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THE CONTROL OF TONIC TENSION BY MEMBRANE POTENTIAL AND INTRACELLULAR SODIUM ACTIVITY IN THE SHEEP CARDIAC PURKINJE FIBRE

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SUMMARY

1. Intracellular Na activity (a^i_{Na}) was measured with recessed-tip, Na-selective micro-electrodes in voltage-clamped sheep cardiac Purkinje fibres. Tension was measured simultaneously. a^i_{Na} was increased reversibly either by exposing the preparation to K-free, Rb-free solution or by adding the cardioactive steroid strophanthidin.

2. An increase of a^i_{Na} produced an increase of tonic tension which was larger at depolarized membrane potentials. At sufficiently negative membrane potentials, changes of a^i_{Na} (over the range 6–30 mM) had no effect on tonic tension. Therefore, both an increase of a^i_{Na} and a depolarization are required to increase tonic tension. It is concluded that either a low level of a^i_{Na} or a large negative membrane potential is sufficient to maintain a low intracellular Ca concentration.

3. Tonic tension was measured as a function of a^i_{Na}. At a given membrane potential the relationship can be described empirically by an equation of the form: tonic tension $= b(a^i_{Na})^y$, where y is a constant and b depends on membrane potential. In five experiments y was found to be $3{\cdot}7 \pm 0{\cdot}7$ (mean $\pm$ S.E.M.) over a range of potentials from -60 to -10 mV.

4. Tonic tension was measured as a function of membrane potential. At a given a^i_{Na} the relationship can be described approximately as: tonic tension $= k \exp(aV)$, where a is a constant and k depends on a^i_{Na}. In five experiments a was found to be $0{\cdot}06 \pm 0{\cdot}01$ mV^{-1} (mean $\pm$ S.E.M.).

5. A depolarization of 10 mV increases tonic tension by the same amount as does an increase of a^i_{Na} that is equivalent to a 3·7 mV change of the Na equilibrium potential, E_{Na}. Hence E_{Na} is nearly 3 times more effective than membrane potential in controlling tonic tension.

6. During a prolonged depolarization (several minutes) the initial increase of tonic tension decays gradually. This is associated with a fall of a^i_{Na}. The relationship between tonic tension and a^i_{Na} is similar to that seen when a^i_{Na} is increased by inhibiting the Na pump. It is concluded that the fall of a^i_{Na} is responsible for the decay of tonic tension.

7. The changes of tonic tension reported in this paper are consistent with the effects of a^{i}_{Na} and membrane potential on a voltage-dependent Na–Ca exchange. The possibility that a voltage-dependent Ca channel contributes to tonic tension is also discussed.

INTRODUCTION

Studies of contraction in cardiac muscle have shown both a twitch and a tonic component of tension (Coraboeuf, 1974). These can be demonstrated by depolarizing the membrane potential for a few seconds. In some preparations this produces a twitch which relaxes completely. In others, notably amphibian cardiac muscle, a tonic component of tension also appears which is maintained throughout the depolarization. Mammalian cardiac muscle generally produces only a twitch and the sheep Purkinje fibre is typical in this respect. However, when intracellular Na is elevated (e.g. by Na pump inhibition) the twitch is followed by an increase of tonic tension (Eisner & Lederer, 1979*a*). It has been suggested that the tonic component produced by depolarization in amphibian cardiac muscle is due to entry of Ca via a voltage-dependent Na–Ca exchange (Horackova & Vassort, 1979; Chapman & Tunstall, 1980, 1981). A similar mechanism in the Purkinje fibre could explain why the tonic component is only seen when intracellular Na is elevated since, under these conditions, depolarization will produce a greater Ca entry via Na–Ca exchange (Mullins & Requena, 1981). However, tonic tension and intracellular Na have not hitherto been measured simultaneously and so it is not clear whether their inter-relationship can be described by Na–Ca exchange.

Although the tonic component does not contribute to the normal heart beat, it is of interest because it gives information about the control of resting Ca in the cell. In the present paper we have therefore studied tonic tension in voltage-clamped Purkinje fibres while simultaneously measuring intracellular Na activity, a^{i}_{Na}. The results show that tonic tension is controlled by both a^{i}_{Na} and membrane potential in a manner consistent with the predictions of a voltage-dependent Na–Ca exchange model, although contributions from a voltage-gated Ca channel cannot be entirely excluded.

Preliminary reports of some of the present results have appeared previously (Eisner, Lederer & Vaughan-Jones, 1982*a*, *b*).

METHODS

The method of measurement of a^{i}_{Na} and tension in a voltage-clamped Purkinje fibre is identical to that described previously (Eisner, Lederer & Vaughan-Jones, 1981*a*). To summarize, free-running Purkinje fibres were dissected from fresh sheep hearts obtained from a local slaughterhouse. They were shortened to < 2 mm and mounted in the experimental bath, one end being coupled to a tension transducer. A two-micro-electrode voltage-clamp was used to control membrane potential, whilst intracellular Na activity was measured with a recessed-tip, Na-sensitive micro-electrode (Thomas, 1978).

Solutions

The modified Tyrode solution consisted of: 145 mM-NaCl, 4 mM-KCl, 2 mM-$CaCl_2$, 1 mM-$MgCl_2$, 10 mM-Tris HCl and 10 mM-glucose, pH 7·40 at 37 °C. Modifications to this solution are indicated in the text: e.g. a solution labelled 4 mM-Rb_o is the standard solution with 4 mM-RbCl isosmotically substituted for KCl. Rb is frequently used instead of K in these experiments. It should be noted

that Rb and K are equivalent in their ability to activate the Na pump (Eisner & Lederer, 1980; Eisner *et al.* 1981*a*; Glitsch, Kampmann & Pusch, 1981).

Tension measurements

It is worth defining the various measurements of tension used in this paper. (i) *Phasic or twitch tension* is the component of tension produced by depolarization which relaxes in less than 500 ms (cf. Fig. 2*A*). (ii) *Tonic tension* is the resting tension of the preparation and can be increased either by depolarization or by an increase of intracellular Na. The increase of tonic tension on depolarization takes a few seconds to reach a maximum value and then decays with prolonged depolarization over several minutes (Fig. 9). The increase of tonic tension on depolarization has previously been referred to as 'voltage-dependent tonic tension' (Eisner & Lederer, 1979*a*). The tonic tension also includes changes of tension produced at a fixed membrane potential (e.g. by a rise of intracellular Na). Previous work has often referred to this as either a contracture or an increase of resting tension. Since the present work shows that this aspect of tonic tension is the same phenomenon as the voltage-dependent component we have referred to both as tonic tension and eschewed the other terms.

RESULTS

Inhibition of the Na pump produces a rise of a^i_{Na} and can lead to an increase of tonic tension at a fixed membrane potential (e.g. Eisner *et al.* 1981*a*). The increase of tension has been attributed to the rise of a^i_{Na} which then produces a rise of intracellular Ca, Ca^{2+}_i, via transmembrane Na–Ca exchange (Baker, Blaustein, Hodgkin & Steinhardt, 1969; Glitsch, Reuter & Scholz, 1970). However, tonic tension is also affected by membrane potential (Eisner & Lederer, 1979*a*, *b*). Fig. 1 shows an experiment designed to investigate whether all of the increase of tonic tension during Na pump inhibition is voltage-dependent. In order to inhibit the Na pump the preparation was superfused with a K-free solution. At the beginning of the recording shown, depolarizing voltage-clamp pulses were applied to elicit the twitch and tonic components of tension. Examples of the tension records are shown in Fig. 2*A* at a faster chart speed: here the distinction between twitch and tonic tension can be seen clearly. The depolarizing pulses were then stopped and the membrane potential stepped through different values. Depolarization increased tonic tension and hyperpolarization decreased it, whilst at the most negative potentials, changing membrane potential had little effect. This point is emphasized in Fig. 2*B* which shows the record of Fig. 1 displayed with tension plotted as a function of membrane potential. It is evident that tonic tension is essentially voltage-independent at potentials negative to −90 mV. The last part of Fig. 1 shows the effects of re-adding 4 mM-Rb_o to reactivate the Na pump. This produced a decrease of a^i_{Na} accompanied by a decrease of tonic tension at the holding potential (−60 mV). Furthermore, at the final low level of a^i_{Na} voltage-dependent tonic tension was also abolished (Fig. 2*Ab*). It should be noted that the level of tonic tension reached at this low a^i_{Na} is virtually the same as that produced by extreme hyperpolarization at high a^i_{Na} (before adding back Rb). In other words changing a^i_{Na} has no effect on tonic tension at sufficiently negative membrane potentials. This means that the increase of tonic tension produced by increased a^i_{Na} is entirely dependent on membrane potential; sufficient hyperpolarization can produce complete relaxation of the muscle, even in the presence of an elevated a^i_{Na}. Thus, in order to increase tonic tension one must have (i) sufficient depolarization and (ii) an increase of a^i_{Na}. In the remainder of this paper we examine the quantitative dependence of tonic tension on both membrane potential and a^i_{Na}.

