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CONTRACTILE ACTIVATION IN MYOTOMES FROM DEVELOPING LARVAE OF *XENOPUS LAEVIS*

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SUMMARY

1. Contractile activation in response to application of different perfusing solutions was observed in developing myotomes from larvae of *Xenopus laevis*.

2. Contractures resulted from treatment with isotonic solutions containing high K^+ concentrations in myotomes from embryos from stage 24 onwards. These developed over 5–10 s and inactivated over 20–30 s. Contractures persisted in curarized preparations.

3. The applied K^+ concentrations at the estimated mechanical threshold in embryos at stages 26 and 34 were about 30 and 20 mM respectively. Maximum activity was achieved at K^+ concentrations of 78 and 48 mM respectively.

4. The lyotropic ion thiocyanate (10 mM) potentiated K^+ contractures, and shifted the threshold K^+ concentration to lower values.

5. Contractures persisted in the presence of 3 mM-Mn^{2+} and in Ca^{2+}-free solutions, with unaltered mechanical thresholds.

6. K^+ contractures were reversibly abolished by 2 mM-tetracaine.

7. Comparisons of resting potentials in 3 and 20 mM-K^+ confirmed that the resting potential remained sensitive to external K^+ concentration, whether in control, tetracaine-containing, or low-Ca^{2+} bathing solutions.

8. Caffeine (10–20 mM) caused sustained contractures from stage 24 onwards. Caffeine sensitivity was greatest at stages 26–28, then appeared to decline at stages 33–36.

9. These observations suggest that mechanisms for excitation–contraction coupling develop in *Xenopus* embryos at the same time as do completed sarcomeres, close to stage 24. Activation of contraction then assumes the adult pattern, involving voltage-dependent release of the intracellular store of activator, independent of entry of extracellular Ca^{2+}.

INTRODUCTION

A substantial amount of evidence suggests that contractile activity in adult skeletal muscle is initiated by a voltage-dependent process, triggered by membrane depolarization (Hodgkin & Horowicz, 1960*a*; Adrian, Chandler & Hodgkin, 1969). This is thought to initiate Ca^{2+} release from intracellular stores (Ashley & Ridgeway, 1970). The process does not require extracellular Ca^{2+} (Luttgau & Spiecker, 1979). Ca^{2+} can also be released from sarcoplasmic reticulum by applying caffeine (Luttgau

& Oetliker, 1968; Caputo, 1976). Furthermore, intramembrane charge movements have been reported in frog skeletal muscle (Schneider & Chandler, 1973). It is possible that at least a component of this charge is causally related to contractile activation (Huang, 1981; Hui, 1983; Vergara & Caputo, 1983).

The situation in embryonic muscle is less clear. One possibility is that the extremely specialized adult mechanisms operate early in development as soon as complete sarcomeres differentiate. Such a mechanism would parallel the early stages in development at which organized sarcomeres, and in particular the sarcoplasmic reticulum and transverse tubular system differentiate (Blackshaw & Warner, 1976; Ezerman & Ishikawa, 1967). However, Blackshaw & Warner (1974) have demonstrated Ca^{2+} action potentials in developing embryonic *Xenopus* myotomes, as has Kano (1975) in embryonic chick skeletal muscle cells. Similarly, developing nerve cells often produce action potentials through activation of Ca^{2+} currents at early stages of development, although these are succeeded in later stages by Na^{+} currents of the adult type (Spitzer, 1979). This pattern occurs in both Rohon–Beard neurones and dorsal root ganglion cells in developing *Xenopus* (Baccaglini & Spitzer, 1977). It is therefore equally possible that contractility in these early stages may depend on entry of extracellular Ca^{2+}, as appears to be the case for adult contractile systems such as cardiac muscle. This paper describes simple observations on contractile responses of myotomes from *Xenopus* larvae, using procedures that parallel classical studies on activation of adult muscle (e.g. Hodgkin & Horowicz, 1960*a*, *b*; Luttgau & Oetliker, 1968; Caputo, 1972). The results suggest that the nature of excitation–contraction coupling in embryonic myotomes resembles that of adult striated muscle, and develops at an early stage in morphogenesis in parallel with sarcomere formation.

