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THE TENSION-DEPOLARIZATION RELATIONSHIP OF FROG ATRIAL TRABECULAE AS DETERMINED BY POTASSIUM CONTRACTURES

BY R. A. CHAPMAN AND J. TUNSTALL

From the Department of Physiology, University of Leicester, Leicester LE1 7RH

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SUMMARY

1. In the presence of extracellular Na ions K contractures evoked from isolated frog atrial trabeculae show an initial phasic and a subsequent tonic contractile response.

2. The phasic response shows a steep dependence on membrane potential, persists in Na-free fluid, but is blocked by Mn ions, D600 and tetracaine. It has an indirect dependence on the $[Ca]_0$ and would seem to be associated with both the secondary inward current and the release of Ca^{2+} from intracellular stores.

3. The tonic component of the K contracture is unaffected by D600 or tetracaine, shows a shallow dependence on membrane potential but is absent in Na-free fluid. Its tension–depolarization curve is immediately affected by alteration of either the $[Ca]_0$ or the $[Na]_0$. The form of the tension–depolarization relationship and the effects of $[Ca]_0$ and $[Na]_0$ are consistent with the strength of the tonic tension being determined by a $3Na^+$ for $1Ca^{2+}$ exchange across the cell membrane.

4. The results agree well with those obtained previously with voltage-clamp experiments on the same tissue, and may also help with the interpretation of Ca-flux experiments.

INTRODUCTION

In a previous paper, results obtained with the Na-withdrawal contractures of isolated frog atrial trabeculae showed that the relationship between $[Ca]_0$, $[Na]_0$ and contractile tension was affected by the membrane potential (Chapman & Tunstall, 1980*a*). To account for this effect it was assumed that contractile tension depends on a second order reaction between the contractile apparatus and intracellular Ca ions and that $[Ca]_i$ is determined, in the living muscle, by a potential dependent exchange of three Na ions for each Ca ion across the sarcolemma. It is therefore possible that tension could result from the activity of this Na–Ca exchange when the sarcolemma is depolarized. A mechanism of this type would be affected by $[Na]_0$, $[Na]_i$ and $[Ca]_0$ in a way similar to that observed in experiments where tension is initiated by depolarization of frog cardiac muscle. Of the reported relationships between membrane potential and contractile force some fit the predictions of the model while others do not (Chapman, 1979). In order to ascertain the role of the potential-dependent Na–Ca exchange in the generation of depolarization-induced contraction, we

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have used K contractures to determine the tension–depolarization relationship of isolated frog atrial trabeculae. This has enabled us to compare the tension–depolarization curves obtained from the living muscle, in normal Ringer, in Na-free Ringer and in the presence of a number of inhibitors, to the predictions of the model used to explain Na-withdrawal contractures.

Part of this work has been already published in brief (Chapman & Tunstall, 1980*b*).

METHODS

TABLE 1. Composition of experimental solutions

	Solution	Tonicity	NaCl (mM)	Tris HCl (mM)	KCl (mM)	Na pyruvate (mM)
A	120 Na Ringer	Isotonic	115·0	5·0	3·0	5·0
B	120 Na 100 K Ringer	Hypertonic	120·0	5·0	100·0	—
C	120 Na K-free Ringer	Hypertonic	120·0	105·0	0·0	—
D	60 Na Ringer	Isotonic	60·0	65·0	3·0	—
E	60 Na 100 K Ringer	Hypertonic	60·0	65·0	100·0	—
F	60 Na K-free Ringer	Hypertonic	60·0	165·0	0·0	—
G	100 Na Ringer	Isotonic	95·0	5·0	3·0	5·0
H	100 Na 100 K Ringer	Hypertonic	100·0	5·0	100·0	—
I	100 Na K-free Ringer	Hypertonic	100·0	105·0	0·0	—
J	50 Na Ringer	Isotonic	50·0	75·0	3·0	—
K	50 Na 100 K Ringer	Hypertonic	50·0	55·0	100·0	—
L	50 Na K-free Ringer	Hypertonic	50·0	155·0	0·0	—
M	Na-free Ringer	Isotonic	0·0	135·0	3·0	—
N	Na-free 100 K Ringer	Isotonic	0·0	38·0	100·0	—
O	Na-free OK Ringer	Isotonic	0·0	138·0	0·0	—

The methods of dissection, mounting and recording the responses of atrial trabeculae isolated from the heart of the frog, *Rana pipiens*, were as described previously (Chapman & Tunstall, 1980*a*). Generally, very fine unbranched trabeculae, between 20 and 100 μm in diameter, were selected for this study.

The composition of the various solutions used in this study are given in Table 1. Variation of the $[K]_0$ was achieved by mixing the appropriate K-free and 100 mM-K fluids in the required ratio. This ensured that the tonicity of the various contracture fluids remained at a constant hypertonicity. In a few experiments the $[K]_0$ was raised above 100 mM, by adding solid KCl to solutions B, E or N. Ca^{2+} was added from 1 M-$CaCl_2$ stock, Mn^{2+} from 1 M-Mn Cl_2 stock, tetracaine as 0·2 M-tetracaine HCl buffered to a pH of 7·2 by Tris HCl, and D600 as a 0·1 M stock in ethanol. In experiments using D600, an equivalent amount of ethanol was added to the other fluids bathing the heart.

The preparation was perfused with an Na-containing Ringer (soln. A or G) and stimulated electrically at a rate between 4 and 12 min^{-1}. K contractures were initiated at intervals of 10–15 min at which time the stimulation was interrupted. When the $[Na]_0$ or $[Ca]_0$ was altered or an inhibitor applied as part of the experiment, the modified Na-Ringer was applied for 15 or 30 sec before the K contracture was initiated. This period is sufficient to exchange the extracellular spaces within the preparation (Chapman, 1971*b*; Page & Niedergerke, 1972). After each contracture the preparation was returned to the standard Na-Ringer. As a control a tension–depolarization curve, in the standard Na-Ringer, was determined before and after those in modified fluids. For the determination of the tension–depolarization relationship in Na-free fluid, the $[K]_0$ was raised after the Na-withdrawal contracture had fully spontaneously relaxed.

The membrane potentials of the muscle cells were measured, for all the experimental solutions used, with intracellular 3 M-KCl-filled micro-electrodes in a separate series of experiments. Curves relating membrane potential to the logarithm of the $[K]_0$ were drawn and used to provide the appropriate membrane potential for the construction of tension–depolarization relationships.

The exponential phases of the tension–depolarization curves (generally between 1 and 10 % of the maximum tension) were regressed using a programmable calculator. The slope of the relationship was determined and the best line fitted to each curve. The shift in the tension–depolarization curves, caused by alteration of the experimental conditions, was read off as the difference between the regression lines. Collected data is expressed as the mean ± the S.E. of the mean, and the number of experiments.