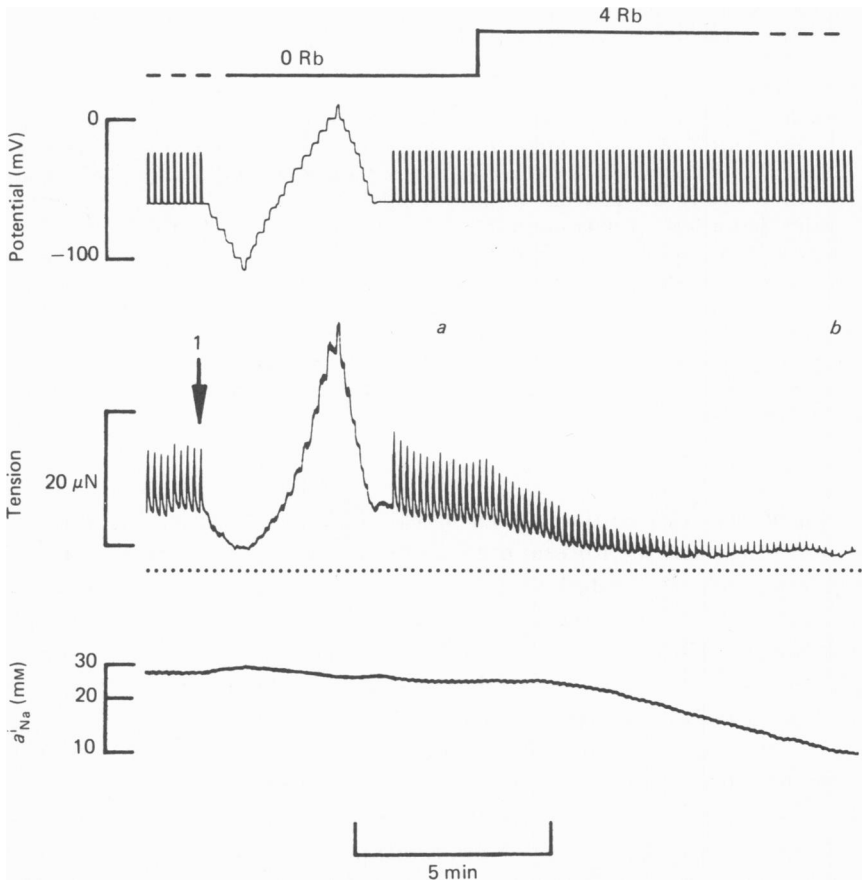

Fig. 1. The effects of membrane potential on resting tension at both high and low a^i_{Na}. Traces show: top, membrane potential; middle, tension; bottom, a^i_{Na}. At the start of the recording shown the preparation had been exposed to K-free, Rb-free solution for 1 h. Voltage-clamp depolarizing pulses of 2 s duration were applied from a holding potential of −60 mV to −20 mV at 0·1 Hz. At arrow 1 the pulses were stopped and the membrane potential was changed in 10 mV steps between −120 and +20 mV (*ca.* 4–5 s at each potential). After this the voltage-clamp depolarizing pulses were restarted. Rb was then added to the superfusing solution to decrease a^i_{Na}. The dotted line has been drawn to facilitate comparison of the tension levels before and after adding Rb. Sample records of tension at *a* and *b* are shown in Fig. 2*A*.

The effects of membrane potential and a^i_{Na} on tonic tension

In the experiment illustrated in Fig. 3 tonic tension was investigated by varying the membrane potential using the protocol illustrated in Fig. 3*B*. Intracellular Na activity was manipulated by removing and then re-adding 4 mM-Rb_o to a K-free solution in order to inhibit and reactivate the Na pump (see Methods). Fig. 3*A* shows the change of a^i_{Na} that this produced. At the times indicated on this record the membrane potential was stepped to different levels and the associated changes of tonic tension were measured. There is no voltage-dependent tonic tension at low a^i_{Na} (i). Records (ii)–(iv) show that an increase of a^i_{Na} is associated with an increase of voltage-dependent tonic tension. a^i_{Na} has increased about 3-fold from (i) to (iv),

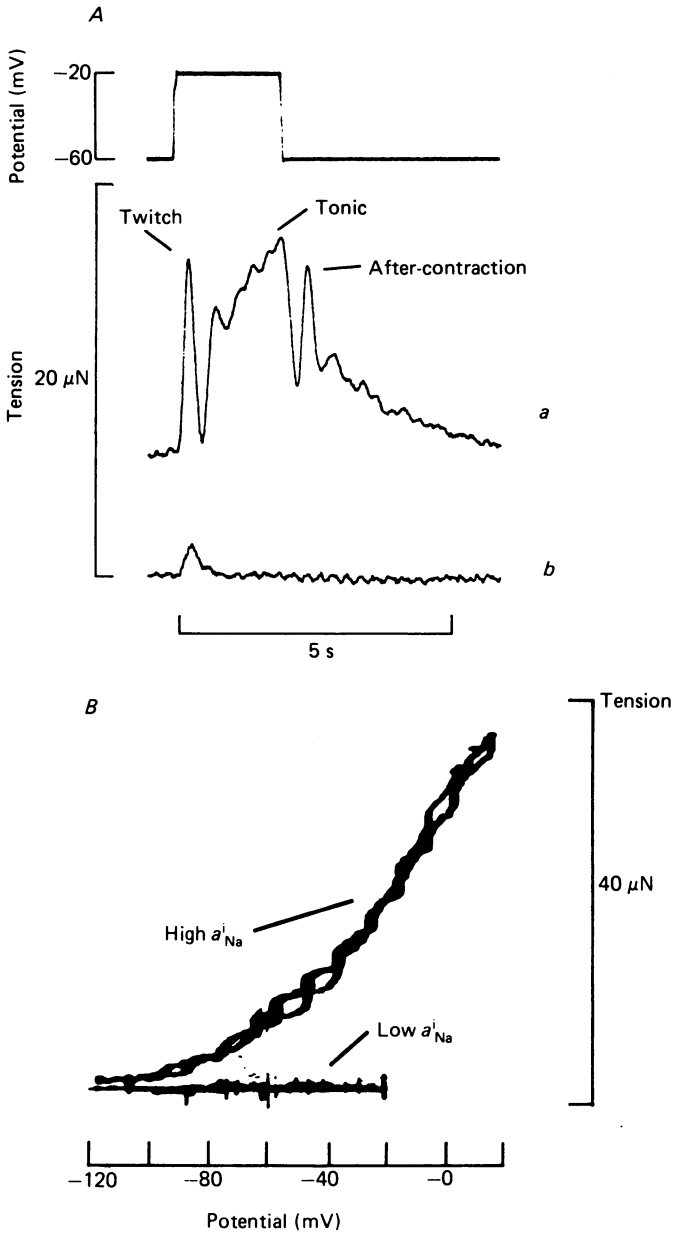

Fig. 2. The effects of a^{i}_{Na} on the relationship between membrane potential and tonic tension. Data from Fig. 1. *A*, voltage-clamp records of tension obtained at the points labelled *a* and *b* on Fig. 1. The upper trace is the membrane potential and the lower records are tension. The vertical position of the two tension traces is as in Fig. 1 (i.e. tonic tension at the holding potential is less at *b* than *a*). The a^{i}_{Na} was 23·4 mM when record *a* was obtained and 9·4 mM when *b* was obtained. *B*, the relationship between tonic tension and membrane potential. The tension and membrane potential records from the steps of potential shown in Fig. 1 have been plotted on the *XY* co-ordinates of a storage oscilloscope. The high a^{i}_{Na} trace (obtained at arrow 1 in Fig. 1) appears as two superimposed lines, showing that the relationship between tension and membrane potential is the same no matter whether the membrane is hyperpolarizing or depolarizing. The record at low a^{i}_{Na} (9 mM) was obtained after the end of the record of Fig. 1.

