LATENCY-RELAXATION IN SINGLE MUSCLE FIBRES

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SUMMARY

1. Latency relaxation and twitch tension were recorded simultaneously in single isolated muscle fibres of *Xenopus laevis*.

2. During low frequency (0·6 or 1 pulse/sec) repetitive stimulation, three successive phases of twitch tension were observed: negative staircase (a slight drop in tension), positive staircase (about 15% increase in tension) and fatigue. At the same time the amplitude of latency relaxation decreased monotonically, and near the peak of positive staircase, the amplitude decreased almost to an undetectable level.

3. The application of caffeine (0·1–1·5 mM) increased peak twitch tension by 15–200%, but decreased the latency relaxation amplitude by 30–93%.

4. The application of Ca-release inhibitors, deuterium oxide and dantrolene sodium, caused a 43–89% decline in peak twitch tension but no change in latency relaxation amplitude.

5. The lack of correlation between changes in peak twitch tension and latency relaxation amplitude suggests that latency relaxation is associated with the mechanism which triggers Ca^{2+} release rather than with Ca^{2+} release itself.

INTRODUCTION

Latency relaxation is the brief relaxation of muscle which begins just before the twitch. Since it coincides with the sequence of events which couples excitation and contraction (E-C coupling), latency relaxation might be directly related to E-C coupling. If this were so, latency relaxation would be a useful tool for studying E-C coupling.

Many theories have been put forward to account for the latency relaxation (Sandow, 1944; 1945; Huxley, 1957; Sandow, 1966; Huxley & Brown, 1967; Hill, 1968; Peachey, 1968; Mulieri, 1972; Haugen & Sten Knudsen, 1976), but the underlying mechanism is still unknown. These theories can be classified into two groups: (1) the latency relaxation is the result of a change in either the compliance or equilibrium length of some elastic structure within the muscle fibres (probably the sarcoplasmic reticulum) and that these changes are caused by a sudden release of Ca^{2+} from the sarcoplasmic reticulum (Sandow, 1966). (2) The latency relaxation

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is the result of some forcible lengthening that occurs in the contractile substance (Sandow, 1947; Haugen & Sten-Knudsen, 1976) or, detachment of long-lasting cross bridges following Ca^{2+} release (Hill, 1968).

In order to test the idea that latency relaxation is directly related to Ca^{2+} release, we attempted to alter Ca^{2+} release from the sarcoplasmic reticulum by various means and observe the effect on both latency relaxation and twitch tension. Low frequency repetitive stimulation and application of caffeine were used as agents to potentiate Ca^{2+} release, D_2O and dantrolene sodium were used as agents to inhibit Ca^{2+} release.

METHODS

Single fibres were isolated from M. flexor brevis digit V of *Xenopus laevis*. Both the major and minor fibre diameters were measured and the geometric mean of these taken as the effective diameter. The tendons were cleared of dead fibre remnants and trimmed to about 1 mm in length. The fibre was tested for twitch response to a propagated action potential. One end of the fibre was held stationary by its tendon in a clamp, the other tendon was tied with fine silver wire to a short glass hook attached to a strain gauge. After mounting, the fibre was stretched to 140% of slack length, which would correspond to a sarcomere length of roughly 3 μm.

Stimulating current (0·05 msec duration) was applied through two platinum plates flanking the fibre along its full length. Twice threshold stimulation was used throughout the experiments in order to elicit the maximum latency relaxation response (Goodall, 1960; Mulieri, 1972). All experiments were performed at room temperature (22–25 °C).

A piezo-resistive strain gauge assembly (Pixie, Endevco, Mountain View, Calif., type 8260) was used in a bridge circuit whose output was proportional to force. The natural resonant frequency of this transducer, under experimental conditions, was approximately 600 Hz, measured from the base line 'noise' of experimental records.