METHODS

Developing larvae of the South African clawed frog *Xenopus laevis* obtained from matings induced by injections of chorionic gonadotrophin (Organon) were staged using the external criteria of Nieuwkoop & Faber (1956). Embryos were transferred to a Sylgard-bottomed experimental chamber, and mounted under magnification by destroying their developing brains with A1 insect pins. The myotomes studied came from embryos between developmental stages 21 and 36. Over this period, blocks of mesoderm cells rotate and differentiate into myotome cells whose long axes parallel the length of the embryo (Hamilton, 1969). Formation of myotome segments begins at the cephalic end of the embryo, and proceeds sequentially towards the caudal end. The ectoderm overlying the developing myotomes was removed with a fine dissecting needle, exposing the most cephalic ten to fifteen segments. At the stages examined these cells are electrically coupled through intercellular junctions; disappearance of this coupling occurs towards stages 36–38 (Blackshaw & Warner, 1976; Armstrong, Turin & Warner, 1983). The exposed myotomes were observed under strong reflected heat-filtered light: small contractures were observed by noting changes in lengths of the developing myotome segments at the anterior end. Larger ones were observed as flexion of the segments towards the side exposed to the test solutions. It was not possible to employ any simple transduction apparatus to measure tension owing to the small size and fragility of the embryos. Consequently only large effects involving changes visible under magnification were examined.

Experiments were performed at room temperature (17–20 °C). Table 1 summarizes the basic solutions employed. Solution A was used to perfuse the preparation in between applications of test solutions in experiments conducted at normal Ca^{2+} concentrations. Solution E was employed between applications in experiments conducted in low $[Ca^{2+}]$. In addition, apart from a few preliminary experiments, 10^{-4} M-*d*-tubocurarine was added to all solutions. The solutions were applied at a rate of about 10 ml/min from a reservoir using a continuous balanced flow system, whose flow was directed over the exposed myotomes in the direction from head to tail. Unless

otherwise stated in the Results, 1–2 min was allowed between applications of testing solutions, to allow full recovery of the preparation. Testing solutions were injected into the flow chamber at a constant rate of close to 20 ml/min using a nozzle whose tip was clamped close to the exposed myotomes directed toward the caudal end. Application of the solution investigated was maintained until no further contractile changes were observed.

TABLE 1. Perfusing solutions: electrolyte compositions. All solutions at pH7; *d*-tubocurarine added. Concentrations in mM

	A	B	C	D	E	F	G
NaCl	115·0	115·0	0	0	115·0	0	115·0
KCl	2·5	0	115·0	0	2·5	115·0	0
Tris Cl	0	0	0	115·0	0	0	0
$CaCl_2$	3·0	3·0	3·0	3·0	0	0	0
$MgCl_2$	2·0	2·0	2·0	2·0	5·0	5·0	5·0
EGTA	0	0	0	0	3·0	3·0	3·0
HEPES	3·0	3·0	3·0	3·0	3·0	3·0	3·0

The test solutions used involved replacement of particular ions, or the addition of drugs (Table 1). When examining simply for the existence of contractures in response to large depolarization, solution C, in which 115 mM-KCl was substituted for NaCl, was used directly (see Hodgkin & Horowicz, 1960*a*). In experiments investigating gradation of contractility with extracellular K^+ concentration, $Tris^+$ was substituted for Na^+ (solution D); this was used in different proportions with solution C in order to vary the level of applied K^+. When examining contractility in Ca^{2+}-free solutions, solution E was used as the perfusing solution between applications of mixtures of solutions F and G in proportions varying to alter the concentration of K^+. The pharmacological agents, used as described in the Results, were added at the following concentrations: *d*-tubocurarine, 10^{-4} M; Mn^{2+}, 3 mM; tetracaine chloride (Sigma, U.K.), 2 mM; caffeine (Sigma, U.K.), 4, 10 or 20 mM.