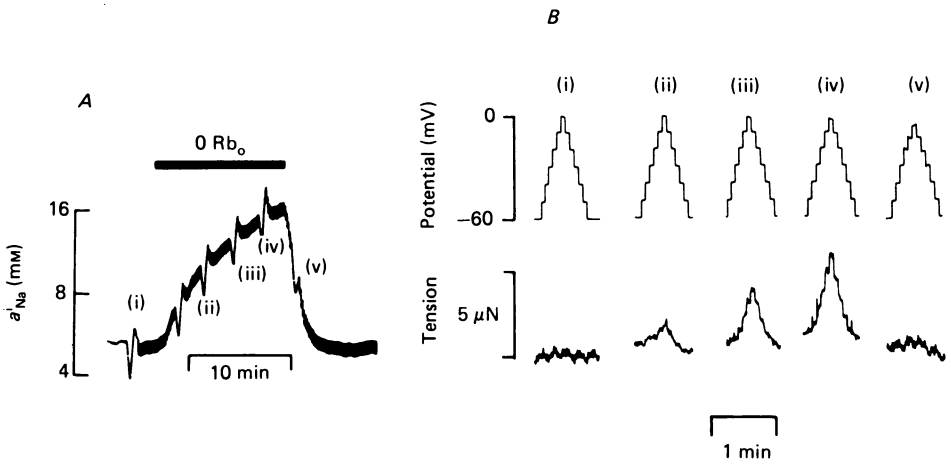

Fig. 3. The effects of a^i_{Na} on the voltage-dependent component of tonic tension. *A*, the change of a^i_{Na} produced by exposure to Rb-free solution for the period shown. The numbered artifacts on the a^i_{Na} trace were produced when the membrane potential was stepped to investigate tonic tension. *B*, the relationship between tonic tension and membrane potential at various a^i_{Na}. The upper trace shows membrane potential and the lower, tension. The records were obtained at the times shown in *A*.

whereas the increase of tonic tension is considerably greater. Tonic tension is therefore very sensitive to small changes of a^i_{Na} over the range investigated.

Fig. 4 shows how the voltage and Na dependence of tonic tension were investigated more quantitatively. In this case strophanthidin (10^{-5} M) was added to elevate a^i_{Na} and a series of short depolarizing pulses was used rather than the longer steps seen in Fig. 3. Shorter pulses are an advantage because depolarization, if prolonged, produces a fall of a^i_{Na} (Eisner, Lederer & Vaughan-Jones, 1981*b*) and a relaxation of tonic tension (Eisner *et al.* 1982*b*). Depolarizing pulses were applied to four different levels. The elevation of a^i_{Na} is associated with a marked increase of twitch tension (cf. Eisner *et al.* 1981*a*; Lee, Kang, Sokol & Lee, 1980). The chart speed is too slow, however, to resolve the tonic component of tension clearly. Therefore in Fig. 5 superimposed tension records at each of the four different membrane potentials have been displayed on an expanded time base, and these are shown for each of the six a^i_{Na} levels indicated in Fig. 4. It is clear that the voltage-dependent component of tonic tension increases markedly with the increase of a^i_{Na}. During the exposure to strophanthidin, after-contractions appear on repolarization and these increase along with the increase of a^i_{Na}. It should be noted that, at the most depolarized potentials and the highest a^i_{Na}, tonic tension has not quite reached a steady value. Consequently in these cases the dependence of tonic tension on a^i_{Na} will be slightly underestimated.

One problem with the analysis of tonic tension is that the relationship between tension and a^i_{Na} is different when a^i_{Na} increases slowly during Na pump inhibition compared with that when a^i_{Na} falls rapidly during Na pump re-activation. Such hysteresis has been reported previously for both twitch and tonic tension (Eisner *et al.* 1981*a*). Comparison of records (ii) and (v) in Fig. 3 shows that a similar hysteresis is seen for the voltage-dependent component of tonic tension. However, we find little hysteresis when a^i_{Na} is changed by adding strophanthidin and then washing it off (not shown). Under these conditions both the rise and subsequent recovery of a^i_{Na} occur slowly (see

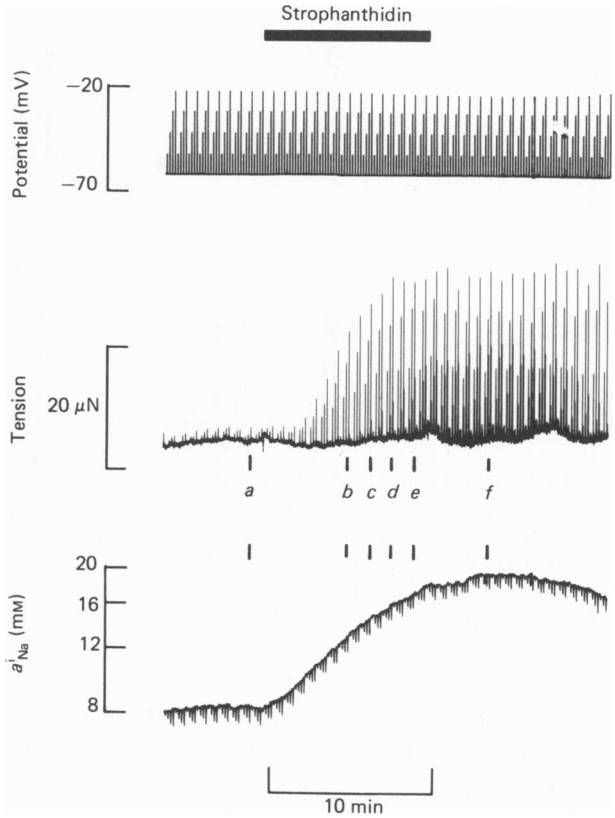

Fig. 4. The effects of strophanthidin on a^i_{Na} and tension. Records show: top, membrane potential; middle, tension; bottom, a^i_{Na}. The membrane potential was held at −63 mV and depolarizing pulses (duration 2 s) were applied every 10 s. The pulses were applied in turn to −53, −43, −33 and −23 mV and then the cycle was repeated. Strophanthidin (10^{-5} M) was applied for the period shown. The final part of the recovery from strophanthidin is not shown.

Fig. 4 and also Deitmer & Ellis, 1978*b*). This suggests that, during the onset of Na pump inhibition, a^i_{Na} may be changing sufficiently slowly for tension to be in equilibrium with a^i_{Na}. It should be noted that the hysteresis phenomenon when it occurs will probably result in an underestimate of the steepness of the relationship between tension and a^i_{Na}.

Data from an experiment similar to that of Fig. 5 are shown in Fig. 6. Voltage-dependent tonic tension is plotted as a function of a^i_{Na} at five different membrane potentials (from −54 to −14 mV) after a^i_{Na} had been elevated by applying strophanthidin (10 μM). Fig. 6*A* shows a plot of tonic tension as a function of a^i_{Na}. Two points deserve attention: (i) at a given membrane potential, an increase of a^i_{Na} increases tonic tension; and (ii) at a given a^i_{Na}, depolarization increases tension. Fig. 6*B* shows that, over the range investigated, there appears to be a linear dependence of log tension on log a^i_{Na}. The relationships at the different membrane potentials are roughly parallel, implying that changing the membrane potential changes tonic tension by a constant fraction irrespective of the level of a^i_{Na}. In five experiments

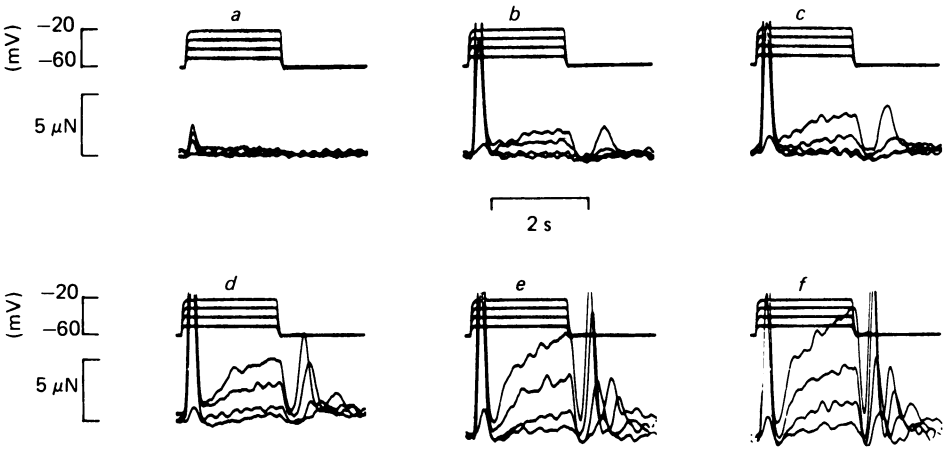

Fig. 5. The effects of strophanthidin on the voltage-dependent component of tonic tension. Data taken from Fig. 4. In each panel the upper trace shows membrane potential and the lower, tension. The letters refer to the points marked on Fig. 4. Each panel shows superimposed records from a cycle of four depolarizing pulses (from −63 mV to −53, −43, −33 and −23 mV). The twitch is off scale in several of the records. In each panel the greater the depolarization the greater the tonic component of tension. Note that as a^i_{Na} is elevated repolarization produces after-contractions.

the mean slope of this logarithmic plot was found to be $3{\cdot}7 \pm 0{\cdot}7$ (mean ± S.E.M.), so that the relationship between tension and a^i_{Na} can be empirically described as:

$$\text{tonic tension} = b(a^i_{Na})^{3{\cdot}7},$$

where b is independent of a^i_{Na} but depends on membrane potential.