The smallest signal that could be detected with the Pixie transducer was about 0·3 μN. This transducer is subject to slow base line drift, probably due to small, local temperature changes. The base line drift problem was overcome by mounting the transducer in a heat sink and also by using an automatic DC balance circuit developed by Nakajima, Gilai & Dingeman (1976). The bridge circuit output was fed into an amplifier (Philbrick, Dedham, Mass., Model 1027) with a gain of 23, then into the automatic DC balancing circuit which positioned the latency relaxation trace at a preselected position on the oscilloscope at the beginning of each sweep.

Resting potentials and action potentials were recorded intracellularly using 2–10 MΩ floating micro-electrodes (Heistracher & Hunt, 1969; Kirsch, Nichols & Nakajima, 1977).

RESULTS

The effect of low frequency repetitive stimulation

During the course of repetitive low frequency stimulation (0·6 or 1 pulse/sec), the amplitude of isometric twitch tension exhibits a phenomenon known as staircase and fatigue. The first few twitches are characterized by a transient decline in amplitude known as negative staircase (Colomo & Rocchi, 1963). Following this decline, twitch height slowly and steadily increased. This increase, known as positive staircase, reached a plateau and then began to decline as the fibre fatigued.

Fig. 1 illustrates single sweep records of latency relaxation and twitch tension evoked by repetitive massive stimulation of a single isolated fibre at 0·6 pulses/sec. Following each stimulus, there is a short delay of approximately 3·4 msec before the onset of positive tension (i.e. the point of which tension recrosses the base line);

this time period is known as the latent period (L_1). Before the development of positive tension, there is a relaxation of tension (latency relaxation) of maximal depth approximately 0.3 mN/mm^2 below resting tension level.

The lower trace in Fig. 1A is a record of tension during the twitch which followed the latency relaxation in the upper trace. The two traces in Fig. 1B illustrate latency relaxation and twitch tension in the same fibre as Fig. 1A, but at the peak of positive staircase. The amplitude of latency relaxation was approximately 40% of control. The latent period, L_1, remained constant at 3.4 msec and the rate of development of latency relaxation (approximated by the slope of the downward
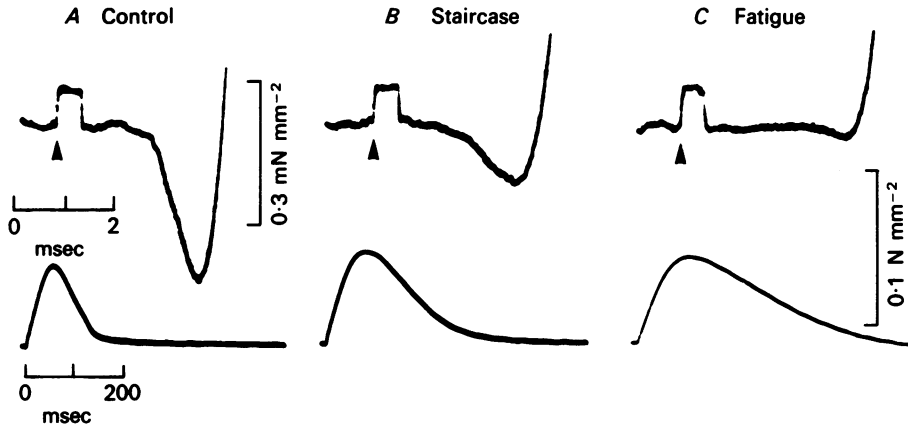

Fig. 1. The effects of low frequency repetitive stimulation on latency relaxation and twitch tension. Uppermost line: single sweeps (fast time base and high gain) records of tension change in response to $2 \times$ threshold massive stimulation (applied at the instant indicated by the arrow together with a 0.5 msec calibration pulse). Latency relaxation shown during three stages of repetitive stimulation: control (A) before repetitive stimulation, staircase (B) peak of twitch potentiation, and fatigue (C) where twitch height is noticeably decreased. Latency relaxation calibration bars in A refer also to B and C. Lower line of traces: twitch tension all at the same slow time base and low gain.

phase of the wave form) appears to be much reduced. Twitch height at the peak of staircase was approximately 115% of control. Fig. 1C shows an example of latency relaxation and twitch height during the beginning of fatigue. Latency relaxation nearly disappeared and L_1 increased by about 0.1 msec. The amplitude of the corresponding twitch decreased to 90% of the level at the peak of staircase.