Resting potential measurements were made at two K^+ concentrations (3 and 20 mM) in myotomes in control solutions, low Ca^{2+} (EGTA-containing) and solutions containing 2 mM-tetracaine. These employed 80–100 MΩ electrodes filled with 0·8 M-potassium citrate, and conventional electronic and display circuitry.

RESULTS

K^+ contractures

Earlier work suggests that the resting membrane potential of developing cells is related through a logarithmic relation to relative concentrations of K^+ on either side of the membrane. This is known to be the case in adult muscle (Adrian, 1956) and may well also be the case in cells in developing systems (Spitzer, 1976). In developing *Xenopus*, Blackshaw & Warner (1974) have demonstrated that membrane potentials of myotomal cells were close to the adult level. Accordingly, perfusing solutions containing high (115 mM) concentrations of K^+, were applied in order to elicit large depolarizations (Hodgkin & Horowicz, 1960*a*). Larvae were studied between developmental stage 21–22, where formation of somites is first observed, and stage 36, where electrical coupling between cells starts to decline, commencing at the cephalic segments (Armstrong *et al.* 1983). If contractile activation is voltage-dependent, contractures should result from application of such solutions, as has been reported earlier in adult skeletal muscle on numerous occasions (e.g. Hodgkin & Horowicz, 1960*a*).

In the presence of 10^{-4} M-*d*-tubocurarine, embryos in low-K^+ solutions did not exhibit spontaneous contractile activity, presumably reflecting blockage of end-plate activation. Mechanical activity occurred in response to application of high-K^+ solutions in myotomes at or beyond stage 24. In contrast, in seven embryos between stages 21 and 23, application of 115 mM-K^+ did not elicit any visible contractile response. This need not exclude contractile activity so slight as to not be visible under even $\times 80$ magnification. However, the relative quiescence of the myotomes from the earlier embryos correlates well with morphological observations that the contractile apparatus is not fully organized at this stage (Blackshaw & Warner, 1976). Furthermore, these results contrast with findings obtained in embryos at or beyond stage 24. Myotomes then showed very striking K^+ contractures, suggesting that the developing cells specifically acquire the ability to contract in response to depolarization close to this stage of development. The K^+ contractures persisted and were similar in character in the presence or absence of *d*-tubocurarine, whether applied at 10^{-6} or 10^{-4} M. They also persisted in the presence of 3 mM-Mn^{2+}. In subsequent experiments 10^{-4} M-*d*-tubocurarine was added to all solutions. Curare did not appear to have any noticeable effect on resting membrane properties. Embryos at stages 26–28 studied in the absence of curare showed mean resting potentials of $-49{\cdot}5 \pm 3{\cdot}1$ mV ($n = 52$), whereas in the presence of curare the resting potential was $-51{\cdot}6 \pm 2{\cdot}4$ mV ($n = 11$). Maximum potentials obtained were -92 and -81 mV respectively: it is likely that these may be closer to the true resting potentials owing to the possibility of electrode leaks resulting from penetration of these small cells (Blackshaw & Warner, 1974).

Mechanical activation in myotomes from embryos in later stage 27–28 (fifteen embryos), stage 33–34 (fourteen embryos) and stage 35–36 (six embryos) all showed similar features. Perfusion with solutions containing 115 mM-K^+ produced sustained contractures developing gradually over 5–10 s. Maintained exposure to the test solutions resulted in a gradual inactivation following the contracture over 20–30 s. Interrupting the contracture by returning the tissue to low-K^+ solution before inactivation caused relaxation over 5–10 s. Contractile inactivation could be reversed by perfusing with the control (low-K^+) solution for 30–60 s, after which K^+ contractures could be elicited again. Although basically qualitative in nature, these findings therefore parallel classical results obtained in adult muscle employing similar experimental procedures (Hodgkin & Horowicz, 1960*a*).

Graded applications of K^+

In adult muscle, the activation process is thought to be regulated steeply, but continuously by voltage (Hodgkin & Horowicz, 1960*a*; Caputo & de Bolanos, 1979). In *Xenopus* embryonic muscle, quantitative measurement of tension development was not possible. Nevertheless, an approximate, visual assessment of the dependence of contractile activation upon voltage was obtained simply by comparing the concentrations of applied K^+ that would just elicit visible mechanical activity, with the levels sufficient to produce a full contracture.