Fig. 7 shows tonic tension plotted as a function of membrane potential at several different levels of a^i_{Na}. Each line corresponds to measurements made over the range of a^i_{Na} indicated. The insert shows the dependence of log tension on membrane potential. Over the range investigated the relationships are again linear and, within the experimental error, are parallel at the different levels of a^i_{Na}. The mean slope of this line was $0{\cdot}06 \pm 0{\cdot}01$ mV^{-1} (mean ± S.E.M.; $n = 5$). Therefore the relationship between tension and membrane potential (in mV) can be empirically described as:

$$\text{tonic tension} = k \exp(0{\cdot}06\ V),$$

where k is independent of membrane potential (V) and depends on a^i_{Na}. The value of the exponent implies that a 17 mV change of membrane potential produces an e-fold change of tension. This is similar to the value of the depolarization required to produce an e-fold increase of tonic tension in frog cardiac muscle (Chapman & Tunstall, 1981). The simple monotonic relationship between tonic tension and membrane potential differs from that reported for cat ventricular muscle (Trautwein, McDonald & Tripathi, 1975) where a biphasic voltage-dependence of tonic tension was seen. No such behaviour has been seen in the present work (e.g. Figs. 2*B* and 7).

So far we have analysed separately the effects on tonic tension of changing either a^i_{Na} or membrane potential. Fig. 8 now examines which of these has the greater

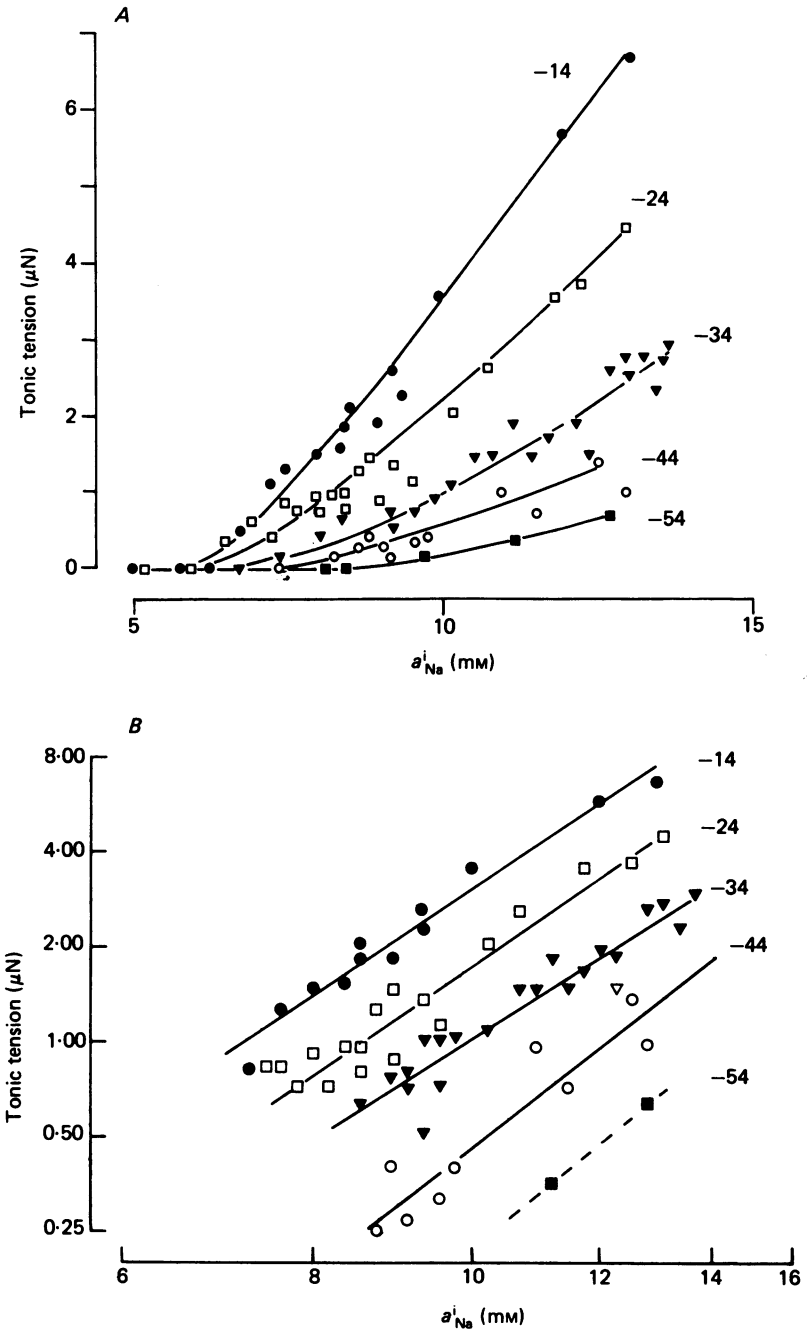

Fig. 6. The dependence of tonic tension on a^i_{Na} at various membrane potentials. The records are taken from an experiment similar to that of Fig. 5. *A*, tonic tension plotted as a function of a^i_{Na} at different membrane potentials: ●, −14 mV; □, −24 mV; ▼, −34 mV; ○, −44 mV; ■, −54 mV. Tonic tension measurements were obtained at the end of a 2 s depolarizing pulse from a holding potential of −64 mV to the potential indicated. *B*, the dependence of log tension on log a^i_{Na}. Symbols and data as in *A*. Only the points above 0·25 μN are shown on these graphs. The continuous lines have been fitted by least squares linear regression.

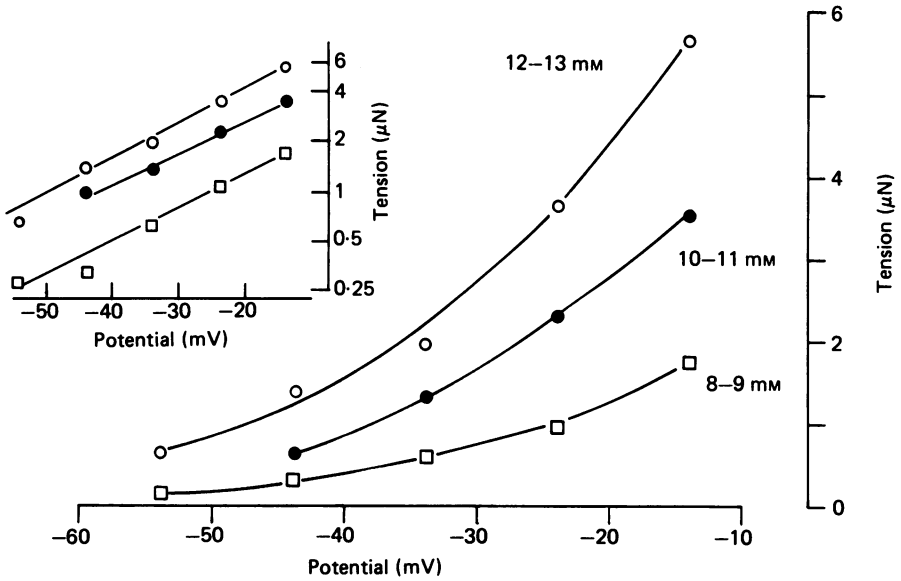

Fig. 7. The dependence of tonic tension on membrane potential at different levels of a^{i}_{Na}. Data from Fig. 6. The points represent the mean level of tension at a given potential for the following ranges of a^{i}_{Na}: ○, 12–13 mM; ●, 10–11 mM; □, 8–9 mM. The inset shows log tension plotted as a function of membrane potential with the same symbols as the main Figure.

influence on tonic tension. We have estimated how much of a change of a^{i}_{Na} has the same effect on tonic tension as a given change of membrane potential. The data can be obtained from experiments similar to that of Fig. 6. The method employed is illustrated in the inset to Fig. 8. At a given potential (say −14 mV), a given level of tonic tension (x) is associated with a certain level of a^{i}_{Na} (y). At −34 mV, however, the same level of tension is associated with a higher level of a^{i}_{Na} (z). Hence a change of membrane potential of 20 mV is equivalent to a change of a^{i}_{Na} from y to z. If the change of a^{i}_{Na} is expressed in terms of a change of E_{Na} (the Na equilibrium potential) then $\Delta E_{Na} = RT/F \ln y/z$. This comparison can be made between a given pair of potentials starting at different levels of membrane potential. Fig. 8 therefore shows the amount of change of E_{Na} (ΔE_{Na}) required to keep tension constant, plotted as a function of the imposed change of membrane potential (ΔE_{m}). The relationship is linear over the 40 mV potential range investigated. The slope of this graph shows that a 3·6 mV change of E_{Na} is required to compensate for a 10 mV change of E_{m}. A similar, linear relationship was found in three other experiments, giving a mean value for the slope of $0{\cdot}37 \pm 0{\cdot}04$ (mean ± S.E.M.; $n = 4$). In other words, E_{Na} is about three times as effective as membrane potential in controlling tonic tension

