). The contraction time of the twitch (20 °C, 43·5 ± 0·7 msec, $n = 11$, S.E. of mean) was prolonged with a Q_{10} of 2·1 and the half-relaxation time (20 °C, 52·9 ± 1·7 msec, S.E. of mean) with a Q_{10} of 2·7 with a decrease in temperature. The maximum tetanic tension at 20 °C was 2086·8 ± 86·7 g/cm² ($n = 11$, S.E. of mean) which was 25 % lower than at 37·5 °C; the decrease in tetanic tension occurred mainly from 30 to 20 °C. During tetani evoked by stimuli to the nerve at 20 °C the tension decreased by 47 ± 5 % (S.E. of mean) and the amplitude of the muscle action potential by 88 ± 3 %. When evoked by stimuli to the muscle the decrement was significantly ($P < 0·02$) lower, −16 ± 5 % ($n = 5$, S.E. of mean). The difference in the decrement of tetani evoked by indirect and direct stimuli was still present at 25 °C and disappeared above that temperature. The decrement in tetani evoked by stimuli directly to the muscle at 20 °C was twice that ($P < 0·05$) at 37·5 °C.

The effect of temperature on the potentiation of twitch tension

The staircase phenomenon. In Fig. 1*A* are shown twitches and action potentials evoked at 20 °C during and one min after the staircase (5/sec, 250 stimuli). Compared with the pre-staircase twitch, the twitch tension during the staircase decreased (negative staircase), the decrement being present already by the second contraction (Fig. 1*B*). The decrement was most marked after 20 stimuli, it decreased gradually to stabilize after 120 stimuli. At 25 and 30 °C the staircase was initially negative, followed by a positive staircase. The potentiation and the increment in twitch tension per stimulus increased with increasing temperature. At 37·5 °C the staircase was positive throughout the train. At all temperatures examined the twitch tension stabilized within the 250 stimuli of the train. Hence, at this level the reduction in the staircase was not due to a delay in the potentiating effect of repetitive stimuli.

During the train of stimuli at 20–30 °C, but not at 37·5 °C, the amplitude of the muscle action potential decreased by 5–10 %. The duration of the negative phase of the action potential was increased after 250 stimuli by 10–40 %, the prolongation during the staircase being most marked at 20 °C. The decrement in the twitch tension was not due to partial block of neuromuscular transmission, because the change in twitch tension at different temperatures was the same with direct stimulation as with stimuli *via* the nerve.

After the staircase, the twitch tension at 20, 25 and 30 °C increased gradually to maximal potentiation followed by a decrease to the pre-staircase level (Fig. 2*A*). At 20 °C the maximal potentiation occurred 3 min after the train and was +30 ± 3 % ($n = 10$, S.E. of mean). The increase in twitch tension was greater and lasted longer the lower the temperature. The rate of increase was lower at 20 than at 30 °C. At 37·5 °C the potentiation decayed quickly after the staircase. On a logarithmic scale, the potentiation decayed linearly with time, the faster the higher the temperature (Fig.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0 50 100 150 200 250
Number of stimuli
C
+10
0
−10
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−30
ΔCT (%)
0 50 100 150 200 250
D
$\Delta \frac{1}{2}RT$ (%)
+50
0
−50
0 50 100 150 200 250
Number of stimuli

2*A*). Also the logarithm of the numerical difference between the experimentally determined change in twitch tension and the extrapolated decay of potentiation (Fig. 2*A*) decayed linearly with time (Fig. 2*B*). As it has been suggested before (Krarup, 1981*a*), the change in twitch tension after the staircase could therefore be described