Most experiments where done between 18 and 21 °C. Some however were done as low as 10 °C and others as high as 25 °C.

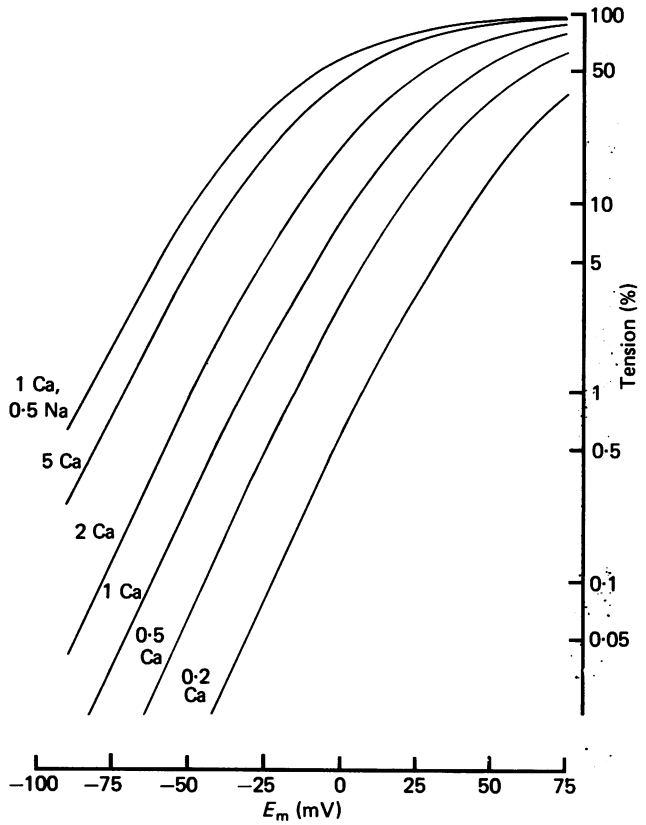

Fig. 1. The theoretical curves derived for the relationships between the logarithm of the relative tension and the membrane potential obtained by solving eqns. (1) and (2) in the text, when $K_{Ca} = 2 \times 10^6$ M^{-1}, $[Na]_i = 7$ mM, $F/RT = 40$ V^{-1}, for a normal $[Na]_0 = 120$ mM, when the $[Ca]_0$ is varied from 0·2 to 5 mM (as indicated on the Figure) and for $[Na]_0$ of 60 mM when $[Ca]_0$ is 1 mM.

Theory

Fig. 1 shows the relationships between membrane potential and the logarithm of the predicted tension obtained by solving eqns. (1) and (2) for different membrane potentials, $[Na]_0$ and $[Ca]_0$. These equations describe the dependence of relative tension on $[Ca]_i$ and in turn the dependence of $[Ca]_i$, on $[Ca]_0$, $[Na]_i$ and membrane potential if a three Na ion for one Ca ion exchange across the cell membrane controls $[Ca]_i$. They were used by Chapman & Tunstall (1980*a*) to fit data obtained with Na-withdrawal contractures in frog atrial muscle. Eqn. (2) was originally derived for comparison with Ca efflux experiments on squid giant axons (Blaustein & Hodgkin, 1969) and has subsequently found a wider application (Blaustein, 1974).

$$\text{Relative tension} = \left[\frac{[Ca]_i K_{Ca}}{1+[Ca]_i K_{Ca}}\right]^2 \quad (1)$$

$$[Ca]_i = \frac{[Ca]_0[Na]_i^3}{[Na]_0^3} \exp E_m F/RT. \quad (2)$$

Where K_{Ca} is the apparent binding constant of the controlling contractile proteins for Ca ions, E_m is the membrane potential and F, R and T have their usual meaning. In Fig. 1, K_{Ca} is 2×10^6 M^{-1} and $[Na]_i$ is 7 mM, i.e. the values derived by fitting eqns. (1) and (2) to data obtained with Na-withdrawal contractures (Chapman & Tunstall, 1980*a*).

Fig. 1 shows that the dependence of the logarithm of tension upon membrane potential is a curve. Over the lower range of tensions which can be accurately measured, using the present experimental method, i.e. 1–10% of the maximum tension, tension increases approximately exponentially with membrane potential, and an e-fold increase in tension occurs for each 14 mV of depolarization. A doubling or halving of $[Ca]_0$ shifts the curve by an amount equivalent to 17·3 mV, a 5 times change in $[Ca]_0$ by 40·5 mV, and a halving of $[Na]_0$ by 52·0 mV, over virtually the whole of the tension range.

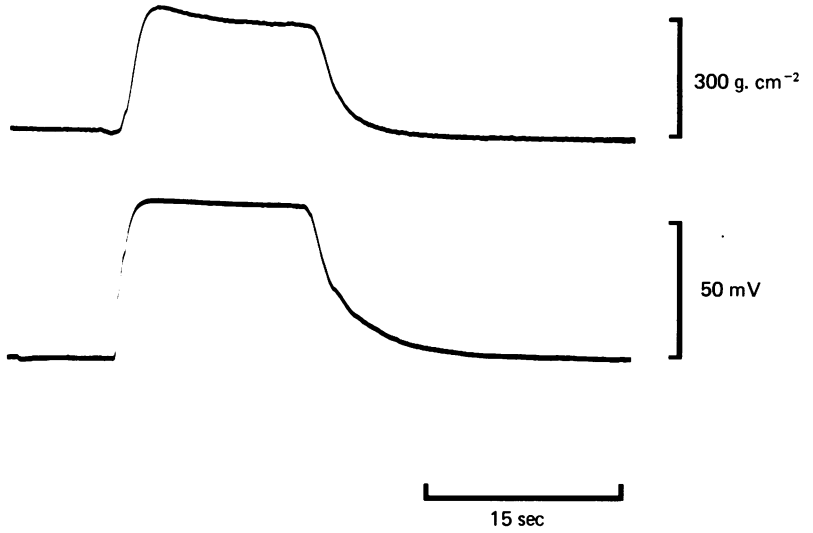

Fig. 2. Simultaneous recording of the tension generated by an isolated frog atrial trabecula (upper trace) and the change in membrane potential of one of the cells (lower trace), when the $[K]_0$ is suddenly raised from 3 to 50 mM. 1 mM-Ca, 20·5 °C.