The effects of prolonged changes of membrane potential on a^{i}_{Na} and tonic tension

The experiments described above have used comparatively brief depolarizations to investigate tonic tension. In another series of experiements we applied voltage-clamp depolarizing pulses lasting for several minutes. One such experiment is shown in Fig. 9. The preparation was superfused with a solution containing 10 μM-strophanthidin

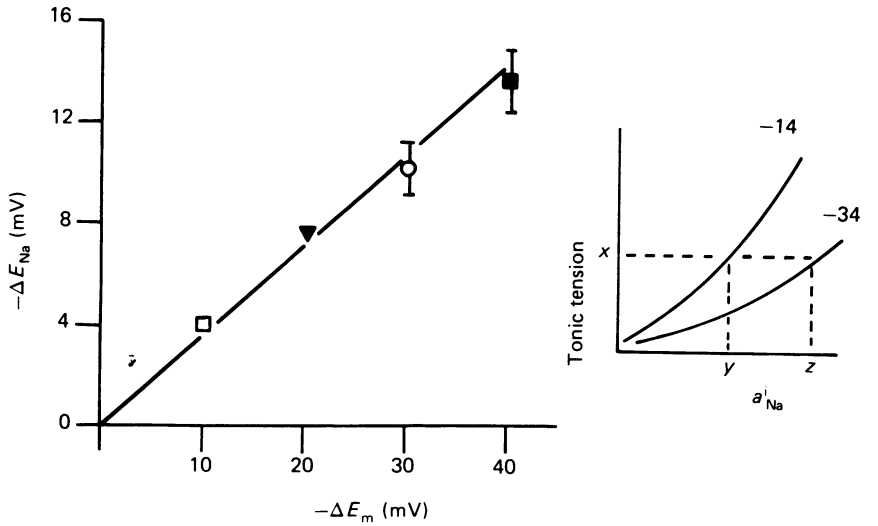

Fig. 8. A comparison of the effects of E_m and E_{Na} on tonic tension. Data from Fig. 6. The method used to obtain the graph is shown in the inset. All data are referred to the points measured at the most positive potential (here −14 mV). A hyperpolarization to another membrane potential would produce a fall of tension. The change of a^i_{Na} required to bring the level of tension back up to the control at −14 mV can be expressed as a change of E_{Na} (ΔE_{Na}). Thus, in the example shown in the inset, a ΔE_{Na} of $RT/F \ln y/z$ is equivalent to a change of E_m (ΔE_m) of 20 mV. Several such comparisons have been made between the points measured for each pair of membrane potentials in Fig. 6 and these are plotted in the main Figure. The original data points have been used to make this comparison. However, when no suitable point existed the −14 mV points were interpolated. The data are shown as mean ± S.E.M. and the number of determinations were: □, 13; ▼, 17; ○, 8; ■, 3. For explanation of symbols see Fig. 6.

to inhibit the Na pump and elevate a^i_{Na}. Consequently, a depolarization from −55 to −20 mV produced an increase of tonic tension. However, after reaching a peak, tension decayed over the next few minutes so that, when the membrane was eventually repolarized, the change of tonic tension was only about 30% of that produced on depolarization. Fig. 9 also shows that the relaxation was accompanied by a simultaneous fall of a^i_{Na}. Such a fall has been observed previously (Eisner *et al.* 1981*b*).

Since, under other conditions, changes of a^i_{Na} affect tonic tension presumably by changing Ca_i^{2+}, it is important to determine whether the present fall of a^i_{Na} is sufficient to account for the relaxation of tonic tension. This can be done by comparing the relationship between tension and a^i_{Na} during the maintained depolarization with that seen during the onset of Na pump inhibition. Such a comparison is made in Fig. 10*A*. Voltage-clamp pulses were applied serially to different levels and external Rb removed to produce an increase of a^i_{Na} which is associated with an increase of both twitch and tonic tension. Fig. 10*B* shows, at a faster chart speed, the tonic tension responses produced by the depolarizing pulses to −20 mV and recorded at the levels of a^i_{Na} indicated in Fig. 10*A*. The pulses were then stopped and a maintained depolarization to −20 mV was applied to decrease a^i_{Na}. We can now compare the relationship between tonic tension and a^i_{Na} when they are changed by

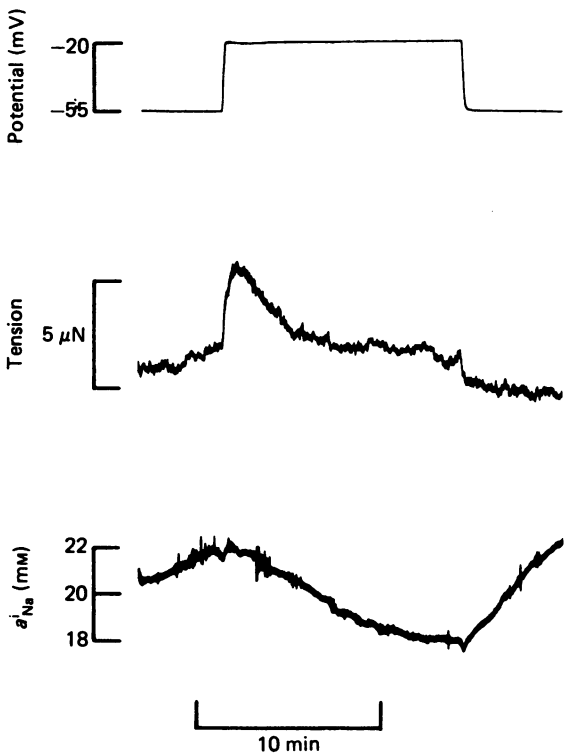

Fig. 9. The effects of depolarization on a^i_{Na} and tonic tension in the presence of strophanthidin (10^{-5} M). The traces show: top, membrane potential; middle, tension; bottom, a^i_{Na}. Strophanthidin was applied for 35 min before the start of the record shown. The membrane was depolarized from −55 to −20 mV for 12·5 min. Initially, a^i_{Na} was increasing because of recovery from a previous depolarization.

these two different methods. Fig. 11 shows the dependence of log tension on log a^i_{Na} for the results of Fig. 10. As previously noted (Fig. 6*B*), there is a linear dependence of log tension on log a^i_{Na} when a^i_{Na} is increased by Na pump inhibition (filled circles). Fig. 11 indicates a similar linear relationship when a^i_{Na} is decreased by prolonged depolarization (open circles). This means that the fall of a^i_{Na} is large enough to account for the relaxation of tonic tension.

In the experiment of Fig. 12, a^i_{Na} was again changed by prolonged depolarization and repolarization. In this experiment, however, brief voltage pulses were also applied so that tonic tension could be investigated at −20 and −60 mV. It is clear that at −20 mV it decays with the fall of a^i_{Na}. Furthermore, tonic tension at −20 mV recovers slowly on repolarization along with the rise of a^i_{Na}. It should also be noted that a decay and recovery of tonic tension is also seen at −60 mV, although the effect is less pronounced than at −20 mV. This result is similar to that of Fig. 6*A* which shows that an elevation of a^i_{Na} produced by Na pump inhibition has much greater effects on tonic tension at more depolarized membrane potentials.

