J. Physiol. (1986), **375**, *pp.* 269–281
With 7 text-figures
Printed in Great Britain

MEASUREMENT OF INTRACELLULAR CALCIUM DURING THE DEVELOPMENT AND RELAXATION OF TONIC TENSION IN SHEEP PURKINJE FIBRES

By D. A. EISNER and M. VALDEOLMILLOS
From the Department of Physiology, University College London, Gower St, London WC1E 6BT

(*Received 24 September 1985*)

SUMMARY

1. The photoprotein aequorin was micro-injected into several cells in a sheep Purkinje fibre. The intracellular Ca concentration ($[Ca^{2+}]_i$) was measured from the resulting light emission.

2. Inhibition of the Na–K pump with strophanthidin resulted in the development of tonic tension which increased on depolarization. This increase was accompanied by an increase of aequorin light. Increasing external Ca concentration ($[Ca^{2+}]_o$) or the magnitude of the depolarization increased both light and tension.

3. If the depolarizing pulse was maintained for several minutes then both tonic tension and aequorin light slowly decayed. The relationship between tension and light was unaffected during this decay. On repolarization the light decayed to below the level before the depolarization before slowly increasing. During this period a test depolarization produced increases of aequorin light and tension which were smaller than control.

4. The application of ryanodine (1–10 μM) abolished all components of tension other than the tonic component. Under these conditions the time course of the increase of tonic tension and aequorin light on depolarization was sufficiently slow to be measured. In most (five out of six) experiments the relationship between light and tension during this development of tonic tension was found to be similar to that during the subsequent spontaneous decay. However, in one experiment the decay of force was greater than could be accounted for by the fall of $[Ca^{2+}]_i$.

5. It is concluded that most of the spontaneous relaxation of tonic tension can be attributed to a fall of $[Ca^{2+}]_i$ rather than to other explanations such as an intracellular acidification or increase of inorganic phosphate concentration.

INTRODUCTION

Under some conditions depolarization of the surface membrane of cardiac muscle produces a tonic component of tension which is maintained throughout depolarizing pulses lasting for several seconds (Eisner & Lederer, 1979). It has been suggested that this tonic tension may be produced by Ca entry into the cell on a voltage-dependent Na–Ca exchange (Chapman & Tunstall, 1980; Eisner, Lederer & Vaughan-Jones,

1983). In the Purkinje fibre tonic tension is a steep function of the intracellular Na concentration ($[Na^+]_i$) which is consistent with it being produced by a Na–Ca exchange (Eisner *et al.* 1983). If this tonic tension is indeed produced by Na–Ca exchange then its time course of development and decay should give information about the contribution of this exchange to regulating internal Ca concentration ($[Ca^{2+}]_i$).

There are, however, two problems with previous studies of tonic tension. First its development during depolarization is superimposed on that of the normal twitch and its relaxation, on repolarization, is obscured by an after-contraction which, like the twitch, appears to be produced by the release of Ca ions from the sarcoplasmic reticulum. Secondly, the measurement of tonic tension is complicated by the presence of spontaneous oscillations of $[Ca^{2+}]_i$ produced by spontaneous release of Ca ions from the sarcoplasmic reticulum (Orchard, Eisner & Allen, 1983; Wier, Kort, Stern, Lakatta & Marban, 1983). These oscillations are out of phase in the different cells of the preparation and the result is that the total tension generated by the preparation may be very different from that expected from the mean value of $[Ca^{2+}]_i$ (Orchard *et al.* 1983; Wier *et al.* 1983; Cannell, Vaughan-Jones & Lederer, 1985). Furthermore the magnitude of the tonic tension will depend, in part, on the properties of the oscillations. In order to remove these problems it is necessary to abolish these Ca oscillations.

If depolarization is maintained for several minutes, tonic tension gradually decays (Gibbons & Fozzard, 1971; Chapman, 1973; Eisner *et al.* 1983). This fall of tension is accompanied by a decrease of $[Na^+]_i$ (Eisner *et al.* 1983) and it has been attributed to an effect of the decrease of $[Na^+]_i$ to decrease Ca entry on Na–Ca exchange. In these experiments changes of $[Ca^{2+}]_i$ were inferred from those of tension. It has, however, been shown that prolonged depolarization can produce an intracellular acidification (Cannell, Lederer & Vaughan-Jones, 1984) which will decrease tension (Fabiato & Fabiato, 1978). It is therefore possible that the fall of tension on prolonged depolarization is produced by the intracellular acidification rather than by a fall of $[Ca^{2+}]_i$. The effects of membrane potential on $[Ca^{2+}]_i$ have only been measured directly in three studies. Sheu & Fozzard (1982) found that depolarization increased $[Ca^{2+}]_i$ as measured with a Ca-selective micro-electrode. Allen, Eisner & Orchard (1984) (using aequorin) and Powell, Tatham & Twist (1984) (using quin 2) found that the initial rise of $[Ca^{2+}]_i$ is followed by a subsequent fall on prolonged depolarization. However, in these studies the membrane potential was changed by elevating extracellular potassium concentration ($[K^+]_o$) and the results may be complicated by direct effects of $[K^+]_o$ rather than of membrane potential (cf. Sjodin & Abercrombie, 1978). A further problem is produced by the presence of spontaneous Ca oscillations: a change in the magnitude of the oscillations rather than the mean level of $[Ca^{2+}]_i$ could account for the spontaneous relaxation of tonic tension.