RESULTS

Comparison of the rate of depolarization with the rate of tension development in atrial trabeculae caused by raising $[K]_0$

To derive accurate tension–depolarization curves using K contractures it is important that the depolarization of the preparation is complete before tension development reaches its peak. Fig. 2 shows the effects of raising $[K]_0$ on both the intracellularly recorded membrane potential and the tension developed by an isolated trabecula. On raising $[K]_0$ the membrane potential changes rapidly, often after the development of an action potential. There is often evidence of a further small but slower creep in the membrane potential. The time taken to depolarize the cells from 10 to 90% of their eventual membrane potential, $2{\cdot}1 \pm 0{\cdot}3$ sec (twenty-seven

experiments), was virtually independent of the size of the change in $[K]_0$. The time to exchange the fluid in the experimental chamber is less than 0·4 sec (Chapman & Tunstall, 1971), so that diffusion into the trabecula would seem to rate limit the depolarization caused by raising $[K]_0$. The time taken for the depolarization of the

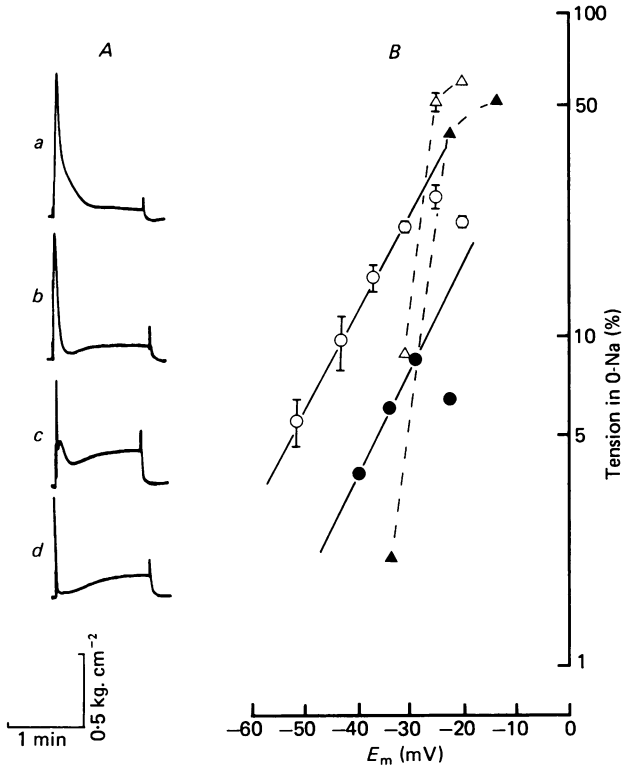

Fig. 3. *A*, traces of K contractures evoked in a 100 mM-Na Ringer, illustrating the contribution of the phasic and tonic components. *a*, 100 mM-K contracture, 0·5 mM-Ca; *b*, 100 mM-K contracture, 0·25 mM-Ca; *c*, 50 mM-K contracture, 0·5 mM-Ca, and *d*, 35 mM-K contracture, 0·5 mM-Ca. 19 °C. *B*, the logarithm of the contracture tension, expressed as a percentage of the tension evoked by perfusion with Na-free fluid, for the phasic (triangles) and tonic (circles) components plotted against the membrane potential. The open symbols are of contractures initiated in 0·5 mM-Ca and the filled symbols for contractures evoked 30 sec after lowering the $[Ca]_0$ to 0·25 mM. The continuous lines are regression lines for the tonic contractures drawn to: tension = 158 exp 0·064 E_m for the data in 0·5 mM-Ca and tension = 84 exp 0·075 E_m for the 0·25 mM experiments ($r > 0·98$ in each case). The bars indicate the range of the determinations done in 0·5 mM-Ca before and after those in 0·25 mM-Ca. 21 °C. *Not* the same experiment as part A.

muscle cells agrees well with the diffusion time into the trabecula calculated from anatomical studies (Page & Niedergerke, 1972). The rate of tension development is strongly dependent on $[Ca]_0$ and $[K]_0$ and may be very rapid. In the analysis of present experiments we have used tensions less than 50% of the tension generated on exposure to Na-free fluid. The time taken for tension to increase from 10 to 90% of its final value was never less than 3·5 sec.

Tension–depolarization relationship in Na-containing Ringer solution

The K contractures evoked from fine atrial trabeculae show a variety of forms which depend upon the $[K]_0$ and hence the membrane potential. When the $[K]_0$ is raised to yield a membrane potential more negative than -40 mV (40 mM-K) the contractile response is composed of a slowly increasing tension which reaches a maximum in 20–50 sec after which tension is well maintained (Fig. 3*A*, *d*). These tonic responses may be preceded by a heart beat. When the membrane is depolarized beyond -30 mV ($\simeq$ 50 mM-K) the initial heart beat is followed by a rapidly developing but phasic contraction which reaches its peak in about 5 sec and then subsides after which the tonic response develops (Fig. 3*A*, *b*, *c*). With the application of strongly depolarizing solutions, the distinction between the phasic and the tonic responses is often unclear, as the tonic component develops more rapidly and is less well sustained it becomes fused into the phasic response (Fig. 3*A*, *a*). The threshold potential for the phasic response is difficult to determine, but the potential at which a phasic response can first be clearly detected was $-30{\cdot}9 \pm 1{\cdot}3$ mV (nineteen experiments).

The logarithm of the strength of the tonic response increases linearly with membrane depolarization (Fig. 3*B*) and regression analysis shows an e-fold increase in tension occurred for a mean depolarization of $15{\cdot}8 \pm 0{\cdot}8$ mV (twenty-six experiments). At large depolarizations, when the tonic tension follows a strong phasic response, the final tonic tension is generally smaller than that obtained with a more moderate depolarization (Fig. 3*B*).

The phasic part of the K contracture shows a steep dependence on the membrane potential. Regression analysis can usually only be done on a few points because the response rapidly saturates, however an e-fold increase in phasic tension occurs for a mean depolarization of $6{\cdot}2 \pm 0{\cdot}6$ mV (nineteen experiments).

The effects of brief changes in $[Ca]_0$ and $[Na]_0$ on the tension–depolarization relationship

Changes in the $[Ca]_0$ and the $[Na]_0$ have complicated effects on the contractility of frog cardiac muscle (Chapman & Niedergerke, 1970; Chapman, 1971*a*). We have attempted to restrict the effects to those which depend immediately and directly upon the extracellular concentration of the ions, by limiting to 30 sec, the period of exposure of the preparation to the altered solution. Fig. 3*B* illustrates the results of a typical experiment where the tension–depolarization curve was determined in the presence of 0·5 mM-Ca before and after a similar determination in 0·25 mM-Ca. The Figure shows that reduction of the $[Ca]_0$ causes a large fall in the size of the tonic tension whereas the phasic tension is only slightly altered. The slopes of the relationships between the logarithm of the strength of two phases of contraction and the membrane potential are unaltered. In experiments of this type a doubling of $[Ca]_0$ shifts the regression lines fitted to the tonic tensions by $15{\cdot}8 \pm 0{\cdot}6$ mV (six experiments) whereas an increase to 5 times the original $[Ca]_0$ shifts the lines by $34{\cdot}0 \pm 2{\cdot}0$ mV (four experiments), in each case towards more negative membrane potentials. Lowering the $[Ca]_0$ produced equivalent shifts in the opposite direction (Fig. 3*B*). Brief alteration of the $[Ca]_0$ has little effect on the phasic tension which was shifted towards more negative membrane potentials by only $3{\cdot}1 \pm 1{\cdot}3$ mV for a doubling of $[Ca]_0$ (six experiments) and by $10{\cdot}2 \pm 2{\cdot}4$ mV (four experiments) when $[Ca]_0$ was increased to 5 times its original value.