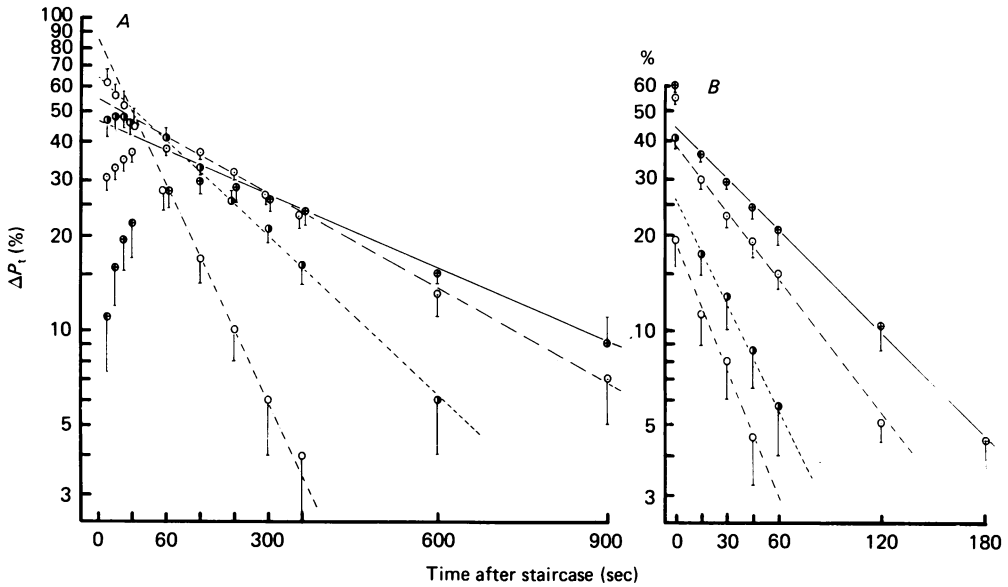

Fig. 2. The effect of temperature (20–37·5 °C) on the change in amplitude of the twitch after the staircase. *A*, the average percentage change in twitch tension (ΔP_t, on a logarithmic scale) as a function of time (15–900 sec) after the staircase. The regression lines were fitted to the linear decay of potentiation ($r = -0{\cdot}9987$ to $-0{\cdot}9999$, $P < 0{\cdot}001$, least squares method). *B*, the average percentage numerical difference (on a logarithmic scale) between the experimentally determined change in twitch tension and the extrapolated decay of potentiation, as a function of time (0–180 sec) after the staircase. The regression lines were fitted to the linear part of the decay ($r = -0{\cdot}9966$ to $-0{\cdot}9991$, $P < 0{\cdot}01$–$0{\cdot}001$, least squares method). At 20–30 °C the extrapolated decay (0 sec) did not fit the points. Symbols as in Fig. 1.

both by the recovery of a process that diminished twitch tension and by the decay of a process causing potentiation:

$$P_{TS}(t) = P_T(0)\exp(-\alpha t) + P_D(0)\exp(-\gamma t) \quad (1)$$

where $P_{TS}(t)$ is the calculated potentiation (in %) as a function of time (t in sec) after the staircase; $P_T(0)$ is the process (in %) at time zero after the staircase that caused

Fig. 1. The effect of temperature (20–37·5 °C) on the amplitude and the time course of the twitch during the staircase (5/sec, 250 stimuli). *A*, superimposed action potentials (upper traces) and twitches (lower traces) to the 1st, 100th, and 250th stimulus in the staircase (left) and to a stimulus 1 min after the staircase (right) at 20 °C. *B*, *C*, *D*, the average percentage change in twitch tension (ΔP_t, *A*), in the contraction time (ΔCT, *B*), and in the half-relaxation time ($\Delta \frac{1}{2}RT$, *D*) as a function of the number of stimuli in the staircase at different temperatures. Symbols: 20 °C, ⊕—⊕; 25 °C, ⊙--⊙; 30 °C, ◑----◑; 37·5 °C, ○- · -○. The points are averages of ten to twelve muscles and the vertical bars denote the S.E. of mean.

potentiation and $P_D(0)$ is the process (in negative %) that caused diminution. The rates of decay of the potentiation, α, and of the process causing diminution, γ, were calculated in sec^{-1}. The time constants of the rates of decay at different temperatures are compiled in Table 1. At 20 °C γ was seven times faster than α; both γ and α increased exponentially with temperature, the Q_{10} of γ being two-thirds of that of α ($P < 0.005$, Table 1).

TABLE 1. The effect of temperature (20–37·5 °C) on the time constants (sec) of the recovery of processes causing diminution (γ, δ) and of the decay of processes causing potentiation (α, β) of twitch tension after the staircase and after the tetanus. Responses were evoked by stimuli to the sciatic nerve. Mean ±S.E. of mean, number of muscles in parentheses

	20 °C	25 °C	30 °C	37·5 °C	Q_{10}
Staircase					
α	544·0±42·0 (10)	402·0±29·5 (12)	249·5±19·1 (10)	108·2±8·7 (10)	2·6±0·1 (11)
γ	79·6±7·4 (10)	67·3±4·7 (12)	41·6±5·5 (10)	30·3±4·8 (8)	1·9±0·2 (11)
Post-tetanus					
α	578·5±63·8 (10)	301·8±25·4 (11)	184·6±12·7 (12)	100·3±6·6 (10)	2·6±0·2 (12)
β	Absent	Absent	*	8·0±1·1 (10)	—
δ	14·9±2·2 (10)	8·1±1·1 (11)	*	Absent	—

* Could not be determined.