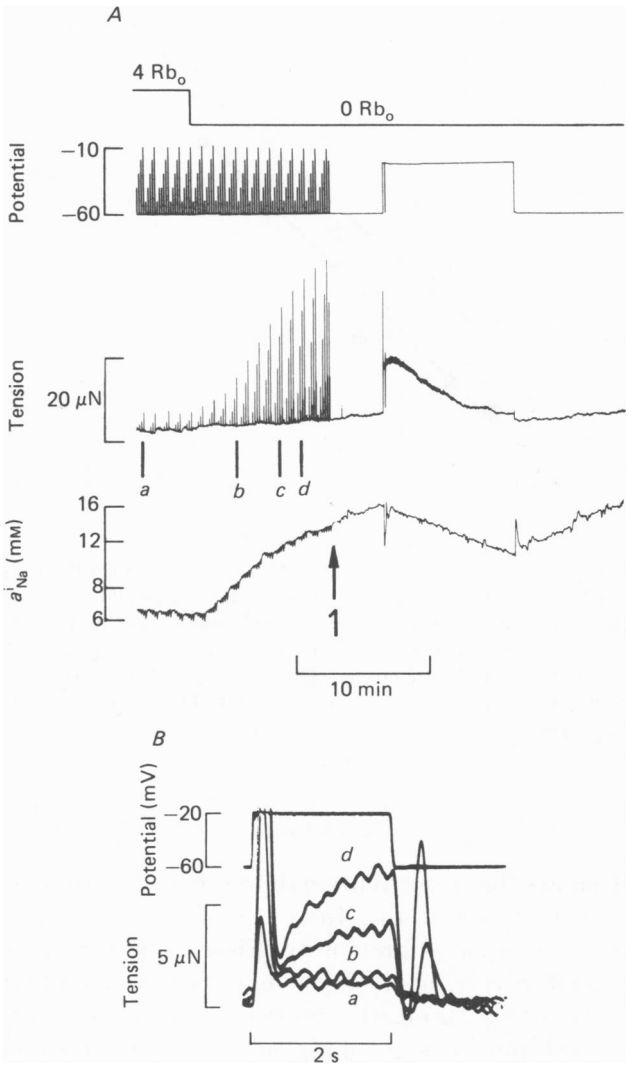

Fig. 10. Comparison of the effects of Na pump inhibition and prolonged depolarization on a^{i}_{Na} and tonic tension. *A*, time course of changes of a^{i}_{Na} and tension. Records show: top, membrane potential; middle, tension; bottom, a^{i}_{Na}. At the beginning of the record shown the preparation was in a solution containing 4 mM-Rb. At the time shown Rb_o was reduced to zero. Throughout this part of the experiment the holding potential was −60 mV. Voltage-clamp depolarizing pulses (duration 2 s) were applied at 0·1 Hz sequentially to the following potentials: −50, −40, −30, −20 and −10 mV. At the time marked by arrow 1 the regular depolarizations were stopped and the membrane was subsequently depolarized for 10 min to −20 mV before being returned to −60 mV. *B*, superimposed tension and potential records obtained in *A* at the times indicated by the letters. Top trace is membrane potential and lower trace is tension. The effects of the depolarizing pulse to −20 mV are shown. Note that the twitches of records *c* and *d* are off scale.

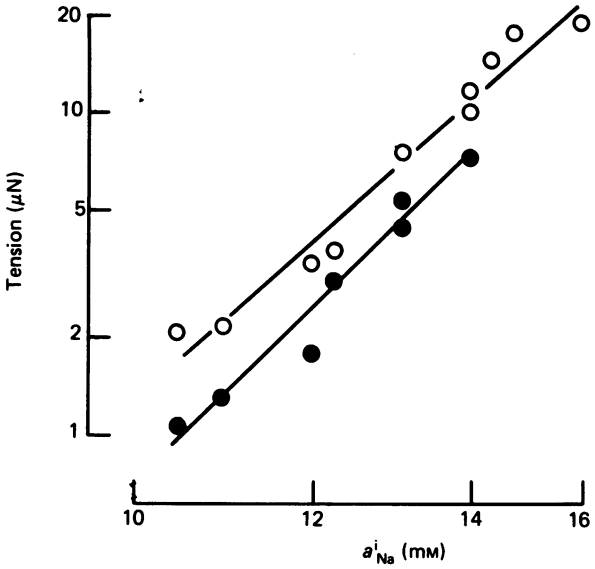

Fig. 11. A quantitative comparison of the dependence of tonic tension on a^i_{Na} during the onset of Na pump inhibition and during long depolarizing pulses. The data are taken from Fig. 10. The ordinate shows tonic tension and the abscissa a^i_{Na} both on logarithmic scales. The open circles (○) denote the relationship between tension and a^i_{Na} during the prolonged depolarization to −20 mV. The filled circles (●) show the tension reached at the end of the depolarizing pulses to −20 mV shown in Fig. 10*B*. The lines have been fitted by a least squares linear regression.

DISCUSSION

The present work shows that tonic tension in the sheep Purkinje fibre is controlled by both membrane potential and a^i_{Na}. Increases of tonic tension associated with increases of a^i_{Na} have been reported previously (Eisner *et al.* 1981*a*). We now see that the magnitude of this effect is critically dependent upon the membrane potential. At depolarized levels a rise of a^i_{Na} produces a large increase of tonic tension whereas at hyperpolarized levels the same change of a^i_{Na} has no effect. This presumably means that hyperpolarization can decrease Ca_i^{2+} to a level below the threshold for activation of the contractile proteins. However, before we can consider how a^i_{Na} and membrane potential interact, we must decide whether the changes of tonic tension measured in the present experiments do indeed reflect changes of Ca_i^{2+}. In the first part of this paper we examined tonic tension during the onset of Na pump inhibition and in the second part looked at the effects of prolonged depolarization. For convenience we will now discuss these two approaches separately although it is probable that in both cases the factors controlling tonic tension are the same.

Tonic tension during Na pump inhibition

Tonic tension as an indication of intracellular Ca activity

The intracellular Ca activity rises slowly during inhibition of the Na pump (e.g. Bers & Ellis, 1982) so that the contractile proteins will eventually be activated. Tonic

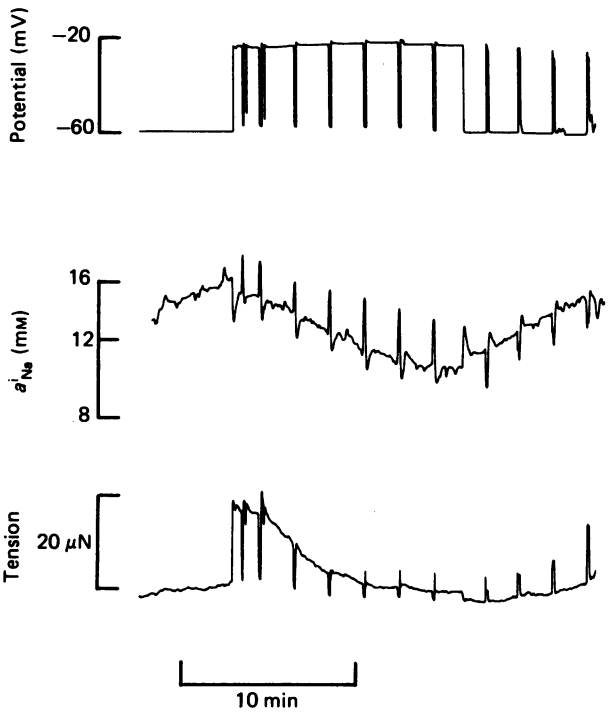

Fig. 12. Inactivation and reactivation of voltage-dependent tonic tension. Records show: top, membrane potential; middle, a^i_{Na}; bottom, tension. The membrane potential was initially held at −60 mV and then a depolarization was applied to −23 mV. Throughout the period of depolarization brief hyperpolarizing pulses were applied back to −60 mV. Next, the holding potential was changed back to −60 mV and then brief depolarizing pulses were applied to −23 mV. It should be noted that the membrane potential droops during these later pulses. If anything this will have led to an underestimate of the re-activation of voltage-dependent tonic tension. The brief changes of membrane potential in this Figure produce artifacts on the a^i_{Na} trace due to the slow electrical time-constant of the Na-selective electrode.

tension should be a better reflexion of Ca_i^{2+} than the twitch since it is maintained for periods which are long in comparison to intracellular diffusion times and the kinetics of cross-bridge formation and detachment. Therefore tonic tension is probably in equilibrium with Ca_i^{2+}. However, one problem still remains. Under the present experimental conditions is tonic tension a unique function of Ca_i^{2+}, or do other factors that influence contraction also change? Perhaps the most important of these is pH_i. Deitmer & Ellis (1980) have shown that Na pump inhibition produces an acidification of 0·1–0·3 pH units in the sheep Purkinje fibre and a decrease of pH_i can decrease the sensitivity of the contractile apparatus to Ca (Fabiato & Fabiato, 1978; Allen, Kurihara & Orchard, 1981). The acidification develops slowly and is most prominent when a^i_{Na} has reached its steady 'plateau' value of about 20 mM. It could therefore produce a significant fall of tension. At present we cannot determine how such changes of pH_i would affect our results. However, the study of Deitmer & Ellis suggests that little or no acidification occurs during the first 15 min of Na pump inhibition (their figs. 7 and 8). The quantitative relationship between tonic tension

and a^i_{Na} derived in the present experiments has usually been measured during this period, the exception being the experiment of Fig. 11 where the analysis is extended for 20 min in K-free solution. Nevertheless, despite this precaution, it is inevitable that the present results will be somewhat affected by changes of pH_i.