In the present study we have therefore measured tension and $[Ca^{2+}]_i$ while the membrane potential was controlled with a voltage clamp. Furthermore, in some experiments, we have used pharmacological methods to remove the Ca oscillations. We have attempted to answer the following questions. (1) What is the response of $[Ca^{2+}]_i$ to maintained depolarization produced by the voltage clamp? (2) Can the

spontaneous relaxation of tonic tension on maintained depolarization be accounted for by changes of mean $[Ca^{2+}]_i$?

A preliminary version of some of these results has been presented to the Physiological Society (Eisner & Valdeolmillos, 1985*a*).

METHODS

The experiments were performed on sheep cardiac Purkinje fibres shortened to 2 mm length and connected to a tension transducer in the experimental bath. A conventional two micro-electrode voltage-clamp system was used in some experiments to control the membrane potential. Aequorin was micro-injected into several (ten to twenty) cells. The general methods used for recording force and aequorin light from voltage-clamp fibres have been described previously (Eisner & Lederer, 1979; Eisner & Valdeolmillos, 1985*b*).

Calibration of aequorin signals

In order to transform aequorin luminescence to $[Ca^{2+}]_i$ we have used the procedure of Allen & Blinks (1979). At the end of the experiment 1% (v/v) Triton X-100 was added to the superfusing solution in order to destroy the cell membranes and therefore react all the aequorin with Ca. The fractional luminescence (L/L_{max}) corresponding to any rate of aequorin light emission in counts per second (c.p.s.) can then be calculated as:

$$L/L_{max} = \text{c.p.s.}/(2{\cdot}8 \times (\text{total no. of counts on exposure to Triton})),$$

where $2{\cdot}8\ s^{-1}$ is the rate constant of aequorin consumption at saturating $[Ca^{2+}]_i$ under the experimental conditions (35 °C). In experiments in which a significant fraction of the aequorin was consumed before adding Triton then this consumption must also be allowed for. To relate L/L_{max} to $[Ca^{2+}]_i$ requires using an appropriate *in vitro* calibration curve. We have used a curve obtained under conditions designed to simulate the intracellular environment (155 mM-K, 3 mM-Mg, pH 7·0, 30 °C). This curve can be described by the following equation (modified from that given by Allen, Blinks & Prendergast, 1977):

$$[Ca^{2+}] = \frac{7 \times 10^{-5}(L/L_{max})^{0{\cdot}33} - 4 \times 10^{-7}}{1 - (L/L_{max})^{0{\cdot}33}}.$$

There is a controversy concerning the exact level of $[Mg^{2+}]$ in muscle cells. The value of 3 mM used in our calibrating solutions comes from the work of Hess, Metzger & Weingart (1982). However, Fry (1986) has recently reported a value of 1·9 mM whereas Blatter & McGuigan (1986) report 0·5 mM. If $[Mg^{2+}]$ is say 0·5 mM then the real values of $[Ca^{2+}]_i$ will be less than that predicted above. However, over the range of concentrations of $[Ca^{2+}]_i$ in the present paper the correction factor is fairly constant and as $[Ca^{2+}]_i$ rises from 0·3 to 30 μM the correction will change from about 2·5 to 2·3. If $[Mg^{2+}]_i$ is intermediate then the correction will be correspondingly less. Therefore the error produced by uncertainty in $[Mg^{2+}]_i$ will scale the level of $[Ca^{2+}]_i$ but will not affect the measured time course of changes of $[Ca^{2+}]_i$.

It has been shown that depolarization produces an intracellular acidification. Acidification is known to produce a small depression of aequorin light output at constant $[Ca^{2+}]$. Published data on this effect (e.g. Allen & Orchard, 1983) would suggest that this effect is trivial. We have reinvestigated this problem. Previous work has used EGTA to buffer $[Ca^{2+}]$. However, EGTA is very pH-dependent and this complicates using it as a Ca buffer when pH is changed. We have therefore used the comparatively pH-insensitive Ca buffer BAPTA (Bis(*o*-aminophenoxyl)ethane-*N*,*N*,*N'*,*N'*,tetraacetic acid). pH was changed from 7·3 to 6·8 at a fixed $[Ca^{2+}]$ of 0·5 μM. This produced less than a 5% change of light emission.

Solutions

The experimental solution contained (mM): NaCl, 145; $CaCl_2$ 5; KCl, 5; $MgCl_2$, 1; glucose, 10. It was buffered to pH 7·3 with 5 mM-Tris-HEPES. Changes in $CaCl_2$ were made without osmotic compensation. All experiments were performed at 35 °C. Strophanthidin (Sigma U.K.) was kept

as a stock solution (10 mM in ethanol). Ryanodine (a generous gift from Dr J. Kenyon: University of Texas at Dallas) was kept frozen in a solution of 10 mM in water.

RESULTS

The experiment illustrated in Fig. 1 shows the effects of inhibiting the Na–K pump with strophanthidin on aequorin light and tension during a depolarizing voltage-clamp pulse. The control record (Fig. 1*A*) shows that depolarization produces a transient rise of aequorin light and also a twitch. The record shown in Fig. 1*B* had been