The reduction of $[Na]_0$ had similar effects to those produced by increasing $[Ca]_0$, a halving of the $[Na]_0$ shifts the regression lines for the tonic tension by $35{\cdot}0 \pm 1{\cdot}8$ mV (four experiments) towards more negative membrane potentials and the phasic tension by $12{\cdot}1 \pm 3{\cdot}7$ mV (four experiments) in the same direction. These results suggest that the K contracture activates two separate mechanisms both of which lead to tension generation.

The tension–depolarization relationship in Na-free fluid

On exposure to Na-free fluid, the muscle develops a strong contracture which relaxes spontaneously to be fully relaxed in 3–6 min. Following the spontaneous relaxation of tension large increases in $[Ca]_0$ have no effect on the tension generated by the muscle or upon the contractures evoked by the application of caffeine, hypertonic fluids or by rapidly cooling the muscle (Chapman & Miller, 1974; Chapman & Ellis, 1974; Chapman, 1978) suggesting that the Na–Ca exchange is now inactivated. However, depolarization of the muscle in Na-free fluid evokes a further contraction (Chapman & Miller, 1974; Chapman & Ellis, 1977). Fig. 4*A* shows a typical tension–depolarization curve determined using K contractures after the spontaneous relaxation of the preceding Na-withdrawal contracture. On semilogarithmic co-ordinates the curve shows only one component which initially increases exponentially with an e-fold increase in tension for a depolarization of $8{\cdot}0 \pm 0{\cdot}4$ mV (ten experiments). The whole tension–depolarization relationship is shifted towards more negative membrane potentials in Na-free fluid (compare Figs. 3*B* and 4). Changing the $[Ca]_0$ for 30 sec before and during depolarization with K-rich Na-free fluid has relatively little effect on the tension depolarization relationship (Fig. 4*A*) in fact an increase in the $[Ca]_0$ to 5 times its initial value shifts the exponential phase of the relationship by $1{\cdot}3 \pm 1{\cdot}9$ mV (five experiments) towards less negative membrane potentials. Lowering the $[Ca]_0$ to 10^{-4} M for 30 sec before the initiation of the K contracture produces a small shift in the exponential phase of the tension–depolarization relationship towards more negative membrane potentials. The application of Ca-free Na-free fluid containing 1 mM-EGTA (free [Ca] $< 10^{-7}$ M) for 30 sec before depolarization has a more substantial effect, shifting the curve towards less negative membrane potentials by an amount equivalent to $40{\cdot}0 \pm 3{\cdot}1$ mV (three experiments).

Alteration of the $[Ca]_0$ before the removal of Na ions from the bathing fluid has a more obvious effect on the tension–depolarization relationship in Na-free fluid (Fig. 4*B*). A 5 times increase or decrease in the $[Ca]_0$ during the Na-withdrawal contracture shifts the tension–depolarization relationship, determined with the $[Ca]_0$ at a constant concentration, by $4{\cdot}6 \pm 0{\cdot}5$ mV (four experiments) raising $[Ca]_0$ causes a shift towards more negative membrane potentials and lowering $[Ca]_0$ having the opposite effect.

The K contractures in Na-free fluid show a slow spontaneous relaxation. A unique feature is that on lowering the $[K]_0$ the rate of relaxation is unchanged, suggesting that relaxation in Na-free fluid does not depend on the membrane potential.

The effect of tetracaine on the tension–depolarization relationships

(i) *In Na-free fluid.* The contractures evoked in Na-free fluid by the application of caffeine, or hypertonic fluid, or by rapid cooling are all strongly antagonized by local anaesthetics (Chapman & Miller, 1974; Chapman & Ellis, 1974; Chapman, 1978). Similarly, the K contractures evoked in the Na-free fluid are inhibited by a brief

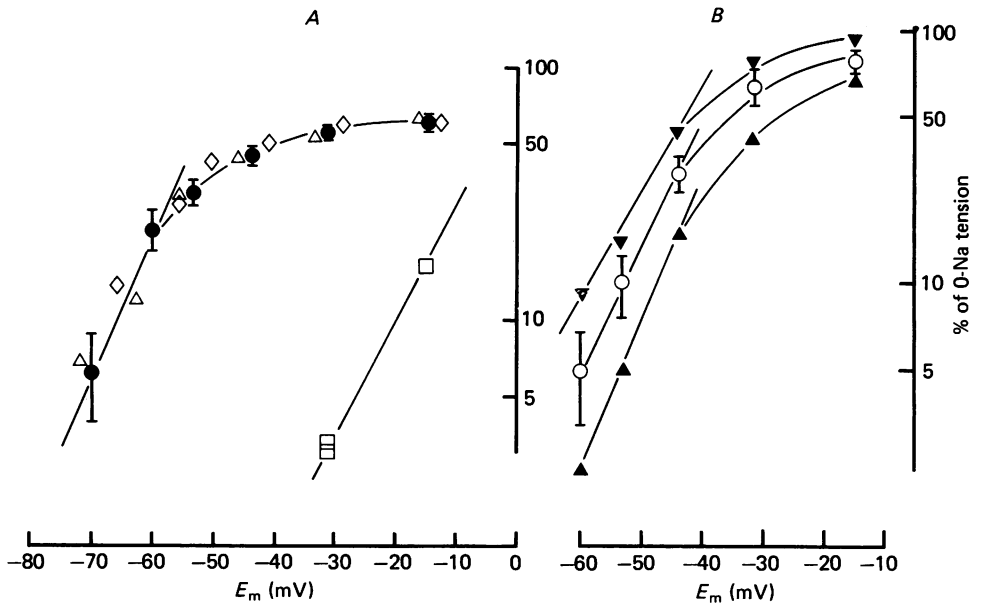

Fig. 4. The tension–depolarization relationship obtained in Na-free fluid after the spontaneous relaxation of the Na-withdrawal contracture. Log tension as a percentage of the Na-free contracture against membrane potential in each case. *A*, the immediate effect of $[Ca]_0$. In this experiment tension–depolarization curves were obtained by exposing the heart to 1 mM-Ca, Na-free fluid for 5·5 min, at which time the spontaneous relaxation was complete, and then raising the $[K]_0$. (●—●), controls in 1 mM-Ca at the beginning and end of the experiment, the bars indicating the range; (◇—◇) 5 min after the Na-withdrawal contracture evoked in 1 mM-Ca, the $[Ca]_0$ was reduced to 0·2 mM-Ca for 30 sec before the $[K]_0$ was increased; (△—△), the $[Ca]_0$ was increased to 5 mM, 30 sec before the initiation of the K-contracture; (□—□), in 1 mM-Ca, 30 sec before the K contracture, 1 mM-tetracaine was added to the perfusing fluid. The straight lines are regression lines; in the absence of tetracaine, tension = 2600 exp 0·125 E_m (r = 0·89) and in the presence of tetracaine, tension = 58 exp 0·11 E_m (r = 0·99). 18 °C.