The steady decline of latency relaxation amplitude during low frequency stimulation was observed in every fibre tested, and this confirms the results obtained with whole sartorius muscles (Sandow, 1945). On the other hand, twitch height followed a triphasic pattern of negative staircase, positive staircase and fatigue. Thus, there seemed to be no simple relationship between the height of twitch and the amplitude of latency relaxation.

The effects of caffeine

It is well known that caffeine, at concentrations of less than 2 mM, potentiates twitch tension without causing contracture. Endo (1975) has shown that the effect can be explained by an enhancement of the Ca-induced Ca release. Based on

experiments with skinned fibres, Endo suggested that in the absence of caffeine, the increase in free myoplasmic Ca during the twitch is not enough to trigger the Ca-induced Ca release mechanism, but in the presence of low levels of caffeine, this mechanism adds a small increment of Ca in addition to that which is normally released by the depolarization-induced Ca-release mechanism (Thorens & Endo, 1975). This extra Ca prolongs the active state and hence, increases the amplitude of the isometric twitch tension. Thus, if latency relaxation depends upon the release of Ca^{2+}, the caffeine potentiation of Ca^{2+} release might be expected to increase its amplitude.
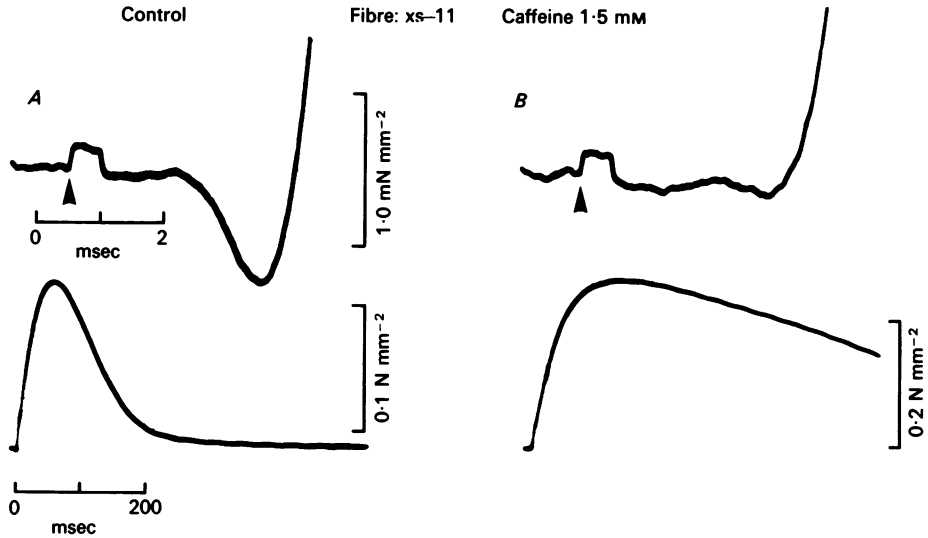

Fig. 2. The effects of low level of caffeine on latency relaxation and twitch. *A*, control records of latency relaxation (upper trace) and twitch (lower trace) in normal Ringer. *B*, latency relaxation and twitch records after caffeine application (1·5 mM). Latency relaxation records at high gain and fast time base. The arrow marks the onset of massive stimulation.

We applied low concentrations (0·1–1·5 mM) of caffeine to observe the effect on parameters of the twitch and latency relaxation. Fig. 2 illustrates the effect of 1·5 mM-caffeine on twitch tension and latency relaxation. The records show that L_1 is shortened by 0·3 msec in 1·5 mM-caffeine. Amplitude of latency relaxation was considerably reduced while twitch height increased to 200 % of the control.