As seen in Fig. 2*B* the size of the process causing diminution at time zero after the staircase (at 20–30, but not at 37·5 °C), as determined by extrapolation, was lower than that expected from subtraction of the extrapolated decay of potentiation from the measured change in twitch tension (Fig. 2*A*). Hence the values of $P_{TS}(0)$ at 20–30 °C calculated from eqn. (1) were greater than the measured change in twitch tension ($\Delta P_t(250)$) at the 250th response in the staircase. Both the calculated and the measured change in twitch tension (Fig. 3*A*) increased linearly with an increase in temperature, and at 37·5 °C they coincided. By contrast, the calculated (P_{TS} (15 sec)) and the measured potentiation (ΔP_t(15 sec)) 15 sec after the staircase were similar (Fig. 3*B*). Assuming that both the recovery of the process causing diminution and the decay of potentiation could be described by single exponential functions also at 20–30 °C, the discrepancy within the first 15 sec after the staircase could be due to the recovery of a second process causing diminution with a very short time constant (about 10 sec at 20 °C). However, a faster stimulus frequency after the staircase than in these experiments (1/15 sec) would be necessary to describe the recovery rate more accurately.

Temperature affected the process causing diminution, P_D, and the process causing potentiation, P_T, differently. At 20 °C the size of the diminution at time zero after the staircase, $P_D(0)$, was -45 ± 2% ($n = 10$, S.E. of mean) and that of potentiation, $P_T(0)$, was $+50\pm4$%. The potentiation increased linearly with increasing temperature; the process causing diminution became less pronounced (Fig. 4*A*). The ratio $|P_D(0)/P_T(0)|$ increased exponentially as the temperature decreased ($Q_{10} = 2·0$, Fig. 4*B*), indicating a relatively larger effect of diminution at low than at high temperature.

Post-tetanic potentiation. Twitches and action potentials evoked before and 2 sec and 1 min after the tetanus (125/sec, 188 stimuli) are shown in Fig. 5*A*. At 20–30 °C

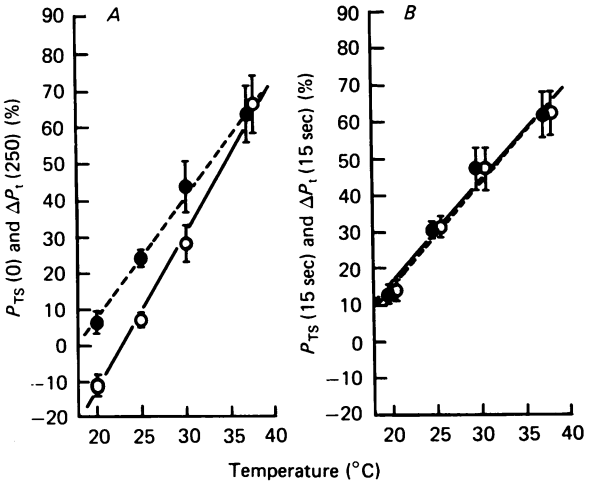

Fig. 3. Calculated (P_{TS}, filled circles, dashed lines) and experimentally determined (ΔP_t, open circles, full lines) percentage changes in twitch tension, *A* for the last twitch in the staircase (5/sec, 250 stimuli), and *B* for the twitch evoked 15 sec after the staircase, as a function of temperature. The regression lines were fitted by the method of least squares ($P < 0{\cdot}01$). The points are averages of ten to twelve muscles and the vertical bars denote the S.E. of mean.

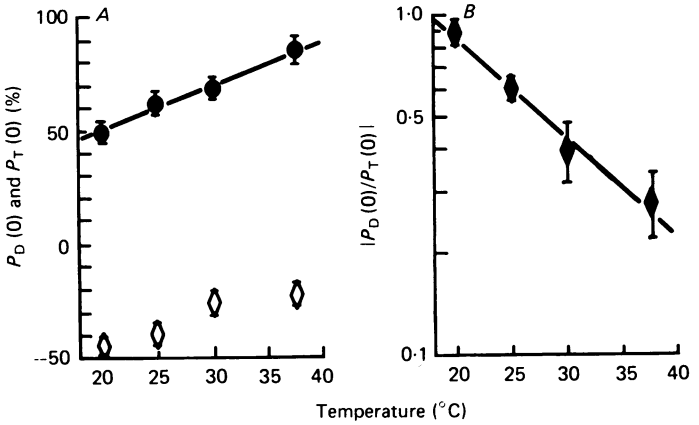

Fig. 4. *A:* calculated events of potentiation ($P_T(0)$, filled circles) and of diminution ($P_D(0)$, open symbols) in per cent at time zero after the staircase as a function of temperature. *B:* the numerical log ratio of $P_D(0)/P_T(0)$ as a function of temperature. The regression lines were calculated by the method of least squares ($P < 0{\cdot}01$). The points are averages of ten to twelve muscles and the vertical bars denote the S.E. of mean.

the twitch tension gradually increased to maximum after the tetanus, followed by a decrease to the pretetanic level. On a logarithmic scale the potentiation decayed linearly with time after the tetanus (Fig. 5*B*). The increase in tension after the tetanus was smaller and shorter in duration than after the staircase. At 20 °C the maximum potentiation occurred 0·5–1 min after the tetanus and was $+16 \pm 1$ % ($n = 10$, S.E. of

mean). After the tetanus at 37·5 °C the post-tetanic potentiation decayed initially rapidly and then more slowly (Fig. 5*B*). On a logarithmic scale, the difference between the post-tetanic potentiation at 37·5 °C and the extrapolated slow decay of potentiation (Fig. 5*B*) 2–10 sec after the tetanus decayed linearly with time (Fig. 5*C*). The decay