B, the effect of the $[Ca]_0$ and during the conditioning Na-withdrawal contracture on the strength of the subsequent K contracture evoked in Na-free fluid containing 0·5 mM-Ca. The Na-withdrawal contractures were elicited either in the presence of 0·5 mM-Ca (○—○); 0·1 mM-Ca (▲—▲); or 2·5 mM-Ca (▼—▼). After 5 min when tension had returned to the resting level the $[Ca]_0$ was changed to 0·5 mM and 30 sec later a K contracture was evoked. The bars are ± the S.E. for three determinations of the K contractures in 0·5 mM-Ca. The straight lines are regression lines for the data between −60 and −40 mV, with r > 0·96 in each case, the lines are drawn to: tension = 4142 exp 0·11 E_m, for the 0·5 mM-Ca data; tension = 4872 exp 0·13 E_m, for the 0·1 mM-Ca data, and tension = 2465 exp 0·094 E_m for the 2·5 mM-Ca data. 18 °C.

application of tetracaine (Fig. 4). Typically, a 30 sec application of 1 mM-tetracaine is sufficient to inhibit the contracture and to shift the tension–depolarization relationship by 42·1 ± 3·3 mV (seven experiments) towards less negative membrane potentials. Lower doses are less effective, while stronger doses (2 mM and over), which may themselves induce a weak contracture, completely inhibit the K contracture even up to $[K]_0$ of 200 mM.

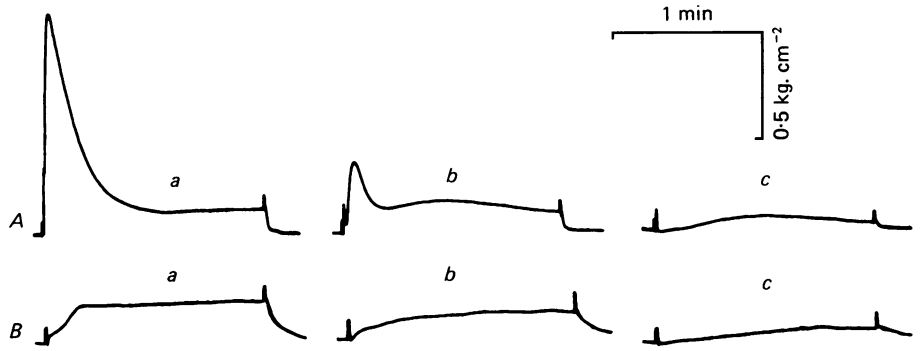

Fig. 5. The effect of 30 sec pretreatment with 1 mM-tetracaine on the K contractures evoked in normal Ringer (120 mM-Na). *A*, control contractures; *B*, contractures evoked in the presence of tetracaine. *a*, 100 mM-K; *b*, 50 mM-K, and *c*, 35 mM-K. 1 mM-Ca. 19 °C.

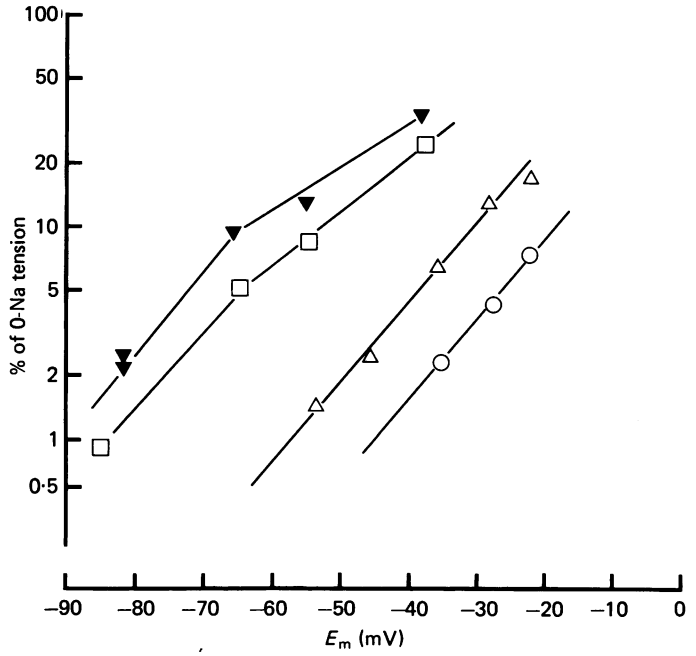

Fig. 6. Tension–depolarization relationship for an atrial trabecula equilibrated in normal Ringer (120 mM-Na, 1 mM-Ca) and pretreated with 1 mM-tetracaine for 30 sec before the initiation of the K contractures. Tension is plotted as the logarithm of the tension, as a percentage of the 0-Na contracture, against the membrane potential. ○—○, 1 mM-Ca throughout; △—△, the $[Ca]_0$ was raised to 2 mM for 30 sec before the K contracture; □—□, the $[Ca]_0$ was raised to 5 mM, 30 sec before the K contracture; ▼—▼, the $[Na]_0$ was reduced to 60 mM, 30 sec before the K contracture. The lines for the 1 and 2 mM-Ca data are regression lines ($r > 0{\cdot}96$) drawn to: tension = 57 exp $0{\cdot}064\ E_m$ for 1 mM-Ca, and tension = 121 exp $0{\cdot}063\ E_m$ for 2 mM-Ca. 20·5 °C.

(ii) *In Na-containing fluid.* A 30 sec exposure to tetracaine, at a concentration of 1 mM or above, has different effects on the phasic and tonic parts of the K contracture evoked in the presence of Na ions. The phasic component is virtually abolished, the tonic component is unaltered or sometimes potentiated (especially at high $[K]_0$) and the rate of relaxation on lowering $[K]_0$ is slowed (Fig. 5). At concentrations less than 1 mM, a 30 sec application of tetracaine produces a partial inhibition of the phasic response. The tension–depolarization curve, in 1 mM-tetracaine shows a single phase when plotted on semilogarithmic co-ordinates (Fig. 6) and an e-fold increment in tension is now produced by a depolarization of $18{\cdot}9 \pm 1{\cdot}0$ mV (twenty-two experiments). The shifts in the tension–depolarization relationship caused by briefly altering the $[Ca]_0$ or $[Na]_0$ in the presence of 1 mM-tetracaine are equivalent to $16{\cdot}2 \pm 1{\cdot}7$ mV (seven experiments) for a doubling of $[Ca]_0$, $44{\cdot}5 \pm 1{\cdot}7$ mV (three experiments) for an increase to 5 times the original $[Ca]_0$, and $45{\cdot}5 \pm 1{\cdot}0$ mV (three experiments) for a halving of the $[Na]_0$. These shifts are all towards more negative membrane potentials (Fig. 6).