The effects of E-C uncouplers

The effect of deuterium oxide (D_2O). Yagi & Endo (1974) have shown that in skinned frog fibres the primary effect of D_2O is to inhibit Ca-induced Ca release from the sarcoplasmic reticulum. But they argue that, in intact fibres, D_2O must inhibit tension by blocking the coupling mechanism which allows excitation of the T-system to be transmitted to the sarcoplasmic reticulum. Also, Kaminer & Kimura (1972) using aequorin, have shown that in barnacle muscle, D_2O blocks Ca^{2+} release.

We tested the effect of 99·8 % D_2O replacement of H_2O in Ringer solution on three fibres and found in each case no apparent reduction of amplitude of latency
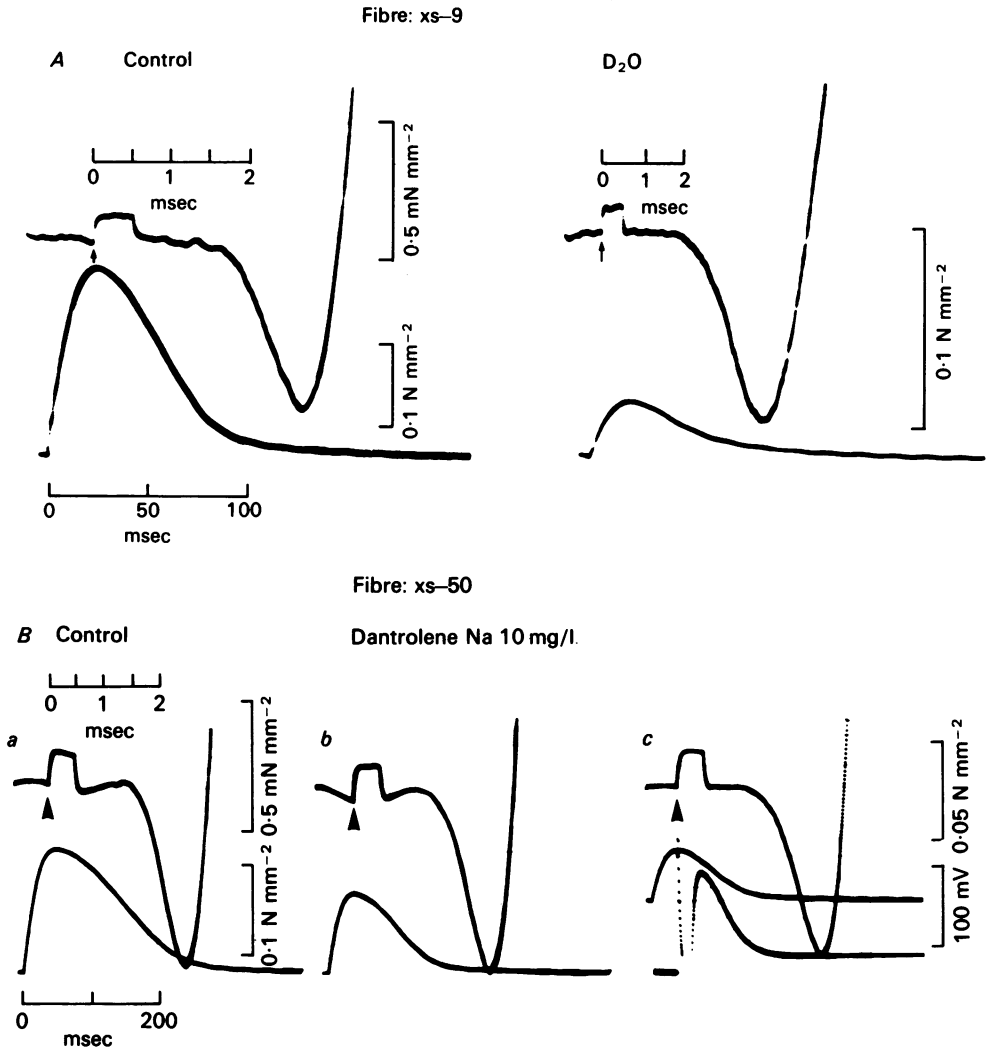
Fig. 3. The effects of E-C uncouplers on latency relaxation and twitch. *A*, effect of D₂O. Left-hand column illustrates the control in normal Ringer; latency relaxation (upper trace; high gain, fast time base) and twitch (lower gain, slow time base). Right-hand column illustrates the effects of D₂O. The latency relaxation record is at the same gain as the control latency relaxation but at a slow time base. The twitch record is at a higher gain as the control twitch but at the same time base. *B*, effects of dantrolene sodium (DaNa) on latency relaxation and twitch. (*a*) control before DaNa application. Upper horizontal time and vertical tension calibration bars refer to latency relaxation record. Lower calibration bars refer to twitch record. (*b*) Tension records in 10 mg/l. DaNa-Ringer solution. Same calibration as in (*a*), (*c*). Tension records (upper and middle traces) and action potential record (lower) in DaNa-Ringer. Same time and latency relaxation tension calibration as (*a*) and (*b*). Twitch tension gain reduced. 100 mV calibration bar refer to the action potential. Action potential time base was the same as the latency relaxation trace. Massive stimulation applied at the point indicated by the arrow.

