Mechanism of Biphasic Contractions in Strontium-Treated Ventricular Muscle

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SUMMARY. Biphasic contractions were produced in dog trabeculae by replacing 90-95% of the calcium in the bathing solution with strontium. These conditions produced prolonged action potentials accompanied by contractions with two distinct phasic components. The early component disappeared slowly when the remaining Ca++ was removed, whereas the late component was eliminated quickly when Sr++ was removed. Manganese ion (0.25 mM) preferentially decreased the late component without changing the action potential, whereas caffeine and ryanodine decreased or eliminated the early component. Ryanodine did not alter the action potential. Isoproterenol rapidly increased the early component and, more slowly and to a lesser degree, increased the late component.

The results suggest that the early component is caused by intracellular release of activator cation, probably from the sarcoplasmic reticulum, whereas the late component is the result of Sr++ entry across the sarcolemma, possibly by way of the slow inward current.


TWO distinct components of contraction, one fast and one slow, have been shown to exist in mammalian ventricular myocardium under certain conditions. They can be seen in rested state contractions, after a 10- to 15-minute stimulation-free period, in the absence (Allen et al., 1976) or in the presence of an adrenergic agonist (Beresewicz and Reuter, 1977; Seibel et al., 1978), in low-temperature, constant frequency contractions under the influence of noradrenaline (Bogdanov et al., 1979), and after 90% replacement of Ca++ with Sr++ (Braveny and Sumbera, 1972). The common characteristic of these conditions is an increase in the duration of the action potential, and, in fact, biphasic contractions can be produced when the plateau of the action potential is lengthened by voltage clamp techniques (Morad and Trautwein, 1968; Braveny and Sumbera, 1970) or by toxins (Coraboeuf et al., 1975; Honerjager and Reiter, 1975). Although it is generally agreed that each component of contraction is due to a specific type of cation release, the site of the cation pools is the subject of some controversy.

A number of proposals of the origin of the two cation release sites have been published, one visualizing Ca release from the same site but by different mechanisms (Allen et al., 1976) and the others hypothesizing contractile activation by two morphologically distinct pools (Braveny and Sumbera, 1972; Beresewicz and Reuter, 1977; Seibel et al., 1978; Bogdanov et al., 1979). The present study was designed to try to resolve the question of the origin of biphasic contractions by using specific pharmacological interventions. Strontium-induced resolution of the two components was used because it allows constant, relatively high frequency (0.5 Hz) stimulation while enabling a quantitative comparison of the effects of adrenergic agonists on the separate components to be made. The results are discussed in relation to the other models of two-component contractions.

Methods

Mechanical Experiments

Mongrel dogs (3-10 kg) of either sex were anesthetized with pentobarbital. The hearts were removed, flushed with 50 ml of cold Krebs-Henseleit solution (KH), and placed in ice-cold, oxygenated KH. Small, thin trabeculae, usually from the right ventricle, were tied at each end with 4.0 silk thread and suspended in a vertical constant temperature tissue bath (20 ml) containing KH solution gassed with 95% O2 and 5% CO2 and maintained at 37°C. One end of the trabecula was attached via a stainless steel wire to a Grass FT 03C isometric force transducer, the other end to the base of the stimulating electrode. Resting tension was increased until the developed twitch tension reached a maximum (Lmax). Stimulation frequency was 0.5 Hz delivered through either punctate or field electrodes at an amplitude 10% above threshold voltage. Preliminary experiments had indicated that this was the maximum frequency at which the two components were clearly separable. Contractions were recorded on either a Grass model 5D polygraph recorder or a Beckman R511A chart recorder.

Electrophysiological Experiments

Muscles were prepared as above but were mounted in a 40-ml horizontal constant temperature tissue bath and stimulated with platinum punctate electrodes. One end of the muscle was pinned to the silicon rubber base of the bath and the other end was attached to a Grass FT 03C transducer. Microelectrodes were filled with 3 M KCl and had resistances of between 2 and 35 MΩ. Contractions, the first derivative of contraction (dF/dt), action potentials, and stimuli were recorded on a Hewlett-Packard model 3960 instrumentation tape recorder, a Hewlett-Packard 4-channel
storage oscilloscope, and a Gould Brush model 440 4-channel chart recorder.