The effects of D600 on the tension–depolarization relationship

The action of D600 on the K contractures is strongly dose-dependent. Unlike tetracaine it is slow to act. This means that, to achieve its maximal effect, the substance must be applied for over 10 min before the first contracture is evoked and then continuously between contractures.

(i) *In Na-free fluid.* D600 at concentrations as high as 0·1 mM has no effect of the Na withdrawal contracture but causes a shift of the tension depolarization relationship towards less negative membrane potentials. The shift is equivalent to $23{\cdot}8 \pm 2{\cdot}7$ mV (four experiments) for 0·1 mM-D600.

(ii) *In Na-containing fluid.* At concentrations between 0·05 and 0·1 mM, D600 inhibits the phasic part of the K contracture evoked in Na-containing Ringer solution, without affecting the tonic component. The remaining tonic component has an exponential phase where an e-fold increase in tension occurs for a $20{\cdot}1 \pm 3{\cdot}7$ mV depolarization (four experiments). This relationship is shifted by $17{\cdot}5 \pm 2{\cdot}5$ mV towards more negative membrane potentials by doubling the $[Ca]_0$ for 30 sec before and during the K contracture.

The effect of Mn^{2+} on the tension–depolarization relationship in Na-free fluid

Chapman & Ellis (1976) have already shown that Mn^{2+} inhibits the K contracture in Na-free fluid and slows the development of this contracture when evoked in otherwise normal Ringer fluid. These workers exposed the heart to relatively high concentrations of Mn^{2+}, so as to inhibit the Na-withdrawal contracture before applying the depolarizing fluid. We find that if the Na-withdrawal contracture is allowed to develop fully and to relax spontaneously, before Mn^{2+} ions are applied, then a brief exposure to Mn^{2+} causes a profound inhibition of the K contracture. Application for 30 sec of a $[Mn]_0$ of twice the $[Ca]_0$ causes the maximum effect and shifts the tension–depolarization curve by $47{\cdot}7 \pm 6{\cdot}3$ mV (four experiments) towards less negative membrane potentials. Further increases in the $[Mn]_0$, up to 20 times the $[Ca]_0$ produce no further change in the tension–depolarization curve. The contractile response that can still be evoked by strong depolarization in Na-free fluid

containing Mn ions is completely inhibited if 2 mM-tetracaine is applied with the Mn ions for 30 sec.

The effects of heart rate on the tension–depolarization relationship in Na-containing Ringer solution

The rate of beating of the heart is known to affect the strength of the subsequent K-contracture (Niedergerke, 1956; Gibbons & Fozzard, 1971). In a few preliminary experiments we have confirmed this observation; the strength of the phasic component and to a lesser extent the tonic component are increased by raising and are depressed by lowering the preceding heart rate.

The effects of temperature on the tension–depolarization relationship in Na-containing Ringer solution

If the temperature of the solution perfusing an atrial trabeculae is lowered from room temperature (17–20 °C) to below 15 °C, the phasic component of the K contracture is reduced and at low temperatures the remaining contracture may be insensitive to tetracaine or D600. On rewarming the preparation to 20 °C the phasic component reappears.

Attempts to compare the tension–depolarization curves at different temperatures were frustrated because the effects of reducing the temperature are slow to develop and are not readily reversible. Cooling the preparation results in a slow rise in the strength of the tonic component which may take over 1 hr to stabilize. In five experiments, where the K contractures were evoked in the presence of 1 mM-tetracaine, the tension–depolarization relationship of the tonic component was measured at 25 °C and again after 1 hr at 15 °C. The effect of reducing the temperature was to shift the exponential phase of the relationship by an amount equivalent to $28{\cdot}4 \pm 6{\cdot}1$ mV towards more negative membrane potentials. The subsequent rewarming of the preparation to 25 °C produced a variable shift in the opposite direction, in two preparations the relationship between tonic tension and membrane potential remained unchanged.

DISCUSSION

The results obtained with K contractures show that depolarization of frog atrial muscle initiates two phases of tension development; two phases of response are also seen in voltage clamp experiments. Table 2 summarizes the differences between the phasic and tonic components as obtained by both methods. These differences, which are both physiological and pharmacological, suggest that the two components originate from separate processes, and the similarities between the data obtained with K contractures and voltage clamp reinforce this view (Table 2). It is most likely that the difference in the timing of the two components found by the two methods is due to the different way in which the membrane potential was altered. However, the relative slowness of the depolarization of the cells caused by perfusion with K-rich fluids, as compared to that achieved with voltage clamp methods, could mean that the phasic contraction is partially inactivated. The notion of there being two separate contractile responses to depolarization of amphibian and mammalian cardiac muscle is not new. It was first suggested iu prolonged action potential experiments, and later with K contractures from mammalian heart and voltage clamp experiments on both tissues (Morad & Trautwein, 1968; Wood, Heppner & Weidmann, 1969; Gibbons & Fozzard, 1971; Vassort & Rougier, 1972; Leoty & Raymond, 1972; Einwächter, Hass & Kern, 1972; Ochi & Trautwein, 1971). The evidence for this division has been carefully assessed elsewhere (Morad & Goldman, 1973) and would seem now to be well established. It is, therefore, reassuring that these results obtained with K contractures on frog atrial muscle now agree with those obtained with voltage clamp. The evidence for a phasic component of contraction in frog ventricle is less strong

TABLE 2. Comparison of the properties of the phasic and tonic components of the K contractures obtained in the present work, which are in turn compared to the results obtained by applying the voltage clamp method to frog cardiac muscle

	Phasic component		Tonic component		
Feature	K contracture	Voltage clamp	K contracture	Voltage clamp	References
Time to peak	5 sec	0·5 sec	20 sec	1 sec	Present study; Leoty & Raymond, 1972; Vassort & Rougier, 1972; Morad & Orkand, 1971
Potential threshold	−30 mV	−40 mV	No discrete threshold, hyperpolarization induces relaxation	No discrete threshold, hyperpolarization induces relaxation	Present study; Vassort & Rougier, 1972; Leoty & Raymond, 1972
Depolarization for an e-fold increase in tension	6–8 mV	6–8 mV	15–20 mV	12–14 mV	Present study; Leoty & Raymond, 1972; Greenspan & Morad, 1972; Goto, Kimoto & Kato, 1971; Morad & Orkand, 1971
Effect of Na-free fluid	Potentiated and threshold reduced	Potentiated with no changes in threshold	Blocked	Blocked	Present study; Vassort, 1973; Benninger, Einwächter, Hass & Kern, 1976
Effect of Mn^{2+}	Blocked	Blocked	No effect	No effect	Present study; Vassort & Rougier, 1972; Leoty & Raymond, 1972; Einwächter, Hass & Kern, 1972; Chapman & Ellis, 1977
Effect of D600	Blocked	Blocked	No effect	No effect	Present study; Einwächter *et al.* 1972; Chapman & Ellis, 1977
Effect of tetracaine	Blocked	Blocked	No effect	No effect	Present study; R. A. Chapman & C. Leoty, (in preparation)
Sensitivity to $[Ca]_0$ and $[Na]_0$	Small and delayed	Small for Ca^{2+} no effect for Na^+	Large and rapid	Large and rapid	Present study; Benninger *et al.* 1976 Einwächter & Brommundt, 1978; Anderson, Hirsch & Kavaler, 1977
Effect of low temperature	Reduced	—	Potentiated	—	Present study