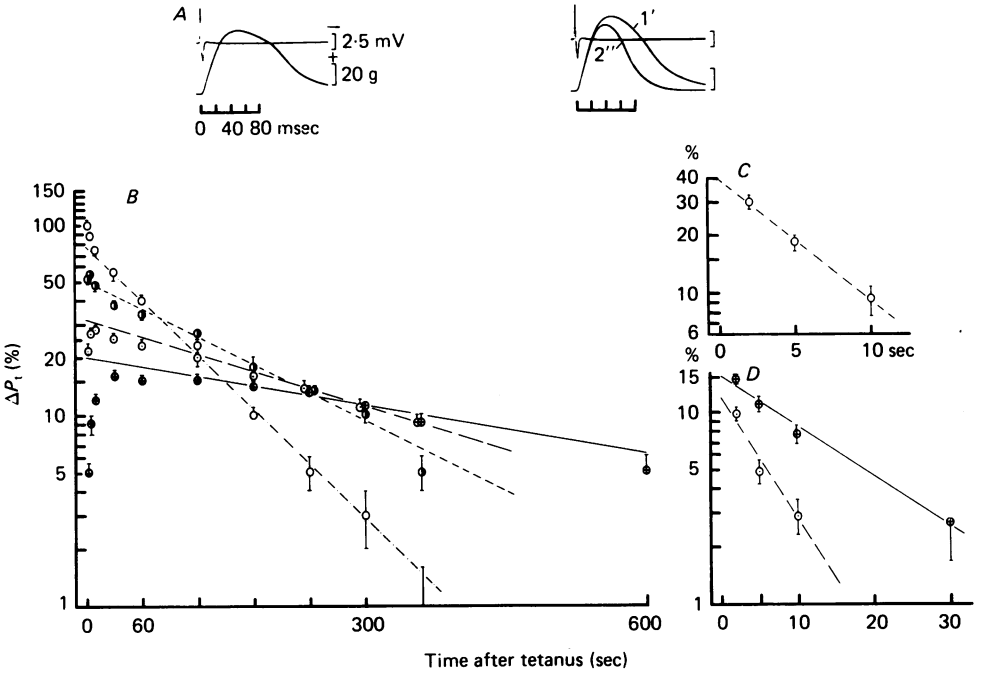

Fig. 5. The effect of temperature (20–37·5 °C) on the twitch after a tetanus (125/sec, 188 stimuli). *A:* action potentials (upper traces) and twitches (lower traces) evoked before (left) and 2 sec and 1 min after (right) the tetanus at 20 °C. *B:* the change in twitch tension (ΔP_t %, on a logarithmic scale) as a function of time after the tetanus. The regression lines were fitted to the linear decay of potentiation ($r = -0{\cdot}9857$ to $-0{\cdot}9979$, $P < 0{\cdot}001$, least squares method). At 25 and 30 °C the potentiation after 600–900 sec decayed faster than expected; not included. *C:* the difference in per cent (logarithmic scale) between the experimentally determined potentiation and the extrapolated decay of potentiation, as a function of time (2–10 sec) after the tetanus at 37·5 °C. The regression line ($r = -0{\cdot}9993$, $P < 0{\cdot}05$) was calculated by the method of least squares. *D:* the numerical difference (%, logarithmic scale) between the experimentally determined potentiation and the extrapolated decay of potentiation at 20 and 25 °C, as a function of time after the tetanus. The regression lines (continuous line, $r = -0{\cdot}9936$, $P < 0{\cdot}01$; dashed line, $r = -0{\cdot}9751$, $P > 0{\cdot}05$) were calculated by the method of least squares. Symbols: 20 °C, ⊕—⊕; 25 °C, ⊙--⊙; 30 °C, ◑---◑; 37·5 °C, ○-·-○. The points are averages from ten to twelve muscles and the vertical bars denote the S.E. of mean.

of post-tetanic potentiation at 37·5 °C could thus be described by two events of potentiation with different rates of decay (Krarup, 1981*a*):

$$P_{TP}(t) = P_{T1}(0)\exp(-\beta t) + P_{T2}(0)\exp(-\alpha t) \qquad (2)$$

where $P_{TP}(t)$ is the calculated potentiation (in %) as a function of time (t in sec) after the tetanus; $P_{T1}(0)$ and $P_{T2}(0)$ are the events of potentiation (in %) at time zero after

the tetanus. The rates of decay of the events of potentiation, β and α, were calculated in sec^{-1}. The time constants of the rates of decay are shown in Table 1 and were similar to those described earlier (Krarup, 1981*a*).