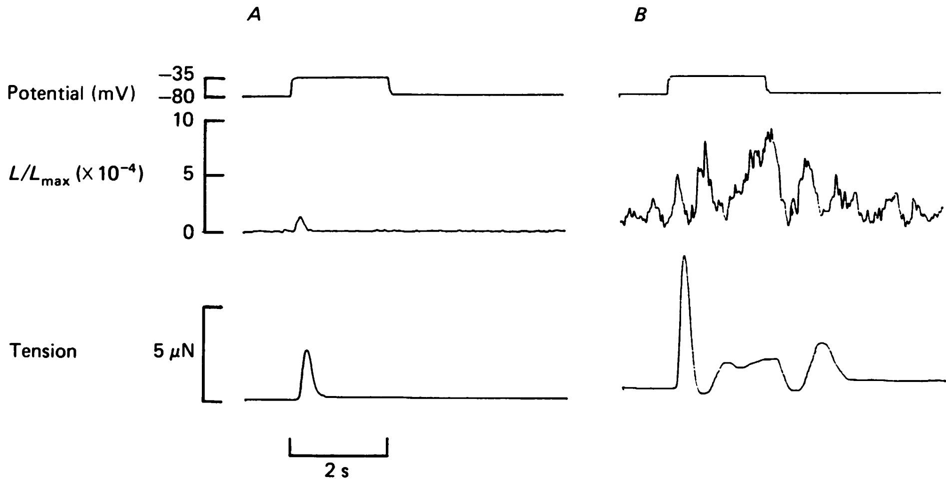


Fig. 1. The effects of strophanthidin on aequorin light and tension. In both panels traces show signal-averaged records of: top, membrane potential; middle, aequorin light (DC-5 Hz); bottom, tension. Sixty-four traces were averaged in *A* and four in *B*. A depolarizing pulse was applied from −80 to −35 mV at 0·17 Hz. Panels show: *A*, control; *B*, 20 min after adding strophanthidin (10 μM).

obtained after exposure to strophanthidin (10 μM). The twitch is considerably increased and an after-contraction is seen on repolarization. Most pertinent to the present paper is the development of tonic tension during the depolarizing pulse. The aequorin light record shows that the tonic tension is accompanied by an increase of aequorin light. Superimposed, however, are oscillations of light. The effects of depolarization to different levels are shown in Fig. 2*A*. It is clear that, the larger the depolarization, the larger the rise of both tension and aequorin light. Fig. 2*B* shows the effects of similar depolarizing pulse after elevating $[Ca^{2+}]_o$ from 5 to 10 mM. At a given potential both tension and aequorin light are greater than in the control. Fig. 3 shows tension plotted as a function of aequorin light for the data of Fig. 2. Although there is more tension and light at the higher $[Ca^{2+}]_o$, the relationship between tension and light does not appear to be markedly affected by elevating $[Ca^{2+}]_o$.

The above experiments have concentrated on pulses which are not much longer than the time taken for force to reach a maximum value. The effects of a longer pulse are shown in Fig. 4. In agreement with previous work (Eisner *et al.* 1983), following the initial rise, tonic tension gradually decays to a lower level. The aequorin record

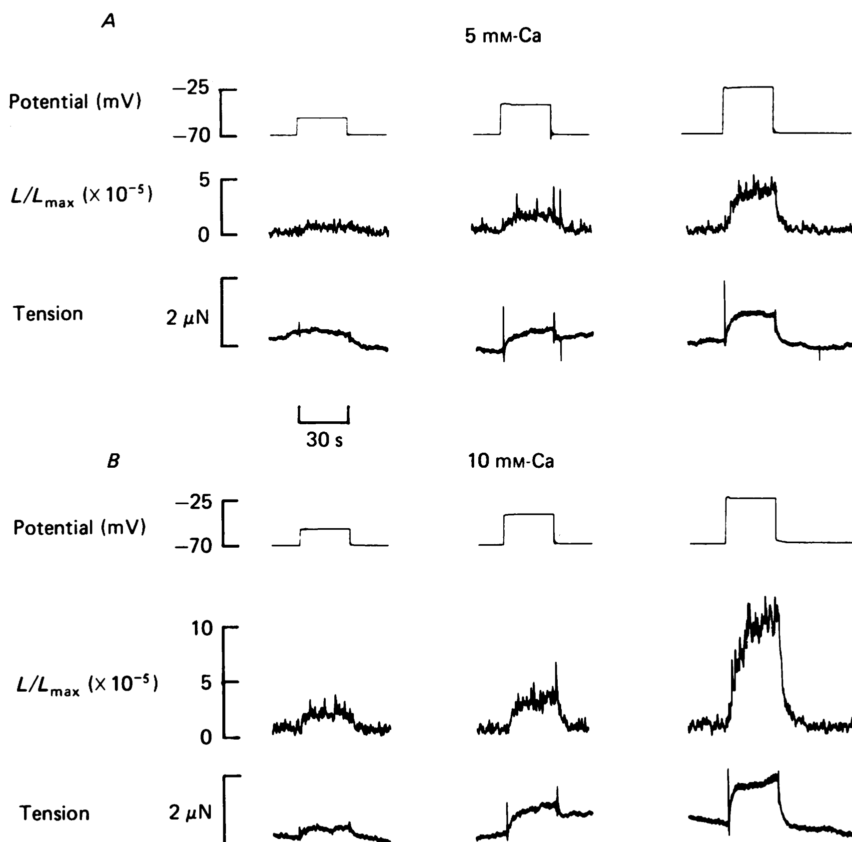


Fig. 2. The effects of changing membrane potential and $[Ca^{2+}]_o$ on aequorin light and tension. In all panels traces show unaveraged records of: top, membrane potential; middle, aequorin light (DC-2 Hz); bottom, tension. The preparation was exposed to 10 μM-strophanthidin throughout. A 30 s depolarizing pulse was applied from −70 mV to the various levels shown. *A*, $[Ca^{2+}]_o$ 5 mM; *B*, $[Ca^{2+}]_o$ 10 mM.

also shows a similar decay. On repolarization both force and light undershoot below the control level and then slowly increase. An important question is whether the fall of $[Ca^{2+}]_i$ can account for the decrease of tension or whether some additional mechanism such as an intracellular acidification must also be invoked. This point was examined by comparing the relationship between tension and light during the decay of tension on prolonged depolarization with that produced by the shorter pulses of Fig. 2. If the fall of force during the long pulse is indeed due to a decay of $[Ca^{2+}]_i$ then the relationship between $[Ca^{2+}]_i$ and force during this decay should be the same as that produced by shorter pulses. On the other hand, if the fall of force is due to some factor other than a decay of $[Ca^{2+}]_i$ then the short pulse should have more force for the same light than the long pulse. The graph of Fig. 3 shows that the relationship between light and tension during the relaxation of the prolonged depolarization is similar to that produced by smaller short pulses.