(compare the results of Goto, Kimoto & Kato (1971) with those of Morad & Orkand (1971)). Differences in the contractile behaviour of amphibian atrial and ventricular muscle may be related to the presence of a larger amount of sarcoplasmic reticulum in atrial as against ventricular muscle cells (Masson-Pevet, 1972; Hillman, 1975).

In isolated frog skeletal muscle fibres two phases of contraction are seen during the development of K contractures. In this case they are probably due to the differential depolarization of the T-system and the surface sarcolemma (Costantin, 1971); in amphibian heart there is little evidence of a T-system (Page & Niedergerke, 1972).

The origin of the tonic contracture: the Na–Ca exchange

The original purpose of this work was to test the ability of the model of the Na–Ca exchange, proposed by Chapman & Tunstall (1980*a*) to predict the relationships for depolarization-induced contraction in frog atrial muscle. Clearly, if there are indeed two processes which are activated by depolarization of the cell membrane and lead to the generation of tension, then excitation–contraction coupling in frog heart cannot be solely due to an electrogenic Na–Ca exchange. Table 3 shows the collected data for the tension–depolarization relationships of the tonic and phasic components of the K contracture in normal Ringer solution, and with tetracaine or D600. The relationship between membrane potential and tonic contractile force, and the effects of $[Ca]_0$ and $[Na]_0$ upon this relationship are very close to the predictions of three Na–one Ca exchange model. The data which best fits the prediction of the model were obtained in the presence of tetracaine or D600. This experimental data is also very similar to that provided by Benninger *et al.* (1976) for the tonic tension observed under voltage clamp (their Table 1). In quantitative terms the predictions of the model are also good, for example; the tension predicted by eqns. (1) and (2) for a 100 mM-K contracture in 1 mM-Ca Ringer is 3 % of the maximum tension, assuming K_{Ca} to be 2×10^6 M^{-1} and $[Na]_i$ to be 7 mM; in five experiments done in the presence of tetracaine or D600, the tonic tension induced by perfusion with Ringer + 100 mM-K was $5{\cdot}6 \pm 0{\cdot}9$ % of the strength of the contracture evoked by perfusion with Na-free fluid. This value, although larger than the predicted tension, would be expected if K_{Ca} were larger by less than 1 S.D. of the mean value found in the Na-withdrawal experiments (Chapman & Tunstall, 1980*a*). Therefore, the dependence of the tonic tension on membrane potential and the effects of $[Ca]_0$ and $[Na]_0$ found in the present work are consistent with $[Ca]_i$ and hence tonic tension, being controlled by an exchange of three Na ions for one Ca ion across the sarcolemma. The effects of cooling the preparation are also in general agreement with this conclusion. If cooling causes a rise in $[Na]_i$ as argued by Chapman & Tunstall (1980*a*), then the shift in the tension–depolarization curve for the tonic tension of 28 mV, caused by a 10 °C cooling, is equivalent to a 50 % rise in $[Na]_i$. Wiggins & Bassett (1978) find that the tonic component of the K contracture in cat ventricular muscle is increased by raising the heart rate and perfusion with the Na ionophore Monensin, and is reduced by perfusion with Na-depleted Tyrode solution.

The origin of the phasic contracture: Ca current or intracellular stores?

It is likely that experiments done in Na-free fluid enable the phasic tension development observed with K contractures or voltage clamp to be studied alone. In these experiments variation of the $[Ca]_0$ over a wide range has little direct effect on

TABLE 3. The collected experimental data for the phasic and tonic components of the K contracture induced in frog atrial trabeculae, in normal Ringer, Na-free Ringer and in the presence of tetracaine or D600, are compared to the predictions of a model for the Na–Ca exchange

	Depolarization for e-fold increase in tension (mV)		Shift in tension–depolarization curve caused by changes in extracellular ion concentrations of					
			× 2 in $[Ca]_o$ (mV)		× 5 in $[Ca]_o$ (mV)		× ½ in $[Na]_o$ (mV)	
Experimental conditions	Phasic response	Tonic response	Phasic response	Tonic response	Phasic response	Tonic response	Phasic response	Tonic response
Normal Ringer	6·2 ± 0·6 (n = 19)	15·8 ± 0·8 (n = 26)	−3·8 ± 1·3 (n = 6)	−15·8 ± 0·6 (n = 6)	−10·2 ± 2·4 (n = 4)	−34·0 ± 2 (n = 4)	−12·1 ± 3·7 (n = 4)	−35·0 ± 1·8 (n = 4)
Normal Ringer + 1 mM tetracaine	—	18·9 ± 1·0 (n = 22)	—	−16·2 ± 1·7 (n = 7)	—	−44·5 ± 1·7 (n = 2)	—	−45·5 ± 1·0 (n = 3)
Normal Ringer + 0·1 mM-D600	—	20·1 ± 3·7 (n = 4)	—	−17·5 ± 2·5 (n = 2)	—	—	—	—
Na-free Ringer	8·0 ± 0·4 (n = 10)	—	—	—	1·2 ± 1·9 (n = 5)	—	—	—
Predictions of $3Na^+$–$1Ca^+$ exchange (eqns. (1) and (2))	14·0		−17·3		−40·5		−50·5	