The experiments involved repeated perfusions with graded concentrations of K^+, in order to produce changes in the membrane potential. In the test solutions, the Na^+ was replaced by $Tris^+$, and 10^{-4} M-curare was added. These changes were made in

order to minimize time-dependent ionic currents due to Na^+, and Ca^{2+} and end-plate activation. The concentrations of K^+ in the test solutions were varied along a logarithmic scale in order to result in changes of membrane voltage in increments of about 10 mV. Between each successive application of test solution, the myotomes were perfused in the low-K^+ control solution for 2–3 min. Even after a run of these ionic replacements, application of the original high-K^+ (115 mM) solution gave normal contractures with which the consequences of applying varying K^+ concentrations could be compared. The effect of each application of external K^+ was therefore reversible.

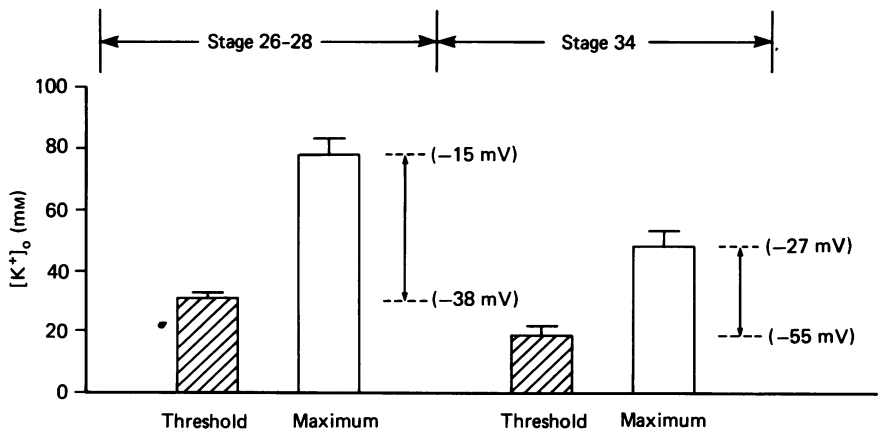

Fig. 1. K^+ concentrations (means ± S.E. of means) corresponding to the contractile threshold and to the achievement of maximum observed contractile activity in myotomes from embryos at stage 26–28 and stage 34. Corresponding Nernst voltages assuming an intracellular K^+ concentration of 140 mM (Adrian, 1956) are shown in parentheses.

Myotomes from embryos between stage 26 and 34 were examined. Fig. 1 summarizes the results obtained. In eight embryos at stages 26–28, the threshold for a barely visible contracture was at an applied $[K^+]$ of 31·3 ± 2·3 mM (mean ± S.E. of the mean). Lower levels of applied K^+ did not elicit contractile activity. Thresholds for contractures could be reliably observed, and gave reproducible results when tested repeatedly in the same embryo. The level of applied K^+ at which maximal contractures were achieved was estimated by comparing visually responses to successive applications of increasing K^+ concentrations with responses to additions of 117 mM-K^+. The concentration at which the activation reached its maximum level, estimated by this approach, was approximately 78 ± 5·4 mM. Between these values, the amount of contractile activity was graded with tension. A very rough estimate of half the maximum contractile level corresponded to a $[K^+]$ of 49 ± 7·4 mM.

Similarly, in four embryos at stage 34, it was found that a just perceptible movement was obtained on perfusing with a $[K^+]$ of 18·8 ± 2·9 mM (mean ± S.E. of the mean). Lower levels of K^+ did not result in visible contractile activity. Higher levels again resulted in contractures of successively increasing size, although detailed quantification through intermediate levels of K^+ was again not possible. An applied

$[K^+]$ of about 48 ± 5 mM was sufficient to induce a contracture indistinguishable from one resulting from applying a test solution containing 117 mM-K^+.