In Fig. 5*D* the logarithm of the numerical difference between the change in twitch tension and the extrapolated decay of potentiation at 20 and 25 °C (Fig. 5*B*) decayed approximately linearly with time. This could suggest that at 20 and 25 °C, as opposed

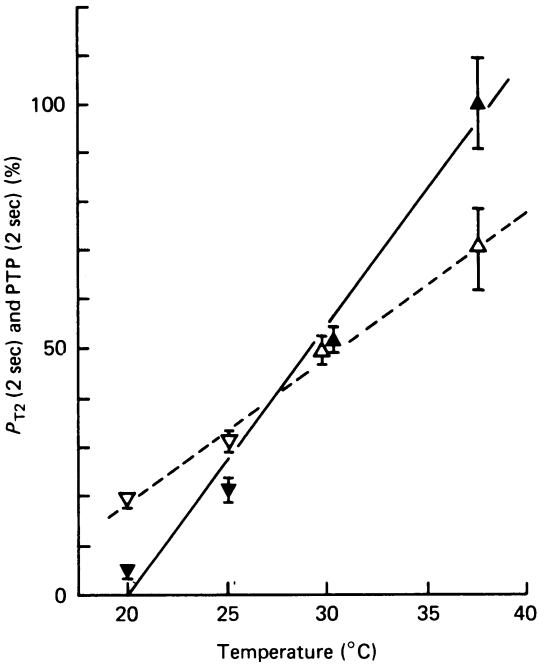

Fig. 6. The calculated event of potentiation (P_{T_2} (2 sec), open symbols, dashed line) and the experimentally determined post-tetanic potentiation (PTP (2 sec), filled symbols, continuous line) 2 sec after the tetanus (125/sec, 188 stimuli) as a function of temperature. The regression lines ($r = 0{\cdot}9917$ and $= 0{\cdot}9972$, $P < 0{\cdot}01$) were calculated by the method of least squares.

to 37·5 °C, the change in twitch tension after the tetanus could be fitted by the sum of the recovery of a process that diminished twitch tension and of the decay of a process causing potentiation:

$$P_{TP}(t) = P_{T_2}(0)\exp(-\alpha t) + P_{D_2}(0)\exp(-\delta t) \quad (3)$$

where $P_{TP}(t)$, $P_{T_2}(0)$, and α have been explained in eqn. (2). $P_{D_2}(0)$ is the process that diminished the twitch tension (in negative %) at time zero after the tetanus, and the rate of decay, δ, was calculated in sec^{-1}. The rate of decay of the event of potentiation, α, was similar after the staircase and after the tetanus and had similar temperature coefficients (Table 1). The rate of decay of the process causing diminution, δ, was about 40 times faster than α ($P < 0{\cdot}001$, Table 1). δ could only be estimated at 20 and 25 °C and the increment of the rate in this temperature range was not significantly different from that of α ($P < 0{\cdot}8$). δ was 5–10 times faster ($P < 0{\cdot}005$–$0{\cdot}001$) than

the rate of decay of the process causing diminution, P_D, after the staircase, γ. The time constants of δ (Table 1) and the size of $P_{D_2}(0)$ (20 °C, -16 ± 1 %; 25 °C, -12 ± 1 %; $n = 10$–11, S.E. of mean) were close to those of the assumed second process causing diminution after the staircase (p. 378). This could suggest that this process was present both after the staircase and after the tetanus at 20 – 30 °C.

The increase in both the calculated event of potentiation, P_{T_2} (2 sec), (eqns. (2) and (3)) and in the experimentally determined post-tetanic potentiation 2 sec after the tetanus could be fitted by linear functions of increasing temperature (Fig. 6) confirming findings by Close & Hoh (1968). The lower post-tetanic potentiation than P_{T_2} (2 sec) at 20 and 25 °C was due to the presence of the process causing diminution, P_{D_2} (2 sec), whereas at 37·5 °C the greater post-tetanic potentiation than P_{T_2} (2 sec) was due to the presence of the event of potentiation with a fast rate of decay, P_{T_1} (2 sec). At 30 °C the three events P_{T_1}, P_{T_2}, and P_{D_2} and their rates of decay contributed to the change in twitch tension.

The effect of temperature on the time course of the potentiated twitch

When compared with the pre-staircase twitch, the time course of the twitch during the staircase was initially shortened, twice as much at 20 as at 37·5 °C ($P < 0{\cdot}001$, Fig. 1*C* and *D*). The maximal shortening occurred at about the 20th contraction (i.e. when the twitch tension at 20 °C was most diminished). The shortened time course was not due to increased synchronization as the action potential was neither shortened in duration nor increased in amplitude; the shortening was also seen when stimuli were given directly to the muscle.

The contraction time. After the maximal shortening the contraction time was gradually prolonged (Fig. 1*C*). However, even at 250 stimuli it was still shortened at 20–30 °C. Also the contraction time of the twitch 2 sec after the tetanus was the more shortened the lower the temperature.