the tension–depolarization relationship (Fig. 4*A*) (Benninger *et al.* 1976). An indirect effect of $[Ca]_0$ on the phasic tension is indicated by Fig. 4*B* and by the after effects of altered heart rate on the K contracture in normal Ringer. This is evidence for a dependence of phasic tension on intracellular rather than extracellular sources of Ca^{2+}. The phasic contracture is inhibited by exposure to D600 or Mn ions and by drastic reduction in the concentration of ionized Ca in the bathing fluid (Table 3), experimental procedures known to inhibit the secondary inward current in frog atrial trabeculae (Vassort & Rougier, 1972; Einwächter *et al.* 1972; Leoty & Raymond, 1972). This suggests that Ca ions carried by the secondary inward current are associated with the initiation of the phasic tension. However, K contractures can still be evoked, in Na-free fluid, in the presence of high concentrations of D600 or Mn ions, or in Ca-free fluid with EGTA, by strong depolarization, i.e. conditions where the secondary inward current should be fully inhibited. This residual contraction is blocked by tetracaine, which alone can fully abolish the depolarization-induced contractures in Na-free fluid. Tetracaine antagonizes the contracture evoked by caffeine, rapid cooling and hypertonity in Na-free fluid (Chapman & Miller, 1974; Chapman & Ellis, 1974; Chapman, 1978), while Mn^{2+} has no effect on the caffeine contracture (Chapman & Ellis, 1977). Although tetracaine blocks the secondary inward current (Eisner, Lederer & Noble, 1979; R. A. Chapman & C. Leoty in preparation), these results suggest that tetracaine has other effects on contraction, which in view of its action on isolated sarcoplasmic reticulum, skinned skeletal muscle fibres and intact skeletal muscle fibres (Endo, 1977; Almers & Best, 1976), are likely to be on the release of Ca^{2+} from intracellular stores. Indeed, low doses of tetracaine inhibit contraction but not the slow inward Ca current in frog skeletal muscle fibres exposed to high $[Ca]_0$ in Cl-free Ringer solution (G. Raymond & D. Porteau, personal communication). This notion is made all the more likely by the differences in the inhibitory kinetics of tetracaine on the secondary inward current and the phasic tension found in voltage clamp experiments of frog atrial trabeculae (R. C. Chapman & C. Leoty, in preparation). The phasic contraction therefore depends on both secondary inward current and the release of Ca^{2+} from intracellular stores. It is possible, therefore, that the Ca current is coupled to the release of Ca^{2+} from these stores. This component of the K contracture would, therefore, seem to have a similar origin to the contractile responses of mammalian heart muscle under voltage clamp as first described by Reuter & Beeler (1969). Indeed, the sensitivity of the contractile response of the mammalian preparation to changes in $[Na]_0$ and $[Ca]_0$ are very similar to those found in frog atrial muscle (Reuter & Beeler, 1969; Beeler & Reuter, 1970; New & Trautwein, 1972).

The observation that tetracaine can produce an inhibition of the K contracture in Na-free fluid which is additional to that induced by Mn ions suggests, that a part of the phasic tension can be evoked in the absence of a secondary Ca inward current and is due to the release of intracellular Ca^{2+} initiated by depolarization of the cell membrane. There is the possibility of a Mn inward current in Na-free fluid (Ochi, 1976), but in the absence of Mn^{2+} the K contracture is not entirely blocked by perfusion with Ca-free fluid containing EGTA or by D600. This means a release of some Ca^{2+} from the intracellular stores may be effected directly by strong depolarization of the cell membrane. A possible route could be by way of the occasional couplings of the

sarcoplasmic reticulum to the sarcolemma found in amphibian heart muscle (Sommer & Johnson, 1969). Another possibility is that contraction is due to a partial reactivation of the Na–Ca exchange by the strong depolarization.

The relative contribution of the phasic and tonic components to the heart beat

Both components of the K contracture, because they depend upon membrane potential, may be activated by the action potential. The phasic component is near to its maximal activation at the peak of the action potential (Vassort & Rougier, 1972; Leoty & Raymond, 1972; Einwächter *et al.* 1972) whereas the tonic component is only at 40% of maximum tension at a membrane potential of +40 mV in 1 mM-Ca Ringer (Fig. 1). The tonic component develops slowly and may therefore not be maximally activated during the relatively brief action potential. This may account for the increased tensions seen when the action potential is artificially prolonged (Morad & Orkand, 1971). However, the strength of the heart beat in frog shows a strong and immediate dependence on both $[Na]_0$ and $[Ca]_0$ (Chapman & Niedergerke 1970; Chapman 1971*a*), suggesting that the Na–Ca exchange affects the strength of the heart beat directly. The results with K contractures in Na-Ringer show that, for depolarizations above a membrane potential of −50 mV, the phasic tension is larger than the tonic tension, i.e. $[Ca]_i$ rises beyond the value at which the Na–Ca exchange is at equilibrium and should favour a net efflux of Ca^{2+} from the cells under these conditions. An efflux of Ca by way of the Na–Ca exchange could be responsible for the rapid effects of changing the $[Ca]_0$ or the $[Na]_0$ on the strength of the heart beat. The slower changes in the strength of the heart beat may be due to an indirect action on the intracellular Ca-stores and idea consistent with the effects of caffeine on these slower changes (Chapman & Miller, 1974).

The possible role of the Na–Ca exchange in the relaxation of tension

If the $[K]_0$ is lowered during the spontaneous relaxation of a K contracture evoked in Na-free fluid, the rate of relaxation is unaltered. By contrast, if Na ions are added to the perfusing fluid relaxation is hastened, and can be very rapid when this $[Na]_0$ is high. In normal Ringer, repolarization of the membrane always leads to a rapid relaxation of tension. This suggests that extracellular Na ions are required for rapid relaxation to take place when the membrane is repolarized and implicates the electrogenic Na–Ca exchange in the relaxation of tension in amphibian heart. A similar conclusion was drawn from voltage clamp experiments of frog heart by Goto, Kimoto, Saito & Wada (1972). This exchange cannot provide the only relaxing system however, because a slow spontaneous relaxation of tension occurs in Na-free fluid, and during K contractures. In the latter case the spontaneous relaxation, in the presence of Na ions, is abolished when both oxidative phosphorylation and glycolysis are inhibited (Chapman, 1973). Under these conditions of metabolic insufficiency, the application of caffeine no longer evokes a contracture, suggesting that the energy consuming relaxation process is Ca-uptake into the sarcoplasmic reticulum.

In a previous paper on Na-withdrawal contractures, we discussed variability in the results reported for the effects of membrane potential (in fact changed $[K]_0$) on the Na-activated ^{45}Ca efflux in cardiac preparations (Chapman & Tunstall, 1980*a*). The existence of the two sources of activating Ca^{2+}, which are mobilized by membrane

depolarization, may go some way towards explaining the ^{45}Ca influx data. Depolarization should result in an increased ^{45}Ca influx by way of the Na–Ca exchange as found by Niedergerke (1963) and a reduction in the ^{45}Ca efflux as found by Wollert (1966) and Reuter & Seitz (1968). However, if the phasic component is also activated and Ca^{2+} is liberated from the intracellular stores, the rise in $[Ca]_i$ could activate an efflux of ^{45}Ca by way of the Na–Ca exchange. One might therefore obtain changes in ^{45}Ca efflux which are complex (Jundt, Porzig, Reuter & Stucki, 1975, their Fig. 9) or simply an increase in ^{45}Ca efflux (Niedergerke, 1963), depending on the experimental conditions and the status of the intracellular stores of Ca.

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