Solutions

Normal Krebs-Henseleit solution had the following composition (mm): NaCl, 118.0; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.4; NaHCO₃, 26.2; glucose, 11.1. Strontium Krebs-Henseleit (SrKH) solution had an identical composition except that it contained no added Ca and 2.5 mM Sr. All chemicals were reagent grade and dissolved in double-distilled, de-ionized water to make stock solutions. The volume of stock solutions of drugs and ions added to the bath never exceeded 1.0% of the bath volume. Drugs and chemicals used were caffeine (Sigma), the sodium salt of ethyleneglycol-bis-(β-aminoethyl ether) N,N'-tetraacetic acid (EGTA, Sigma), isoproterenol hydrochloride (Sigma), manganese chloride (Fisher), and ryanodine.

Statistics

Unless otherwise stated, data were analyzed using a completely random design analysis of variance with multiple comparisons by Duncan’s test (Steel and Torrie, 1960). The levels of significance are as follows: * P < 0.05; ** P < 0.01; *** P < 0.001.

Results

Effects of Complete Replacement of Ca by Sr

Trabeculae were equilibrated in KH solution before Ca replacement by stimulation at 0.5 Hz for 1 hour. Replacing the bathing medium with SrKH produced a series of changes in the shape of the contractions (Fig. 1). Peak tension decreased and the contraction duration increased within 2 minutes in most tissues. A hump appeared during relaxation and became progressively larger until there was a partial relaxation seen in Figure Id but, instead, demonstrated either prolonged and rounded or prolonged and flat peaks.

The changes in P1 and P2 continued until, after 20–40 minutes in SrKH, P1 completely disappeared. The remaining P2 contraction characteristically had a long latency between stimulation and the onset of contraction. The developed tension was greater than in KH solution within 60 minutes of washing with SrKH, as previously shown in cat papillary muscle (Weyne, 1966a; Brutsaert, 1967; Henderson and Catteill, 1976) and was made up of a P2 twitch followed by a plateau phase (P3). Tension was maintained at a slowly declining level (the plateau) until the membrane repolarized. Although the steady state contractions in SrKH without Ca had no discernible P1, after a rest period of 1–2 minutes, the first contraction had a small P1 followed by P2 and a very long P3. This may account for the results of Verdonck and Carmeliet (1971), who showed that a tissue in Na-free SrKH stimulated at 0.5 to 1.0/min had a small fast-rising phase that was eliminated by caffeine or ryanodine.

The development of biphasic contractions in SrKH could be greatly speeded up by repeated washings or, especially, by adding 0.2 to 1.0 mM EGTA to the bath. Typically, the sequence of contraction changes shown in Figure 1 was complete within 3 minutes after EGTA treatment. This observation was used in a number of experiments to facilitate the production of biphasic contractions, particularly in the relatively uncommon experiments where repeated washing with SrKH did not produce a progressive decline of P1.

During Ca replacement by Sr, the action potential configuration also changed appreciably (Fig. 2). The most prominent effect was on the duration, which increased from 255 ± 9 msec (n = 6) in KH solution to 2 seconds or more in some muscles. In addition, as seen in Figure 2, phase 1 repolarization disappeared and phase 2 became long and flat. Final repolarization to the resting level occurred abruptly and was always accompanied by relaxation of the muscle if a P3 component of contraction was present prior to the repolarization. When restitution of the membrane potential occurred before P2 had fully developed, tension was able to continue to increase for a short time even after complete repolarization.

Maintenance and Characteristics of Biphasic Contractions

After incubation in SrKH, when the contraction consisted of two peaks, the loss of P1 and the prolongation of P2 could be prevented by the addition of...
small amounts of Ca to the bath. At Ca concentrations of between 0.1 and 0.2 mM (with 2.5 mM Sr present), the biphasic contractions became stable, with P1 having about the same tension as P2. The relative tensions of P1 and P2 could be adjusted by small changes in the Ca concentration.

The action potentials seen during maintained biphasic contractions had reduced but, in most cases, not eliminated, phase 1 repolarizations. Prolonged, flat plateaus were followed by abrupt phase 3 repolarizations (Fig. 2b). As was the case with the P2 contractions seen in the absence of Ca (Fig. 2a), relaxation usually began during phase 3 repolarization. Occasionally, however, P2 tension was able to continue developing after complete repolarization in some tissues in which the action potential duration was somewhat less than average (Table 1). The possibility of asynchronous contraction of the muscle due to a decreased conduction velocity in the presence of Sr (Weyne, 1966b) was ruled out, since repeated microelectrode impalements over the length of the preparation showed a similar temporal relationship between the action potential and contraction.