After the staircase the contraction time was gradually prolonged at 20–30 °C, the more the lower the temperature. At 20 °C the maximum prolongation ($+19 \pm 3$ %, $n = 10$, S.E. of mean) occurred 3–4 min after the staircase when the potentiation was maximal. When compared to the maximal potentiation at 37·5 °C (at the 250th twitch in the staircase) the prolongation of the contraction time was 42 ± 4 % ($n = 8$, S.E. of mean) greater ($P < 0{\cdot}001$) per unit potentiation at 20 than at 37·5 °C.

When the contraction time was most shortened and the twitch tension most diminished during the staircase at 20 °C, the rate of force development was only diminished by half that of the twitch tension. At the 100th stimulus it was even slightly increased (about 5 %) though the tension was decreased. At 37·5 °C the rate of force development was increased to nearly the same extent as the twitch tension. At 20 °C the increase in the rate of force development after the staircase was about 10 % less than that of the twitch tension. Two sec after the tetanus at 20 °C the increase in the rate of force development was three times greater than that of the twitch tension. However, 1 min after the tetanus, when the twitch tension was maximal, the increase in both the tension and in the rate of force development was equal, and the rate of force development was only slightly greater than 2 sec after the tetanus. As illustrated by the example in Fig. 5*A*, the increase in post-tetanic potentiation from 2 sec to 1 min after the tetanus was associated with a prolongation of the twitch.

It was suggested in a previous report that the change in the contraction time of the twitch during the staircase and 2 sec after the tetanus was a function of both the degree of potentiation of the twitch and of the number of repetitive stimuli in the train (Krarup, 1981*a*). As seen in Fig. 7*A*, the change in the contraction time could be described by linear functions of the potentiation attained at different temperatures both at the 250th contraction in the staircase (5/sec) and 2 sec after the tetanus (188 stimuli, 125/sec). At a given level of potentiation the contraction time was more prolonged in the staircase than in the tetanus, presumably due to the greater number of stimuli in the staircase.

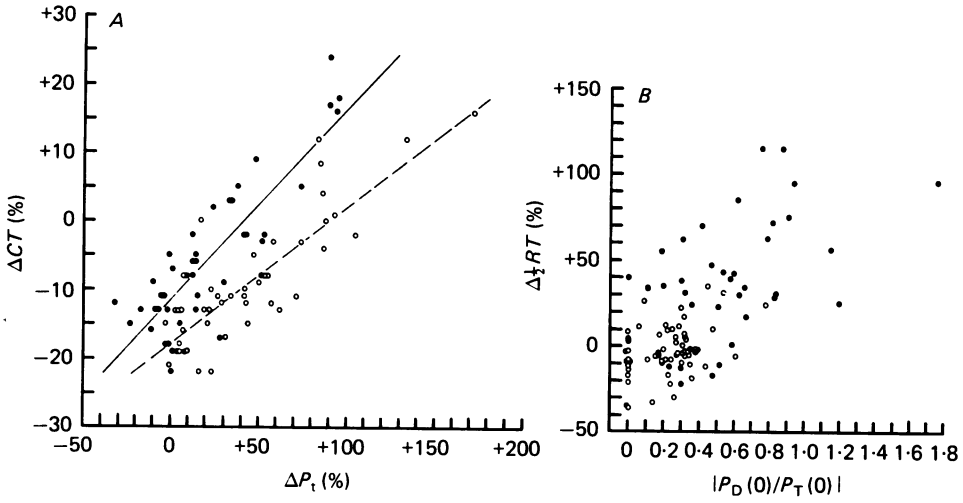

Fig. 7. *A:* the percentage change in the contraction time (ΔCT) as a function of the change in twitch tension (ΔP_t, %) at the 250th response in the staircase (filled circles, continuous line, $r = 0{\cdot}8513$, $n = 42$, $P < 0{\cdot}001$) and 2 sec after the tetanus (open circles, dashed line, $r = 0{\cdot}8440$, $n = 45$, $P < 0{\cdot}001$). The points are from thirteen different muscles at different temperatures (20–37·5 °C). The regression lines were calculated by the least squares method. *B:* the percentage change in the half-relaxation time ($\Delta\frac{1}{2}RT$) of the twitch at the 250th response in the staircase, as a function of the numerical ratio of $P_D(0)/P_T(0)$. The open circles are from 2, 3, and 5/sec staircases in twenty-one muscles (from Krarup, 1981*a*) and the filled circles from thirteen muscles at different temperatures (20–37·5 °C). By the least squares method the relationship was significant ($r = 0{\cdot}6453$, $n = 97$, $P < 0{\cdot}001$).