relaxation. Representative results are illustrated in Fig. $3A$. The lower traces show that D_2O reduced twitch height to 12 % of control. The amplitude of latency relaxation, in contrast, was nearly unchanged compared to control. D_2O altered the length of the latent period; L_1 increased from 2·8 to 4·7 msec. Similar results, obtained from other fibres, are presented in Table 1. These results agree with those obtained by Sandow, Pagala & Sphicas (1976) from whole muscle treated with D_2O.

TABLE 1. The effect of repetitive low frequency stimulation
and pharmacological agents on contraction

Treatment		Control		Treated fibres	
		Twitch amplitude $(10^{-2}\ N\ mm^{-2})$	Latency relaxation amplitude $(10^{-5}\ N\ mm^{-2})$	Twitch amplitude $(10^{-2}\ N\ mm^{-2})$	Latency relaxation amplitude $(10^{-5}\ N\ mm^{-2})$
Stimulation at 1/sec	Mean ± S.D.	11·0 ± 2·9	56·8 ± 13·1	12·7 ± 1·8	6·8 ± 6·0
Stimulation at 0·6/sec	Mean ± S.D.	9·5 ± 3·5	56·3 ± 18·9	10·8 ± 2·7	16·3 ± 6·0
Caffeine (1·5 mM)		13·4	61·4	27·7	10·8
Ringer D_2O (99·8%)	Mean ± S.D.	15·3 ± 6·2	60·1 ± 29·2	1·8 ± 0·74	57·7 ± 24·3
DaNa (10 mg/l.)	Mean ± S.D.	12·4 ± 1·0	57·4 ± 10·3	7·6 ± 0·2	52·6 ± 9·1

The effect of dantrolene Na (DaNa). Evidence accumulated by many investigators (e.g. Gilly & Costantin, 1974; Hainaut & Desmedt, 1974; Brocklehurst, 1975; Van Winkle, 1976; Morgan & Bryant, 1977) indicates that DaNa reduces twitch tension by specifically inhibiting Ca^{2+} release without affecting the rate of Ca^{2+} uptake or the contractile proteins. Based on the hypothesis that latency relaxation is related to Ca^{2+} release, DaNa should inhibit it. The results of an experiment to test the effect of DaNa on latency relaxation and twitch tension is presented in Fig. $3B$. Whereas twitch tension (lower trace in Fig. $3B$) declines to approximately 60 % of control in DaNa, latency relaxation amplitude (upper trace in Fig. $3B$) remained nearly constant. Furthermore, DaNa appeared to have no significant effect on the duration of the latent period, as compared to the control. Qualitatively, there appeared to be no change in the rate of latency relaxation development. The bottom trace in Fig. $3B$ shown an intracellular recording of the action potential in DaNa which appears to be normal in amplitude and shape. The initial rise of the action potential is obscured by the stimulus artifact caused by massive stimulation.

Table 1 summarizes the effects of Ca^{2+} release potentiators and inhbitors on twitch tension, and the amplitude of latency relaxation, showing that in all cases an increase in twitch was accompanied by a decrease in latency relaxation amplitude.