The influence of a^i_{Na} and membrane potential on tonic tension

The fact that tonic tension and therefore Ca_i^{2+} is influenced by a^i_{Na} can be explained in terms of transmembrane Na–Ca exchange (Reuter & Seitz, 1968; Baker *et al.* 1969; Glitsch *et al.* 1970). However, tonic tension is also sensitive to membrane potential and this can be explained in one of two ways: (i) the Na–Ca exchange is voltage-sensitive so that depolarization increases Ca entry or decreases Ca efflux by influencing this exchange directly (cf. Chapman & Tunstall, 1980; Mullins, 1981); (ii) depolarization increases net Ca entry by affecting some additional mechanism such as a Ca channel. A third possibility, that membrane potential influences the uptake and release of Ca from intracellular stores, seems less likely since caffeine, a drug known to impair Ca metabolism in the sarcoplasmic reticulum, does not decrease tonic tension (Eisner & Lederer, 1982).

There is much evidence from work on squid axons that Na–Ca exchange can be affected by membrane potential (Blaustein, Russell & De Weer, 1974; Mullins & Brinley, 1975; Baker & McNaughton, 1976) and recent studies have shown that the increase of Ca_i^{2+} produced by depolarization depends on Na_i (Mullins & Requena, 1981). In cardiac muscle there is also some evidence in favour of a voltage-dependent Na–Ca exchange in as much as depolarization increases the Na_i-dependent Ca uptake by cardiac sarcolemmal vesicles (Philipson & Nishimoto, 1980). However, there is insufficient evidence at present to allow one to ascribe the voltage-dependence of tonic tension unequivocally to a Na–Ca exchange. We will therefore consider both of the above possibilities.

(i) *Voltage-dependent Na–Ca exchange.* The simplest possible model assumes that the Na–Ca exchange is at equilibrium. This assumption is unlikely to hold strictly since, when the Na pump is inhibited, Na–Ca exchange probably produces a net efflux of Na from the cell (Deitmer & Ellis, 1978*a*), thus explaining why a^i_{Na} does not rise above about 30 mM (see, e.g. Fig. 1). Nevertheless, a consideration of the equilibrium condition provides an insight into the present results. For the equilibrium Ca concentration to depend on membrane potential, the carrier must transport more than two Na ions per Ca ion. If n Na ions are transported per Ca ion, the equilibrium condition can be obtained (Blaustein & Hodgkin, 1969):

$$Ca_i = Ca_o\,(Na_i/Na_o)^n \exp\,(n-2)\,VF/RT, \tag{1}$$

$$\ln Ca_i = \ln Ca_o + (n-2)\,VF/RT + n \ln (Na_i/Na_o), \tag{2}$$

$$\ln Ca_i = \ln Ca_o + F/RT\,[(n-2)\,V - nE_{Na}], \tag{3}$$

where V, E_{Na}, F, R and T have their usual meaning.

Eqn. (3) implies that a change of E_{Na} of $(n-2)/n$ mV will have the same effects on Ca_i and hence on tension as a 1 mV change of membrane potential. Our experiments (Fig. 8) give a value of 0·37 for the slope of the line relating E_{Na} and membrane potential. This gives a value of 3·2 for n. Bearing in mind the equilibrium

assumption, this result is consistent with a Na–Ca exchange which transports about three Na ions per Ca ion. This agrees with the conclusions of other work using a variety of different techniques (Pitts, 1979; Reeves & Sutko, 1979; Chapman & Tunstall, 1980; Bers & Ellis, 1982; Sheu & Fozzard, 1982).

(ii) *Voltage-dependent Ca channel.* On a scheme in which Ca_i^{2+} is determined by a voltage-independent Na–Ca exchange in parallel with a voltage-dependent Ca channel the exchange will not be at equilibrium. Therefore the magnitude of the flux through the exchange cannot be predicted from thermodynamic considerations and will depend on the exact mechanism of the exchange. We will consider an arbitrary description of Na–Ca exchange and show that, when combined with an appropriate voltage-dependent Ca channel, the observed results can be reproduced.

Let the net Ca efflux through the Na–Ca exchange be given by:

$$\text{net Ca efflux} = a\ Ca_i/Na_i^n - b\ Ca_o/Na_o^n,$$

where a and b are constants. The first term on the right-hand side represents the Ca efflux, the second the Ca influx through the exchange.

Let the Ca influx through the channel be a function of membrane potential $g(V)$. Therefore in the steady state:

$$g(V) = a\ Ca_i/Na_i^n - b\ Ca_o/Na_o^n.$$

Therefore

$$Ca_i = [g(V) + b\ Ca_o/Na_o^n]Na_i^n/a,$$

or

$$\ln Ca_i = \ln [g(V) + b\ Ca_o/Na_o^n] + n \ln Na_i - \ln a.$$

If $g(V)$ is greater than $b\ Ca_o/Na_o^n$ (in other words, the channel Ca influx is greater than that through Na–Ca exchange) and we assume that $g(V) = \exp kV$ where k is a constant then:

$$\ln Ca_i = kV + n \ln Na_i - \ln a. \tag{4}$$

Under our experimental conditions (i.e. Ca_o and Na_o are constant), this equation is formally identical to that derived above (eqn. 2) for a voltage-dependent Na–Ca exchange model. It must therefore be concluded that the present results are equally consistent with either scheme (i) or scheme (ii). If a different formalism had been used to describe Na–Ca exchange then a different dependence of the channel influx on membrane potential would be required to reproduce the results. In general, then, given a particular voltage-independent Na–Ca exchange in combination with a Ca channel of appropriate properties one can reproduce the present results.

Neither scheme (i) nor scheme (ii) makes explicit mention of other active Ca extrusion mechanisms. Although there is evidence for such Ca pumps in cardiac muscle (Caroni & Carafoli, 1980), they have not been included for simplicity. However, unless they are voltage-sensitive, their presence will not alter our conclusions. They could help to maintain a low Ca_i^{2+} but the Na- and voltage-sensitivity of Ca_i^{2+} would still reside in the properties of Na–Ca exchange (or the exchange plus a Ca channel).

Although our analysis cannot positively identify a voltage-sensitive Na–Ca exchange (scheme (i)), it should be noted that the conventional slow inward current I_{si} does not have the properties required of the Ca channel invoked for scheme (ii). This is

because (*a*) significant changes of resting tension can be seen at potentials negative to the activation range of I_{si} (cf. Eisner & Lederer (1979*a*) for further discussion of this point), and (*b*) Mn and D600 in concentrations which abolish I_{si} do not significantly affect the voltage-dependence of tonic tension (D. A. Eisner, W. J. Lederer & R. D. Vaughan-Jones, unpublished observations). Therefore, scheme (ii) requires the existence of a so-far undiscovered Ca channel which is affected by depolarization. It is undoubtedly simpler to interpret the voltage-dependence of tonic tension in terms of a voltage-dependent Na–Ca exchange.

Relaxation of tonic tension during prolonged depolarization

The present work shows that, after an initial rapid rise, tonic tension decays slowly during prolonged depolarization. Once again one must consider whether the decay of tonic tension reflects a fall of Ca_i^{2+} or whether other factors (e.g. a fall of pH_i) are involved. It is uncertain whether depolarization affects pH_i. Ellis & Thomas (1976) and Vaughan-Jones (1979) find no effect whilst De Hemptinne (1981) finds a small acidification (0·05 pH units) which may affect contraction. Furthermore it is not known how membrane potential affects pH_i during Na pump inhibition, so that an influence of pH_i cannot be excluded. On the other hand, direct Ca measurements in ferret ventricular muscle (using aequorin as a Ca indicator) show that depolarization (produced by increasing K_o at constant Na_o) leads to a rise of Ca_i^{2+} which then partially decays (D. G. Allen, D. A. Eisner & C. H. Orchard, unpublished observations). Therefore, at least in ventricular muscle, Ca_i^{2+} rises and then declines during prolonged depolarization.

A fall of a^i_{Na} accompanies the decay of tonic tension and this may mediate the relaxation. The fall could result from a decreased Na entry through membrane channels allowing Na extrusion mechanisms to lower a^i_{Na} (Eisner *et al.* 1981*b*). Intracellular Ca activity would then be decreased via Na–Ca exchange. Another possibility is that depolarization promotes a Na efflux and Ca influx through a voltage-dependent Na–Ca exchange. As a^i_{Na} falls the Ca influx would decrease and other Ca extrusion mechanisms would lower Ca_i^{2+}. The present results do not allow a separation between these two possibilities but, in either case, it is the decline of a^i_{Na} which eventually allows the decline of Ca_i^{2+} and tension.

It might be argued that a^i_{Na} does not control the fall of tension. Rather, some other process such as increased uptake of Ca by the sarcoplasmic reticulum decreases Ca_i^{2+} which, by Na–Ca exchange, then decreases a^i_{Na}. This possibility can be discounted because, although Ca_i^{2+} decays during prolonged depolarization, it will nevertheless be greater than before the depolarization. The a^i_{Na} would then *increase* rather than decrease if it were controlled by Ca_i^{2+}.