These simple findings suggest that contractile activation is initiated only with depolarization beyond a particular threshold voltage, and is then graded with the degree of depolarization. Fig. 1 shows the Nernst voltages (in parentheses) at the relevant K^+ concentrations applied if one assumes a value of intracellular $[K^+]$ of about 140 mM, as was demonstrated for adult muscle (Adrian, 1956). Comparing resting membrane potentials in myotomes in 3 and 20 mM-extracellular K^+ yielded maximum resting potentials of -81 mV ($n = 11$) and -31 mV ($n = 19$) respectively. This confirms that the membrane potential was sensitive to extracellular K^+ concentration, although predicting a higher relative permeability to ions other than K^+ than in adult muscle. The Nernst voltages for threshold and maximum contractures in stage 34 embryos were -55 and -25 mV respectively, suggesting a steepness in the voltage dependence of mechanical activation in embryonic myotomes similar to the situation in adult muscle. However, the corresponding range of voltages in earlier stage 26 embryos fell between -38 mV (threshold) and -15 mV (maximum activation). Thus the approximate steepness of the predicted activation–voltage relation in the two cases was similar, but their position differed; the precise mechanism for the difference remains to be determined.

Effect of lyotropic ions

Hodgkin & Horowicz (1960*b*) demonstrated that anions such as thiocyanate (SCN^-) and nitrate (NO_3^-) shifted the contractile activation curve in adult muscle in the hyperpolarizing direction. If contractile activation in embryonic myotomes is similarly dependent upon a process sensitive to membrane field, then parallel effects should occur in developing muscle. The effect of thiocyanate was investigated as follows.

(1) By exposing the myotomes to varying concentrations of extracellular K^+, it was possible to determine a subthreshold concentration of added K^+. When four embryos at stage 32 were exposed to an applied K^+ concentration of $17{\cdot}9 \pm 1{\cdot}85$ mM contraction did not occur. However, when the same concentration was applied in the presence of 10 mM-SCN^- there were marked contractures. This is consistent with potentiation of the contractile process through a shift in the contractile threshold towards more hyperpolarized voltages.

(2) The threshold for a sustained K^+ contracture was compared in the presence and absence of SCN^- in eight embryos at stage 26. SCN^- reduced the threshold K^+ from $31{\cdot}34 \pm 2{\cdot}26$ to $11{\cdot}3 \pm 1{\cdot}16$ mM. Assuming an intracellular $[K^+]$ of 140 mM (Adrian, 1956), this corresponds to a shift of the activation curve along the voltage axis by about 25 mV (see Fig. 2).

Independence of extracellular Ca^{2+}

The previous experiments suggested that contractile activation in embryonic muscle cells is regulated by a voltage-dependent process. However, this could as likely reflect an entry of extracellular Ca^{2+} into these small cells, as it could an activation mechanism of the adult type. Thus Ca^{2+} spikes have been reported to occur in embryonic *Xenopus* (Blackshaw & Warner, 1974), and Ca^{2+} currents do occur in adult

striated muscle (Beaty & Stefani, 1976; Stanfield, 1977). However, contractures continued to be produced in response to 117 mM-K^+ in twenty-three embryos covering stages 25–36 in the presence of 3 mM-Mn^{2+}, which was reported to block Ca^{2+} spikes. Furthermore, the effect of high-K^+ solutions was also investigated using Ca^{2+}-free solutions containing 3 mM-EGTA and 5 mM-Mg^{2+}. Application of these continued to elicit full contractures.