It is difficult to quantify the levels of $[Ca^{2+}]_i$ measured in the previous experiments

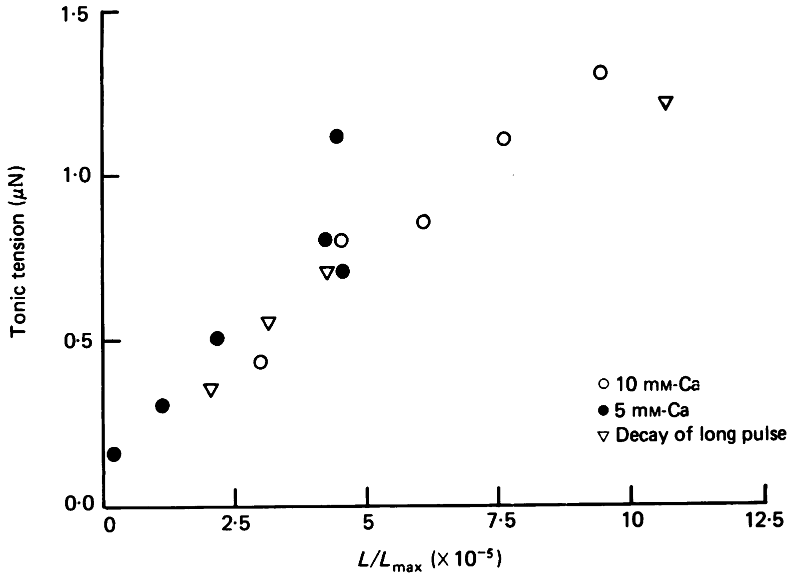


Fig. 3. The relationship between aequorin light and tonic tension. The graph shows tonic tension (ordinate) plotted as a function of aequorin light. Measurements were made at the end of a 30 s duration depolarizing pulse. The data are taken from the experiment of Fig. 2. and also include pulses not shown in Fig. 2. The different points were obtained by depolarizing to various levels. Symbols denote: (●), $[Ca^{2+}]_o$ 5 mM; (○) $[Ca^{2+}]_o$ 10 mM. The points indicated (▽) were obtained from the relaxation of tension shown in the experiment of Fig. 4.

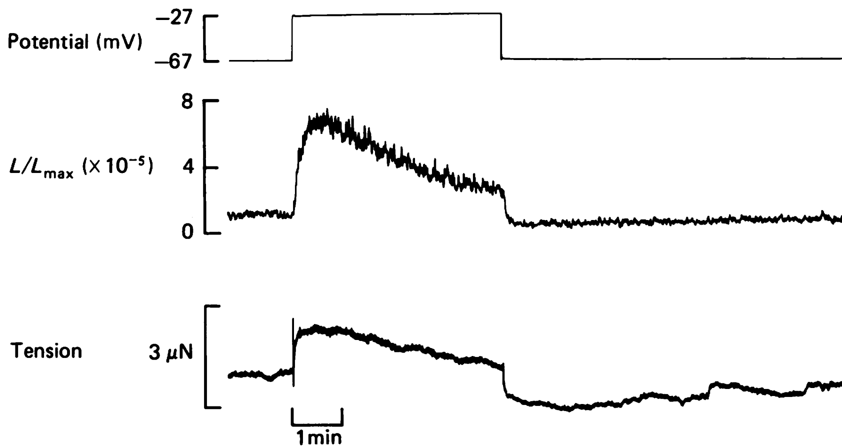


Fig. 4. The effects of prolonged depolarization on aequorin light and tension. Traces show: top, membrane potential; middle, aequorin light (DC-2 Hz); bottom, tension. The preparation had been exposed to strophanthidin (10 μM) for 40 min. It was depolarized from −67 to −27 mV for the period shown. The relation between aequorin light and tension for the decay of tonic tension during depolarization is shown in Fig. 3.

because of the presence of spontaneous oscillations of $[Ca^{2+}]_i$. For this reason in the rest of the paper we have added ryanodine to remove Ca oscillations (Wier *et al.* 1983; Eisner, Orchard & Allen, 1984). The addition of ryanodine also has two other advantages. First it abolishes the twitch and after-contractions (Sutko & Kenyon, 1983) which therefore makes it possible to study the time course of tonic tension. Secondly it slows the development of tonic tension (Sutko & Kenyon, 1983) which (see below) makes it easier to investigate the relationship between tension and $[Ca^{2+}]_i$.