DISCUSSION

The purpose of these experiments was to test the hypothesis that latency relaxation is a mechanical signal resulting from the release of activator Ca^{2+} from the sarcoplasmic reticulum (Sandow, 1945, 1947, 1966; Multieri, 1972; Herbst & Piontek, 1975). We found that twitch height could be altered by agents which interfere with Ca^{2+} release, but that there was an inverse relationship between twitch and latency relaxation amplitudes.

It is believed (Sandow, 1944, 1973; Lowy & Sten-Knudsen, 1963; Mulieri, 1972) that latency relaxation amplitude is determined by opposing processes; one which generates negative tension and another which generates positive tension. According to Sandow, the positive tension-generating process, which is responsible for the twitch, begins soon after the onset of latency relaxation. Because of this overlap, any decrease in the latent period may 'mask' the expression of the negative tension-generating process and thus, reduce latency relaxation amplitude. However, when one looks at Fig. 2 (the effect of caffeine), it would be difficult, although not impossible, to explain the large reduction of latency relaxation by a very small decrease of latent period. During repetitive low frequency stimulation in normal Ringer, we did not find a progressive shortening of the latent period which might have explained the observed monotonic decline of latency relaxation amplitude. Also, at the beginning of fatigue, when twitch height was still 80 % of normal (Fig. 1*C*), latency relaxation was completely absent and the latent period was actually longer than control. Thus, the onset of twitch development could not have masked the latency relaxation in this case.

Another result which is not explained by the 'masking' hypothesis has recently been observed by Sandow and his coworkers (Taylor, Preiser & Sandow, 1972; Pagala & Sandow, 1976). They found that the drug physostigmine, which is known to cause prolongation of the action potential, 'decreases the latency relaxation amplitude in striking opposition to the associated increase in twitch tension and peak time'. This result also cannot be explained by the 'masking effect' of twitch onset.

In the case of D_2O, an 'unmasking effect' is not likely to explain the observation that latency relaxation amplitude was unchanged since the suppression of twitch development rate would have to be exactly offset by the suppression of the development of latency relaxation rate. A more reasonable explanation is that latency relaxation is not directly related to Ca^{2+} release.

We found that after the application of DaNa, twitch height declined by about 40 % and both latency relaxation amplitude and latent period remained nearly constant. In this case, an unmasking effect cannot account for the observation that latency relaxation amplitude did not change when Ca^{2+} release was inhibited, since the latent period appeared to be unaffected.

In conclusion, our results do not support the idea that latency relaxation is caused by Ca^{2+} release from the sarcoplasmic reticulum. Furthermore, our experiments with the Ca^{2+} release inhibitors appear to contradict this hypothesis. If the sarcomere elongation process as proposed by Haugen & Sten-Knudsen (1976), were caused by Ca^{2+} binding to the thin filaments, then elongation, and hence, the latency relaxation ought to be very much reduced by D_2O. Likewise, if Ca^{2+} binding

to thin filaments suddenly increased the recycling rate of cross bridges as proposed by Hill (1968), the rate of increase should be inhibited in D_2O since Ca^{2+} release is reduced. These theories predict a decrease in latency relaxation after D_2O application, in contradiction to our finding. However, it should be mentioned that these theories are not altogether ruled out by the present results since Ca^{2+} release in the presence of DaNa or D_2O was not totally abolished.

A possible hypothesis of the origin of latency relaxation which is consistent with our results, simply requires the latency relaxation to be associated with the mechanism which triggers Ca^{2+} release rather than with Ca^{2+} release itself. According to this scheme, Ca^{2+} release inhibitors such as DaNa and D_2O affect the E-C coupling step which occurs after latency relaxation or a triggering process has already taken place, whereas caffeine affects the E-C coupling step before the development of latency relaxation. This hypothesis could explain our observation that Ca^{2+} release inhibitors do not change latency relaxation but affect the twitch tension, and that caffeine affects both the latency relaxation as well as the twitch.

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