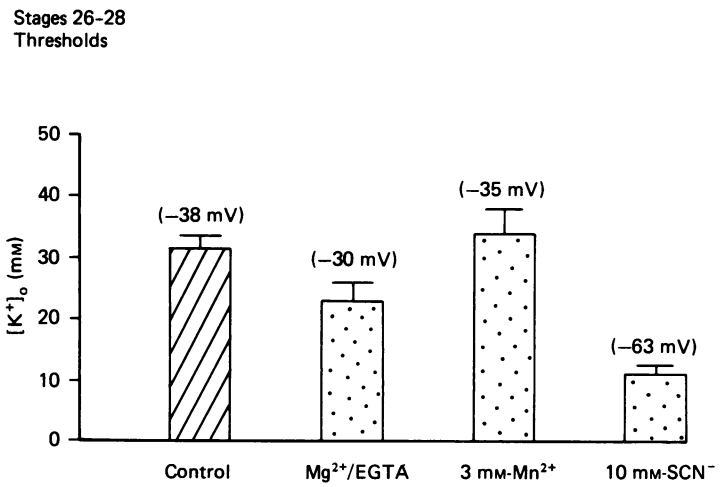

Fig. 2. K^+ concentrations (means ± S.E. of means) corresponding to a just detectable, sustained contracture in curarized myotomes from embryos between stages 26 and 28 in (i) controls; (ii) low-Ca^{2+} EGTA-containing solutions; (iii) solutions containing 3 mM-Mn^{2+}; (iv) solutions containing 10 mM-thiocyanate. Corresponding Nernst voltages assuming an intracellular K^+ concentration of 140 mM (Adrian, 1956) are shown in parentheses.

These findings were supplemented by applications of graded concentrations of extracellular K^+ in the presence of curare in order to determine the contractile threshold in either Mn^{2+}, or in Ca^{2+}-free media. All the embryos examined fell between stages 26 and 28. Threshold was defined as corresponding to that K^+ concentration that would reproducibly elicit a just visible, but sustained contracture. Control myotomes had a threshold of 31·3 ± 2·3 mM-K^+. This corresponds to a Nernst potential for K^+ of about −38 mV, assuming an intracellular K^+ concentration of 140 mM. In the absence of Ca^{2+} (Mg EGTA-containing solution), the threshold K^+ concentration was lower (23·4 ± 3·1 mM). This corresponds to a shift of the activation curve in the hyperpolarizing direction of about 8 mV, and is not the result one would expect had contractile activity been dependent on extracellular Ca^{2+} entry. Rather, the size and direction of the change suggests a system whose dependence upon Ca^{2+} was similar to that of adult muscle (see Luttgau & Spiecker, 1979). Addition of 3 mM-Mn^{2+} resulted in only a small (not significant) increase in the threshold K^+ concentration to 34·2 ± 3·9 mM. Again this is not consistent with a dependence of contractile activation upon influx of Ca^{2+} through Ca^{2+} channels, as these would have been blocked by Mn^{2+}.

These findings are consistent with full mechanical activation in such early embryos being independent of an extracellular supply of Ca^{2+}.

Effects of tetracaine and caffeine

The experiments here demonstrated similarities in the pharmacological effects of tetracaine and caffeine between embryonic and adult muscle (Luttgau & Oetliker, 1968; Caputo, 1972). Fig. 3 shows measurements of the resting potential at two concentrations of extracellular K^+ (3 and 20 mM) in embryos at stages 26–28. The experiments were performed in embryos slightly cooled to 15 °C. This enhanced