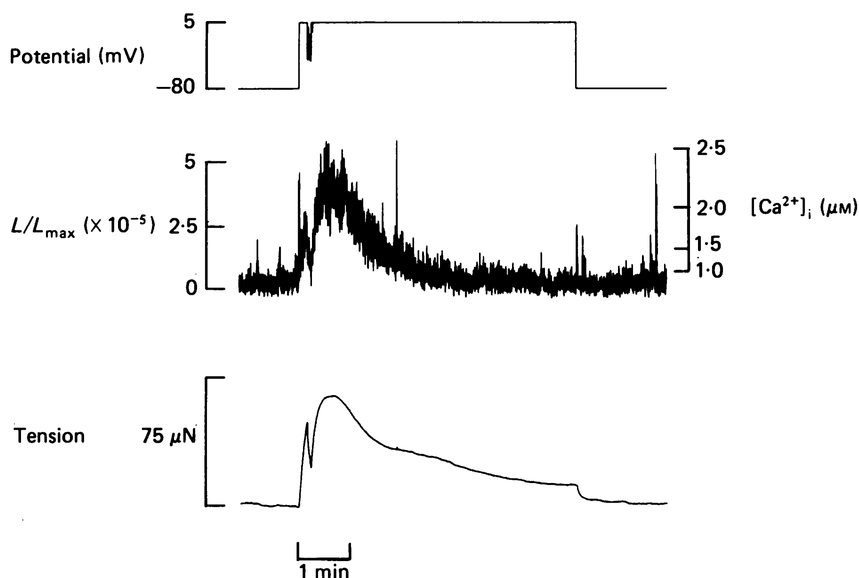


Fig. 5. The effects of prolonged depolarization on aequorin light and tension in the presence of ryanodine. The preparation had been exposed to strophanthidin (10 μM) for 50 min and to ryanodine (1 μM) for 15 min before the record was obtained. Traces show: top, membrane potential; middle, aequorin light (DC-1·5 Hz); bottom, tension. The preparation was depolarized from −80 to −5 mV for the period shown apart from a brief period when it was partly repolarized. The aequorin light record is noisier in this than in other records because considerably less aequorin was injected.

Tonic tension and $[Ca^{2+}]_i$ *in the absence of Ca oscillations*

The response of tension and $[Ca^{2+}]_i$ to prolonged depolarization in the presence of ryanodine is shown in Fig. 5. The results are qualitatively similar to those obtained in the absence of ryanodine: depolarization produces an initial increase followed by a slow decrease of tonic tension. It is clear that the initial rise of tonic tension is slow. In this experiment a brief repolarizing pulse was applied shortly after beginning the depolarization. The relaxation of tension and aequorin light produced by repolarization is considerably faster than those of the spontaneous falls seen on prolonged depolarization.

In the experiment of Fig. 6, the preparation was again depolarized, and $[Ca^{2+}]_i$ and tension rose to a peak level before spontaneously decaying. The membrane was then repolarized. A test depolarization applied after about 30 s produced no

detectable increase of aequorin light and a very small increase of tension. However, after a further 5 min period at −70 mV, depolarization produced a larger increase of $[Ca^{2+}]_i$ and tension. The increase of $[Ca^{2+}]_i$ is, however, still smaller than control. Finally, after a further 5 min, depolarization produced an increase of $[Ca^{2+}]_i$ which was back to control. The slow repriming of tonic tension following a test depolarization has been described previously (Eisner *et al.* 1983) and has been shown to be associated with an increase of $[Na^{+}]_i$. The present work shows that it is accompanied by a slow repriming of $[Ca^{2+}]_i$.

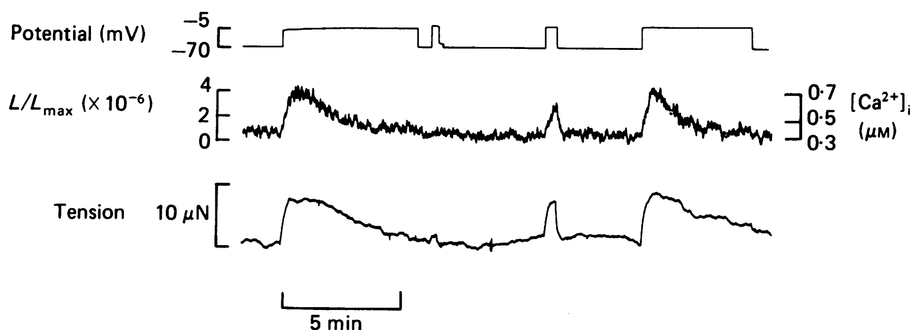


Fig. 6. The repriming of aequorin light and tonic tension following a prolonged depolarization. The preparation had been exposed to strophanthidin (10 µM) for 40 min and to ryanodine (1 µM) for 10 min before the record was obtained. Traces show: top, membrane potential; middle, aequorin light (DC-1·5 Hz); bottom, tension.

The test pulses in Fig. 6 are considerably shorter than the initial control depolarization. This was done to minimize the changes of $[Na^{+}]_i$ produced by long pulses. However, the attenuation of the rise of aequorin light cannot be due to the fact that the pulses are shorter since the initial rates of rise of light are smaller in the test pulses than in the initial control depolarization.

The fact that the initial rises of both tension and $[Ca^{2+}]_i$ are slowed by ryanodine allows one to compare the relationship between $[Ca^{2+}]_i$ and tension both during the development of force on depolarization and during the spontaneous relaxation. This comparison cannot be made in the absence of ryanodine since the fast kinetics of the twitch mean that force may not be in equilibrium with $[Ca^{2+}]_i$. Such a comparison has been made for the experiment of Fig. 7*A* which shows the effects of prolonged depolarization in the presence of ryanodine. In this Figure the aequorin light records have also been converted to $[Ca^{2+}]_i$. Fig. 7*B* shows that the relationship between tension and aequorin light during the development of tonic tension is similar to that seen during the spontaneous relaxation.

In five out of six preparations studied, results similar to that of Fig. 7 were obtained. However, in the other preparation, only 60% of the decay of force could be accounted for by a fall of $[Ca^{2+}]_i$.