The half-relaxation time. In contrast to the contraction time, the half-relaxation time at the end of the staircase at 20 °C was 10 times more prolonged ($P < 0{\cdot}02$) than at 37·5 °C (Fig. 1*D*). After the staircase the prolonged half-relaxation time slowly returned to the pre-staircase level. The prolongation at the 250th twitch at different temperatures was found to be a function of the size of the process causing diminution, $P_D(0)$, at time zero after the staircase ($P < 0{\cdot}05$, least-squares method), confirming previous findings at 37–38 °C (Krarup, 1981*a*). In Fig. 7*B* the change in the half-relaxation time is plotted as a function of the numerical ratio of $P_D(0)/P_T(0)$. The plot includes both observations at different temperatures and data from twenty-one muscles previously reported (Krarup, 1981*a*). Although there was a wide scatter (presumably due to the extrapolations involved in determining $P_D(0)$ and $P_T(0)$) the significant ($P < 0{\cdot}001$) relationship could suggest that the increase in the process

causing diminution, due to the decrease in temperature, caused the increased prolongation in the half-relaxation time. By contrast, 2 sec after the tetanus the half-relaxation at 20 °C was shortened by 47 ± 1 % (n = 10, S.E. of mean) which was significantly more ($P < 0.005$) than at 37·5 °C (30 ± 3 %).

DISCUSSION

A model has been proposed which described potentiation of twitch tension at 37–38 °C in terms of processes with opposite effects (Krarup, 1981*a*). Thus, the degree of increase in the twitch tension in the staircase is determined by a process causing potentiation and by the simultaneous occurrence of a process that diminishes the twitch. The decay of the increased twitch tension after the staircase is the result of the rate of decay of the process causing potentiation and the recovery of the process causing diminution. The post-tetanic potentiation on the other hand is described in terms of two events of potentiation with markedly different rates of decay. A two-compartment model of post-tetanic potentiation was proposed, with only the compartment with a slow rate of decay being activated during the staircase. This model could also describe the findings in this study.

To investigate whether different processes are indeed involved in determining the size of the isometric twitch, the staircase and the post-tetanic potentiation were studied at different temperatures to obtain further temporal separation between the processes.

It is well known that the post-tetanic potentiation decreases when the muscle is cooled and Close & Hoh (1968) and Hoh (1974) found it to be absent in the extensor digitorum longus (EDL) at 20 °C. This was confirmed in this study, and the twitch was even decreased during the staircase. However, after the train of stimuli the muscle gradually developed potentiation, more delayed and more marked after the staircase than after the tetanus. In fact, when extrapolated to time zero the potentiation at 20 °C was about + 20 % after the tetanus and + 50 % after the staircase, suggesting that potentiation was produced in the train of stimuli but that the twitch tension was decreased by the event of diminution. The potentiation associated with a slow rate of decay at 37·5 °C decreased in size with cooling of the muscle but was present both after the staircase and after the tetanus at 20–37·5 °C. The rates of decay had similar Q_{10}. The potentiation with a fast rate of decay, present after the tetanus but not after the staircase at 37·5 °C, was absent below 30 °C. This may suggest that the processes responsible for the two compartments, assumed to be related to the excitation-contraction coupling (Krarup, 1981*a*), have different sensitivity to low temperature.

The absence of post-tetanic potentiation at 20 °C was attributed to more complete activation of fast-twitch fibres at low temperatures (Close & Hoh, 1968; Hoh, 1974). A greater activation at low temperature in cat muscle was also proposed by Ranatunga (1977*b*) and has in amphibian muscle been attributed to a longer duration of the action potential (Bastian & Nakajima, 1974; Costantin, 1975), to more easily released activator calcium at low temperature (Sakai, 1965), and to a delay in the reuptake of calcium by the sarcoplasmic reticulum (Podolsky & Stephenson, 1977). The potentiation in frog muscle at 0 °C was associated with a prolongation of the

contraction time and ascribed to a prolongation of the time during which the muscle was completely activated (Ritchie & Wilkie, 1955). In the EDL the contraction time was more prolonged per unit potentiation at 20 than at 37·5 °C which is compatible with potentiation at low temperature being due to a prolongation of the time during which the muscle is more fully activated. This suggestion is supported by the smaller increase in the rate of force development than in twitch tension. The contraction time in cat fast-twitch muscle was also more prolonged per unit increase in tension during double-responses at low than at high temperature (Table 1 of Ranatunga, 1977*b*), but the increase in the contraction time was attributed to the greater tension.

It was suggested in a previous report that repetitive contractions *per se*, independently of potentiation, may cause a shortening in the time course of the twitch (Krarup, 1981*a*). Maximal relative shortening of the contraction time occurred at about the 20th contraction in the staircase at all temperatures; the amount of shortening was decreased by the greater potentiation after fewer stimuli at higher temperatures. That the changes in twitch tension and in the contraction time did not occur in parallel may indicate that the two phenomena are separate. The shortening of the contraction time and the half-relaxation time could be due to a shortening of the time during which the muscle was activated, supported by the rate of force development at 20 °C being only slightly diminished during the staircase. At the end of the staircase and 2 sec after the tetanus the contraction time was increasingly prolonged the greater the potentiation. This could be attributed to the increased activation (Ranatunga, 1977*b*) or to a prolongation of the time during which the muscle was activated.