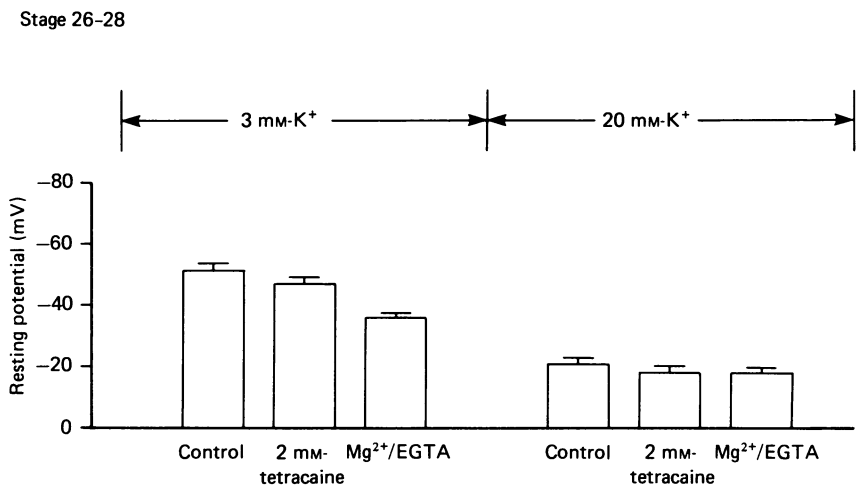

Fig. 3. Resting potentials (means ± S.E. of means) measured in anterior myotomes of embryos at stages 26–28, at two K^+ concentrations (3 and 20 mM) under three conditions: controls; in the presence of 2 mM-tetracaine; and in low-Ca^{2+} (EGTA-containing) solutions. Maximum (single) values of the resting potential at an extracellular K^+ concentration of 3 mM were −81, −70 and −55 mV respectively. At an extracellular K^+ concentration of 20 mM the maximum potentials were −31, −28 and −24 mV respectively.

embryo survival under the pharmacological conditions tested. Only stable penetrations were examined. Tetracaine did not greatly affect the resting potential at the concentrations of K^+ tested; at an external K^+ of 3 mM, the resting potentials in control and tetracaine-treated myotomes were $51{\cdot}6 \pm 2{\cdot}4$ mV ($n = 11$; maximum value 81 mV) and $47{\cdot}1 \pm 2{\cdot}0$ mV ($n = 23$; maximum value 70 mV) respectively. Increasing the extracellular K^+ concentration to 20 mM altered the resting potential to the similar values of $20{\cdot}9 \pm 1{\cdot}5$ ($n = 19$; maximum value 31 mV) and $18{\cdot}0 \pm 1{\cdot}3$ mV ($n = 22$; maximum value 28 mV) respectively. Fig. 3 also shows results from embryos in Ca^{2+}-free media through the same K^+ concentrations. Penetrations were less stable, but nevertheless the measured voltages suggested that cells maintained an adequate resting potential which fell on addition of 20 mM-K^+ to similar levels as in controls. Despite a persistent dependence of membrane voltage upon extracellular K^+, 2 mM-tetracaine in test and control solutions abolished contractile activation in response to applied solutions containing 115 mM-K^+, in twenty-three embryos between stages 24 and 36. This effect was reversible: normal K^+ contractures were restored within 30 s of washing out the tetracaine.

Caffeine is known to release intracellular activator stores in adult muscle causing

contractures independent of membrane potential changes (e.g. Luttgau & Oetliker, 1968). The effect of caffeine was tested in three groups of embryos at stages 23–24, 26–28 and 33–36 respectively. In each group, the effect of successively increasing concentrations of caffeine of 4, 10 and 20 mM were tested. These concentrations are substantially higher than the 2·5 mM sufficient to cause full contractures in adult muscle (Caputo, 1972). Fig. 4 summarizes the results obtained.

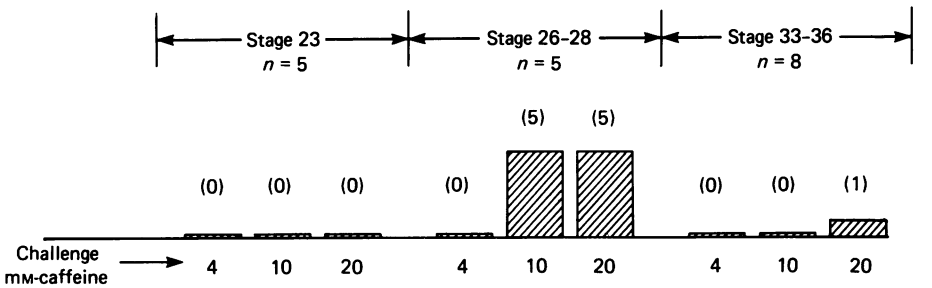

Fig. 4. Schematic representation of the effect of challenging myotomes from embryos at stages 23, 26–28, and 33–36 respectively with successively higher concentrations of applied caffeine: 4, 10 and 20 mM. Stage 23 embryos were unresponsive to caffeine, even at 20 mM, but embryos became more responsive at stages 26–28. However, at stages 33–36, sensitivity to caffeine again declined. The figures in parentheses give the number of embryos out of the total tested in each group that responded to caffeine; this is represented by the height of each box.