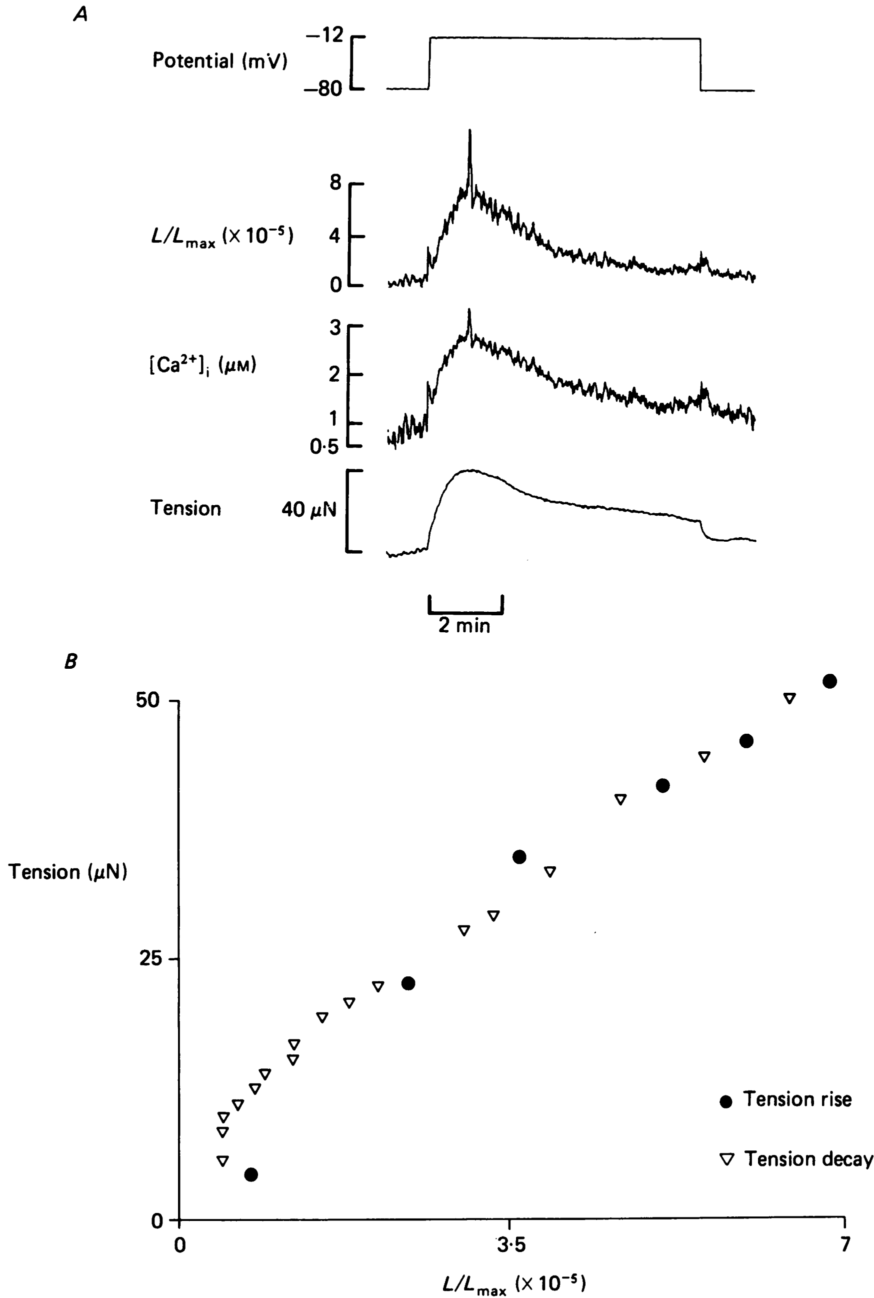


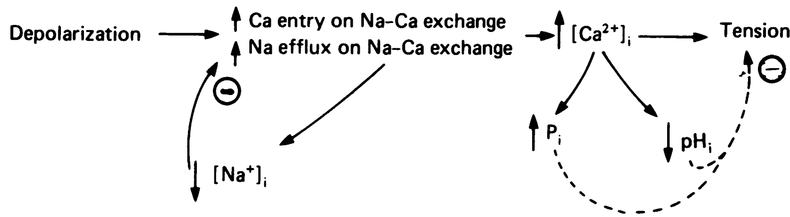
Fig. 7. The relationship between aequorin light and tonic tension during both the development and the spontaneous relaxation of tonic tension. *A*, original record. Traces show (from top to bottom): membrane potential; aequorin light (DC-1·5 Hz); calculated $[Ca^{2+}]_i$; tension. The preparation had been exposed to strophanthidin (10 μM) for 45 min and to ryanodine (1 μM) for 20 min. A depolarizing pulse was applied from −80 to −12 mV. *B*, graph of tension as a function of aequorin light. Data taken from *A*. The symbols denote: (●), during the development of tonic tension; (▽) during the spontaneous relaxation.