Whatever the mechanism, it is important to note that the change of a^i_{Na} is large enough to account for the observed change of tension: the relationship between tension and a^i_{Na} is similar no matter whether a^i_{Na} is changed by varying the Na pump activity or by a prolonged depolarizing pulse. Furthermore, a reduction of a^i_{Na} during prolonged depolarization is associated with a decrease of voltage-dependent tonic tension (Fig. 12). The control of tonic tension by membrane potential and a^i_{Na} under these conditions therefore resembles that observed during the onset of Na pump inhibition.

Relaxation of tonic tension during prolonged depolarization has been reported in other cardiac preparations although the depolarization has more usually been produced by raising external K (e.g. Gibbons & Fozzard, 1971; Chapman, 1973). In these cases the relaxation appears to be a complex process which is poorly understood, although an energy-dependent uptake of Ca^{2+} into the sarcoplasmic reticulum has been suggested in amphibian heart (Chapman, 1973). However, it is not known whether a^i_{Na} falls with depolarization in these preparations, and the possibility that this may contribute to relaxation is worth considering.

CONCLUSIONS

The present paper shows that membrane potential and a^i_{Na} interact to control tonic tension and therefore a^i_{Ca}. Internal Na can be changed by inhibiting the Na pump and it can then be changed by prolonged depolarization and repolarization: in either case an increase of a^i_{Na} produces an increase of tonic tension, the magnitude of which depends on the prevailing membrane potential. Such results are consistent with a transmembrane Na–Ca exchange which is voltage-sensitive. At equilibrium, a system exchanging three Na for one Ca describes the data well although the possible existence of other Ca pumps and leaks could mean that the exchange is not close to equilibrium so that the exact coupling ratio would remain in doubt. Alternatively, the voltage- and Na-sensitivity of tonic tension could result from a voltage-insensitive Na–Ca exchange in parallel with a voltage-gated Ca channel. However, such a channel would have to be entirely novel since the conventional slow inward current, I_{si}, cannot account for the results.

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REFERENCES

ALLEN, D. G., KURIHARA, S. & ORCHARD, C. H. (1981). The effects of reducing extracellular carbon dioxide concentration on intracellular calcium transients in mammalian cardiac muscle. *J. Physiol.* **317**, 52*P*.

BAKER, P. F., BLAUSTEIN, M. P., HODGKIN, A. L. & STEINHARDT, R. A. (1969). The influence of calcium on sodium efflux in squid axons. *J. Physiol.* **200**, 431–458.

BAKER, P. F. & MCNAUGHTON, P. A. (1976). Kinetics and energetics of calcium efflux from intact squid giant axons. *J. Physiol.* **259**, 103–144.

BERS, D. M. & ELLIS, D. (1982). Intracellular calcium and sodium activity in sheep heart Purkinje fibres. Effects of changes of external sodium and intracellular pH. *Pflügers Arch.* **393**, 171–178.

BLAUSTEIN, M. P. & HODGKIN, A. L. (1969). The effect of cyanide on the efflux of calcium from squid axons. *J. Physiol.* **200**, 497–528.

BLAUSTEIN, M. P., RUSSELL, J. M. & DE WEER, P. (1974). Calcium efflux from internally dialysed squid axons: the influence of external and internal cations. *J. supramol. Struct.* **2**, 558–581.

CARONI, P. & CARAFOLI, E. (1980). An ATP-dependent Ca^{2+}-pumping system in dog heart sarcolemma. *Nature, Lond.* **283**, 765–767.

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*. *J. Physiol.* **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. *J. Physiol.* **305**, 109–123.

CHAPMAN, R. A. & TUNSTALL, J. (1981). The tension–depolarization relationship of frog atrial trabeculae as determined by potassium contractures. *J. Physiol.* **310**, 97–115.

CORABOEUF, E. (1974). Membrane electrical activity and double component contraction in cardiac tissue. *J. molec. cell. Cardiol.* **6**, 215–225.

DE HEMPTINNE, A. (1981). Has the membrane potential an influence on the regulation of the intracellular pH in cardiac Purkinje fibres? *Arch. int. Physiol. Biochem.* **89**, 23–24.

DEITMER, J. W. & ELLIS, D. (1978*a*). Changes in the intracellular sodium activity of sheep heart Purkinje fibres produced by calcium and other divalent cations. *J. Physiol.* **277**, 437–453.

DEITMER, J. W. & ELLIS, D. (1978*b*). The intracellular sodium activity of cardiac Purkinje fibres during inhibition and re-activation of the Na–K pump. *J. Physiol.* **284**, 241–259.

DEITMER, J. W. & ELLIS, D. (1980). Interactions between the regulation of the intracellular pH and sodium activity of sheep cardiac Purkinje fibres. *J. Physiol.* **304**, 471–488.

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

EISNER, D. A. & LEDERER, W. J. (1979*b*). The role of the sodium pump in the effects of potassium depleted solutions on mammalian cardiac muscle. *J. Physiol.* **294**, 279–301.

EISNER, D. A. & LEDERER, W. J. (1980). The relationship between sodium pump activity and twitch tension in cardiac Purkinje fibres. *J. Physiol.* **303**, 475–494.

EISNER, D. A. & LEDERER, W. J. (1982). Effects of caffeine on the transient inward current in cardiac Purkinje fibres. *J. Physiol.* **322**, 48*P*–49*P*.

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1981*a*). The dependence of sodium pumping and tension on intracellular sodium activity in voltage-clamped sheep Purkinje fibres. *J. Physiol.* **317**, 163–187.

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

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1982*a*). The relationship between intracellular Na activity and tonic tension in sheep cardiac Purkinje fibres. *J. Physiol.* **326**, 67*P*–68*P*.

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1982*b*). A fall of intracellular Na activity accompanies the decay of tonic tension during prolonged depolarization in sheep cardiac muscle. *J. Physiol.* **328**, 21–22*P*.

ELLIS, D. & THOMAS, R. C. (1976). Direct measurement of the intracellular pH of mammalian cardiac muscle. *J. Physiol.* **262**, 755–771.

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

GIBBONS, W. R. & FOZZARD, H. A. (1971). High potassium and low sodium contractures in sheep cardiac muscle. *J. gen. Physiol.* **58**, 483–510.

GLITSCH, H. G., KAMPMANN, W. & PUSCH, H. (1981). Activation of active Na transport in sheep Purkinje fibres by external K or Rb ions. *Pflügers Arch.* **391**, 28–34.

GLITSCH, H. G., REUTER, H. & SCHOLZ, H. (1970). The effect of the internal sodium concentration on calcium fluxes in isolated guinea-pig auricles. *J. Physiol.* **204**, 25–43.

HORACKOVA, M. & VASSORT, G. (1979). Sodium–calcium exchange in regulation of cardiac contractility. Evidence for an electrogenic, voltage-dependent mechanism. *J. gen. Physiol.* **73**, 403–424.

LEE, C. O., KANG, D. H., SOKOL, J. H. & LEE, K. S. (1980). Relation between intracellular Na ion activity and tension of sheep cardiac Purkinje fibers exposed to dihydro-ouabain. *Biophys. J.* **29**, 315–330.

MULLINS, L. J. (1981). *Ion Transport in Heart.* New York: Raven Press.

MULLINS, L. J. & BRINLEY, F. J. JR (1975). Sensitivity of calcium efflux from squid axons to change in membrane potential. *J. gen. Physiol.* **65**, 135–152.

MULLINS, L. J. & REQUENA, J. (1981). The 'late' Ca channel in squid axons. *J. gen. Physiol.* **78**, 683–700.

PHILIPSON, K. D. & NISHIMOTO, A. Y. (1980). Na^+–Ca^{2+} exchange is affected by membrane potential in cardiac sarcolemmal vesicles. *J. biol. Chem.* **255**, 6880–6882.

PITTS, B. J. R. (1979). Stoichiometry of sodium–calcium exchange in cardiac sarcolemmal vesicles. *J. biol. Chem.* **254**, 6232–6235.

REEVES, J. P. & SUTKO, J. L. (1979). Sodium–calcium ion exchange in cardiac sarcolemmal vesicles. *Proc. natn. Acad. Sci. U.S.A.* **76**, 590–594.

REUTER, H. & SEITZ, N. (1968). The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. *J. Physiol.* **195**, 451–470.

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

THOMAS, R. C. (1978). *Ion-Sensitive intracellular microelectrodes.* New York & London: Academic Press.

TRAUTWEIN, T., MCDONALD, T. F. & TRIPATHI, O. (1975). Calcium conductance and tension in mammalian ventricular muscle. *Pflügers Arch.* **354**, 55–74.

VAUGHAN-JONES, R. D. (1979). Regulation of chloride in quiescent sheep-heart Purkinje fibres studied using intracellular chloride and pH sensitive micro-electrodes. *J. Physiol.* **295**, 111–137.