After the staircase at 20–30 °C the twitch tension increased initially rapidly and then at a slower rate. After the tetanus the twitch tension increased rapidly but there was no slow increase corresponding to that after the staircase.

The slow phase of increase after the staircase was attributed to the recovery of a process causing the twitch to be diminished. Cooling of the muscle slowed the recovery rate but at 20 °C it was still seven times faster than the rate of decay of potentiation. At 20 °C the diminution was clearly separated from the potentiation. At 37·5 °C the recovery rate was about three times faster than the rate of decay of potentiation, both having shorter time constants than at 20 °C. Thus, at 37·5 °C the process causing diminution was only present as a 'shoulder' on the decay curve of potentiation. The size of the process causing diminution was markedly pronounced by cooling. At 20 °C it was about 90 % of the size of the event of potentiation, whereas at 37·5 °C it was 25–30 %. This process of diminution has been suggested to be due to fatigue (Krarup, 1981*a*), a conclusion which was supported by the half-relaxation time of the twitch being increasingly prolonged the greater the diminution. After the tetanus the diminution was absent and the half-relaxation time was the more shortened the lower the temperature. The decline in twitch tension due to fatigue in frog muscle was shown to be associated with an increase in the depolarization necessary to bring about mechanical tension (Grabowski, Lobsiger & Lüttgau, 1972). These authors also found that the number of twitches necessary to cause fatigue was about three times less at low than at high temperature. The greater diminution at low temperature in the EDL may be related to the much larger tension-time area of the twitch at 20 than at 37·5 °C.

The rapid increase in twitch tension both after the staircase and after the tetanus at 20–30 °C could be due to the recovery of another process causing diminution, P_{D2}, with a time constant at 20 °C of 10–15 sec; that is 5–10 times faster than the recovery of the process causing diminution, P_D, only present after the staircase. At 20 °C the size of P_{D2} was only about one third that of P_D. The initial rapid recovery of twitch tension occurred at temperatures where the twitch tension decreased initially in the staircase. The tetanic tension at 20 °C also showed a decrement which was not due to failure of neuromuscular transmission. It could be suggested that the rapid recovery of twitch tension after the train of stimuli reflected the decrement in the staircase and the tetanus. The initial negative staircase, associated with a shortening in the time course of the twitch, could be due to a shortening of the activation time or possibly due to partial failure of propagation in the transverse tubular system. Partial failure of excitation could in itself cause shortening of the contraction time. Both the negative staircase and the initial rapid recovery were absent at 37·5 °C, perhaps due to either the absence of the deterioration in the contractile tension or to the diminution being minimized by potentiation. This latter possibility could explain the small decrement in twitch tension initially in the staircase at 37–38 °C after administration of Dantrolene Na (Krarup, 1981*b*).

The depolarization along the transverse tubular system (T-tubules) in frog muscle is a regenerative process (Costantin, 1970), and the action potential in the T-tubules probably has a lower safety factor (Huxley, 1974) and a slower propagation velocity than the potential along the surface membrane (Gonzáles-Serratos, 1971; Adrian & Peachey, 1973). A gradual deterioration of excitation along T-tubules may be due to the decreased rate of depolarization at low temperature or to a change in the ion concentration in the T-tubules by the repetitive depolarizations. A gradual deterioration of excitation of T-tubules at 20–30 °C in rat muscle may be analogous to the arrest of propagation of the action potential along the surface membrane in repetitively stimulated frog muscle fibres below 8 °C (Grabowski *et al.* 1972). The compound muscle action potential in the EDL was only slightly changed despite the marked decrement in twitch tension, indicating that partial block of propagation in the surface membrane was not the main cause of the deterioration of contractile tension at 20 °C. This confirms earlier findings in rat fast-twitch fibres where the intracellular action potential behaved qualitatively similar during trains of stimuli at 22 and 37–38 °C (Hanson, 1974).

The present study does not yield direct information as to the cause of the process causing diminution, P_{D2}. The event of diminution is assumed to be present throughout the staircase at 20–30 °C; in spite of the diminution the twitch was found to be potentiated. Potentiation may occur in the presence of gradual deterioration of the depolarization of the T-tubules, assuming that the membrane sites which are activated are also potentiated. This concept is at variance with the assumption that the increase in post-tetanic potentiation after the tetanus (Connolly, Gough & Winegrad, 1971) is caused by the transport of Ca from 'relaxation' to 'releasing' sites in the sarcoplasmic reticulum (Winegrad, 1970). A limitation in potentiation set by the transport of Ca is not compatible with the simultaneous occurrence during the staircase of processes with opposite effects, potentiation and diminution.

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