DISCUSSION

The present work has shown that depolarization produces an increase of $[Ca^{2+}]_i$ which develops over a few seconds and then decays over a few minutes. On repolarization the capacity to develop a rise of $[Ca^{2+}]_i$ 'reprimes' slowly. The demonstration of the spontaneous relaxation of $[Ca^{2+}]_i$ confirms the result of previous work in which membrane potential was changed by varying $[K^+]_o$ (Allen *et al.* 1984*a*; Powell *et al.* 1984). Prolonged depolarization has been shown to result in a secondary decay of tonic tension in a variety of cardiac preparations (Chapman, 1973; Eisner *et al.* 1983; Allen *et al.* 1984). Measurements with Na-sensitive micro-electrodes have found that depolarization decreases $[Na^+]_i$ (Eisner, Lederer & Vaughan-Jones, 1981, 1983; January & Fozzard, 1984) and it was therefore suggested that this fall of $[Na^+]_i$ might, by acting on Na–Ca exchange, decrease $[Ca^{2+}]_i$ and thence tension. However, as pointed out previously (Eisner *et al.* 1983), it was possible that the secondary fall of force could be produced by some factor other than a decrease of $[Ca^{2+}]_i$. This alternative explanation gained support from the demonstration of an intracellular acidification during prolonged depolarization (Cannell *et al.* 1984). Such an acidification would be expected to decrease the amount of force produced by a given $[Ca^{2+}]_i$ (Fabiato & Fabiato, 1978). The origin of the acidification is unclear. It may be a direct consequence of the increase of $[Ca^{2+}]_i$ (Vaughan-Jones, Lederer & Eisner, 1983) or, alternatively, be secondary to an increase of lactic acid production by anaerobic glycolysis as a consequence of the increased ATPase activity of the contractile apparatus (Allen, Eisner, Morris, Pirolo & Smith, 1986). Furthermore, manoeuvres which increase $[Ca^{2+}]_i$ also increase the intracellular inorganic phosphate concentration (Hoerter, Miceli, Jacobus, Renlund, Gerstenblith & Lakatta, 1983; Allen *et al.* 1986) which will also decrease force (Kentish, 1986).

In order to decide whether the fall of tension is produced by (i) a decrease of $[Ca^{2+}]_i$ or (ii) some other factor we have compared the time course of change of aequorin light with that of tension. Fig. 3 showed that the relationship between tension and light during the decay of force on prolonged depolarization was similar to that produced by varying the magnitude of briefer pulses. This result suggests that the fall of $[Ca^{2+}]_i$ (as measured by aequorin) is responsible for the fall of force. Two problems should, however, be discussed here. (1) It is possible that some factor other than a fall of $[Ca^{2+}]_i$ is responsible for the fall of aequorin light during prolonged depolarization. The only factor which is both known to affect aequorin and to change during prolonged depolarization is the intracellular pH. However (see Methods) the effect is not large enough to contaminate the aequorin records significantly. (2) A more serious problem is produced by the presence of spontaneous Ca oscillations. It is therefore difficult to exclude the possibility that the fall of tension and light is produced, at least in part, by a change of the magnitude of the oscillations of $[Ca^{2+}]_i$ rather than the mean level. However, even when the oscillations had been abolished by ryanodine, the relaxation of force could be accounted for by a fall of $[Ca^{2+}]_i$. In one experiment, however, the fall of aequorin light was insufficient to account for all the decay of tension. In this last experiment it is possible that other factors contributed.

The events occurring on depolarization are consistent with the following scheme (Eisner *et al.* 1983; Allen *et al.* 1984; Eisner, Allen & Orchard, 1985):



On this model depolarization acts directly on the Na–Ca exchange to increase Ca entry and thence increases $[Ca^{2+}]_i$. However, this effect on Na–Ca exchange will also decrease $[Na^+]_i$ which will, in turn, decrease Ca entry on Na–Ca exchange thus producing the secondary relaxation of $[Ca^{2+}]_i$ on maintained depolarization. It should be noted that the decrease of $[Na^+]_i$ could also be produced by an effect on a passive Na channel (Eisner, Lederer & Vaughan-Jones, 1981; January & Fozzard, 1984). However, the Na–Ca exchange scheme above has at least the advantage of economy inasmuch as it can explain not only the fall of $[Na^+]_i$ but also the increase of $[Ca^{2+}]_i$ (Eisner *et al.* 1985). In one preparation the fall of force could not be accounted for by changes of $[Ca^{2+}]_i$ and here it is possible that the other factors indicated by the dotted lines may cause the relaxation.

In conclusion the spontaneous relaxation of force during a maintained depolarization can be accounted for by a fall of $[Ca^{2+}]_i$.

This work was supported by grants from the Medical Research Council and the British Heart Foundation. M. V. was supported by a M. E. C. Fleming award. We are grateful to Dr J. R. Blinks for providing us with aequorin which was purified in his laboratory with support from N.I.H. grant number HL12186. We thank D. G. Allen, J. C. Kentish, G. L. Smith & S. C. Wray for comments on an earlier version of this manuscript.

REFERENCES

ALLEN, D. G. & BLINKS, J. R. (1979). The interpretation of light signals from aequorin injected skeletal and cardiac muscle cells – a new method of calibration. In *Detection and Measurement of Free Calcium Ions in Cells*, ed. ASHLEY, C. C. & CAMPBELL, A. K., pp. 159–174. Amsterdam: Elsevier/North-Holland.

ALLEN, D. G., BLINKS, J. R. & PRENDERGAST, F. G. (1977). Aequorin luminescence: relation of light emission to calcium concentration – a calcium-independent component. *Science* **196**, 996–998.

ALLEN, D. G., EISNER, D. A., MORRIS, P. G., PIROLO, J. S. & SMITH, G. L. (1986). Metabolic consequences of increasing intracellular calcium and force production in perfused ferret hearts. *Journal of Physiology* (in the Press).

ALLEN, D. G., EISNER, D. A. & ORCHARD, C. H. (1984). Factors affecting free intracellular calcium concentration in quiescent ferret ventricular muscle. *Journal of Physiology* **350**, 615–630.

ALLEN, D. G. & ORCHARD, C. H. (1983). The effects of changes of pH on intracellular calcium transients in mammalian cardiac muscle. *Journal of Physiology* **335**, 555–567.