Caffeine did not elicit observable contractures in five embryos at stages 22–23, whether observed at high or low power, at all three applied concentrations. Myotomes were first responsive to caffeine close to stages 24–25, and sensitivity was greatest between stages 26 and 28. Unlike K^+ contractures, caffeine contractures persisted as long as test solution was applied, and did not inactivate. Thus myotomal segments from four embryos at stages 26–28 produced steady flexions developing over 10–20 s with application of either 10 or 20 mM-caffeine, but not with 4 mM-caffeine. Surprisingly, in embryos at stages 34–36, 4 or 10 mM-caffeine had no effect and even concentrations of 20 mM elicited sustained contractures only in one out of eight embryos tested. The effects of caffeine on myotomes and adult muscle are therefore similar around developmental stage 26, although higher concentrations appear to be required for effective action. However, the decreased caffeine susceptibility at later stages 33–36 does represent a pharmacological difference between developing and adult muscle, but its significance is uncertain.

DISCUSSION

The experiments described here examined the basic features of contractile activation of embryonic myotomes of *Xenopus*, in order to assess similarities and differences between embryonic and adult muscle. On the one hand the early differentiation of ultrastructure associated with contractility might reflect early acquisition of mechanisms akin to adult muscle. On the other hand, the existence of marked Ca^{2+}

currents at such stages, and the situation in systems such as (adult) cardiac muscle, might reflect an importance of extracellular Ca^{2+} entry in the activation process. The experimental procedures adopted here paralleled the approach used by Hodgkin & Horowicz (1960*a*) who examined activation of adult muscle elicited by applications of solutions containing elevated concentrations of K^+, thereby altering the membrane potentials of the fibres concerned. Even the simple observation of mechanical contractures described here revealed features of interest concerning the process of excitation–contraction coupling.

Blackshaw & Warner (1976) have demonstrated myofibrils, but not complete sarcomeres in electron microscopy of myotomes of developing *Xenopus* at stage 22. However, at stage 24 sarcomeres were fully differentiated and this correlated with spontaneous flexing movements in response to end-plate potential activity. The experiments here show that initiation of contractile activity in response to depolarization, or to applied caffeine is absent at stage 22, but is acquired close to stage 24. It would therefore appear that the mechanisms by which membrane voltage regulates excitation–contraction coupling develop at much the same time as assembly of complete sarcomeres.

The features of the activation process in the early embryo paralleled findings in adult muscle. The degree of activation was graded with the imposed depolarization, and enhanced by the lyotropic anion SCN^-. Although Ca^{2+} action potentials have been demonstrated in *Xenopus* myotomes (Blackshaw & Warner, 1974), it is unlikely that the contractile activation is dependent upon such extracellular Ca^{2+} entry. Thus contractures were not affected by the presence of Mn^{2+}, which blocks Ca^{2+} currents, and persisted in the absence of extracellular Ca^{2+} using EGTA-containing solutions. The threshold voltages for contractile activation under conditions where entry of extracellular Ca^{2+} was compromised were similar to those in controls. Thus there is probably an adequate source of intracellular Ca^{2+} to initiate contraction even at early stages of development.

Further parallels with adult muscle were demonstrated by investigating the effects of the pharmacological agents caffeine and tetracaine. Application of caffeine beyond stage 24 resulted in sustained contractures, suggesting that intracellular Ca^{2+} is released in a manner similar to that already shown in adult muscle (Caputo, 1976). However, sensitivity to caffeine was lower than in adult muscle and varied with developmental stage. It could first be demonstrated once contractures were present beyond stage 24, but sensitivity declined at stages 33–36. Tetracaine reversibly blocked the excitation–contraction process, again in parallel with its effect on the mechanical properties of adult muscle (Luttgau & Oetliker, 1968).

These findings suggest that the development of mechanisms regulating excitation–contraction coupling is complete close to the time of formation of complete sarcomeres, at stage 24. Once developed, this process assumes the highly specialized features found in adult muscle, involving voltage-dependent release of sufficient internal activator stores to be independent of entry of extracellular Ca^{2+}.

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