BLATTER, L. A. & MCGUIGAN, J. A. S. (1986). Free intracellular magnesium concentration in ferret ventricular muscle measured with ion-selective microelectrodes. *Quarterly Journal of Experimental Physiology* **71** (in the Press).

CANNELL, M. B., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1984). The effect of membrane potential on intracellular pH in sheep cardiac Purkinje fibres. *Journal of Physiology* **349**, 45*P*.

CANNELL, M. B., VAUGHAN-JONES, R. D. & LEDERER, W. J. (1985). The influence of intracellular calcium oscillations on tension in heart muscle: The effect of Ryanodine on the tension–sodium relationship in sheep cardiac Purkinje fibers. *Federation Proceedings* **44**, 2964–2969.

CHAPMAN, R. A. (1973). The effects of temperature and metabolic inhibitors on the spontaneous relaxation of the potassium contracture of the heart of the frog. *Rana pipiens*. *Journal of Physiology* **231**, 233–249.

CHAPMAN, R. A. & TUNSTALL, J. (1980). The interaction of sodium and calcium ions at the cell membrane and the control of contractile strength in frog atrial muscle. *Journal of Physiology* **305**, 109–123.

EISNER, D. A., ALLEN, D. G. & ORCHARD, C. H. (1985). The regulation of resting calcium concentration in cardiac muscle. In *Control and Manipulation of Calcium Movement*, ed. PARRATT, J. R., pp. 65–86. London: Raven Press.

EISNER, D. A. & LEDERER, W. J. (1979). Inotropic and arrhythmogenic effects of potassium depleted solutions on mammalian cardiac muscle. *Journal of Physiology* **294**, 255–277.

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1981). The effects of rubidium ions and membrane potential on the intracellular sodium activity of sheep Purkinje fibres. *Journal of Physiology* **317**, 189–205.

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1983). The control of tonic tension by membrane potential and intracellular Na activity in the sheep cardiac Purkinje fibre. *Journal of Physiology* **335**, 723–743.

EISNER, D. A., ORCHARD, C. H. & ALLEN, D. G. (1984). Control of intracellular ionized calcium concentration by sarcolemmal and intracellular mechanisms. *Journal of Molecular and Cellular Cardiology* **16**, 137–146.

EISNER, D. A. & VALDEOLMILLOS, M. (1985*a*). Relaxation of tonic tension and $[Ca^{2+}]_i$ in voltage-clamped sheep cardiac Purkinje fibres. *Journal of Physiology* **360**, 61*P*.

EISNER, D. A. & VALDEOLMILLOS, M. (1985*b*). The mechanism of the increase of tonic tension produced by caffeine in sheep cardiac Purkinje fibres. *Journal of Physiology* **364**, 313–326.

FABIATO, A. & FABIATO, F. (1978). Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *Journal of Physiology* **276**, 233–255.

FRY, C. H. (1986). The actions of extracellular magnesium on mammalian ventricular myocardium. *Journal of Physiology* **372**, 32*P*.

GIBBONS, W. R. & FOZZARD, H. A. (1971). High potassium and low sodium contractures in sheep cardiac muscle. *Journal of General Physiology* **58**, 483–510.

HESS, P., METZGER, P. & WEINGART, R. (1982). Free magnesium in sheep, ferret and frog striated muscle at rest measured with ion-selective micro-electrodes. *Journal of Physiology* **333**, 173–188.

HOERTER, J. A., MICELI, M. V., JACOBUS, W. E., RENLUND, D. G., GERSTENBLITH, G. & LAKATTA, E. G. (1983). *Journal of Physiology* **334**, 105*P*.

JANUARY, C. T. & FOZZARD, H. A. (1984). The effects of membrane potential, extracellular potassium, and tetrodotoxin on the intracellular sodium ions activity of sheep cardiac muscle. *Circulation Research* **54**, 652–665.

KENTISH, J. C. (1986). The effects of inorganic phosphate and creatine phosphate on force production in skinned muscles from rat ventricle. *Journal of Physiology* **370**, 585–604.

ORCHARD, C. H., EISNER, D. A. & ALLEN, D. G. (1983). Oscillations of intracellular Ca^{2+} in mammalian cardiac muscle. *Nature* **304**, 735–738.

POWELL, T., TATHAM, P. E. R. & TWIST, V. W. (1984). Cytoplasmic free calcium measured by quin2 fluorescence in isolated ventricular myocytes at rest and during potassium-depolarization. *Biochemical and Biophysical Research Communications* **122**, 1012–1020.

SHEU, S.-S. & FOZZARD, H. A. (1982). Transmembrane Na^+ and Ca^{2+} electrochemical gradients in cardiac muscle and their relationship to force development. *Journal of General Physiology* **80**, 325–351.

SJODIN, R. A. & ABERCROMBIE, R. F. (1978). The influence of external cations and membrane potential on Ca-activated Na eflux in *Myxicola* giant axons. *Journal of General Physiology* **71**, 453–466.

SUTKO, J. L. & KENYON, J. L. (1983). Ryanodine modification of cardiac muscle responses to potassium-free solutions. Evidence for inhibition of sarcoplasmic reticulum calcium release. *Journal of General Physiology* **82**, 385–404.

VAUGHAN-JONES, R. D., LEDERER, W. J. & EISNER, D. A. (1983). Ca^{2+} ions can affect intracellular pH in mammalian cardiac muscle. *Nature* **301**, 522–524.

WIER, W. G., KORT, A. A., STERN, M. D., LAKATTA, E. G. & MARBAN, E. (1983). Cellular calcium fluctuations in mammalian heart: direct evidence from noise analysis of aequorin signals in Purkinje fibers. *Proceedings of the National Academy of Sciences of the U.S.A.* **80**, 7367–7371.