Figure 3 illustrates the time-to-peak tension of contractions in KH solution and of P1 and P2 in 28 preparations. The time-to-peak P1 was found to be greater than the time-to-peak in KH (203 and 171 msec, respectively). Time-to-peak P2 (441 msec) was greater than either KH contractions or P1.

Effects of Ca and Sr on Biphasic Contractions

The dependence of P2 on extracellular Sr is shown in Figure 4A. In a trabecula which was bathed in SrKH in the absence of any Ca and which was contracting biphasically, P2 was decreased in the second contraction after a wash with Ca-free, Sr-free KH solution and eliminated within five contractions. The time required to abolish P2 may be ascribed to the time needed for extracellular equilibration. The contraction remaining appeared to be P1-like in its time course and was unaffected by the wash with 0 Ca, 0 Sr KH in the time shown, indicating that the cation pool responsible is tightly bound either to the membrane or to some intracellular structure. When 2.5 mM Sr was added to the bath at this point, P2 reappeared on the next contraction and reached a constant level in three contractions (5 sec). Furthermore, the added Sr did not affect P1.

In Figure 4B, the effects of adding 1.0 mM Ca to the bath, in which a muscle was showing only a P2 contraction in Ca-free SrKH, are illustrated. The second contraction following the addition of the Ca exhibited a marked depression of peak tension without the appearance of a P1 component. The fourth contraction had a hump on the rising portion which progressively increased until, by the seventh contraction, P1 was about equal to P2. Eventually, P1 tension became much greater than P2 tension. The rapid decline of P2 after adding Ca may be interpreted according to the well-described competition between Ca and Sr on the magnitude of the slow inward

![Figure 2](http://circres.ahajournals.org/)

**FIGURE 2.** Panel A: Action potential (top trace) and tension (bottom trace) changes after 68 minutes in SrKH at 0.5 Hz. Short AP and contraction: KH control. Long AP and contraction: SrK. Panel B: AP and tension of a muscle contracting biphasically in SrKH.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Contraction and Action Potential Durations</th>
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<tr>
<td>KH Control</td>
<td>Sr Biphasic</td>
</tr>
<tr>
<td>Contraction duration (msec)</td>
<td>353 ± 12</td>
</tr>
<tr>
<td>Action potential (msec)</td>
<td></td>
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<tr>
<td>Duration</td>
<td>253 ± 9</td>
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<tr>
<td>Time to 50% Repol.</td>
<td>219 ± 11</td>
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Significance determined by a paired t-test (2-sided). Values are means ± SEM. ** * * p < 0.001.
**FIGURE 3.** Time-to-peak tension of control and biphasic contractions. Time was measured from the moment of stimulation to maximum developed tension. KH: control contractions. P1: the early component of biphasic contractions. P2: the late component of biphasic contractions. For all groups, n = 28. The time-to-peak P1 may have been increased due to the buildup of P2 tension just prior to the onset of P1 relaxation.

Current (Vereecke and Carmeliet, 1971a; Bass et al., 1975; Linden and Brooker, 1980). The requirement for replenishment of an intracellular store of Ca by Ca entry through the Ca channel could be the cause of the delay between Ca addition and the development of P1.

Calcium removal by EGTA had an opposite effect to that of Ca addition (Fig. 5). It is possible to selectively remove Ca with EGTA, since the stability constant of the Ca-EGTA complex is more than two orders of magnitude greater than that for Sr-EGTA (Holloway and Reilly, 1960). After 0.4 mM EGTA was added to a preparation contracting biphasically with 0.1 mM Ca and 2.5 mM Sr, P2 rapidly increased without an accompanying change in P1 tension. With time (2–3 minutes), however, P1 declined until it was no longer detectable. In one experiment, addition of a higher concentration of EGTA (0.8 mM) decreased both P1 and P2 within two contractions. P2 reached a nadir after six contractions and subsequently increased to greater than pre-EGTA levels, while P1 continued its decline. The lower concentration of EGTA therefore seems to easily chelate the Ca which competes with Sr for P2 but has more difficulty removing the Ca involved in the genesis of P1.

**FIGURE 4.** Dependence of P1 and P2 on Ca and Sr. Panel A: after the contractions of the muscle had progressed to the biphasic stage in SrKH (as in Fig. 1A), a wash with Ca-free, Sr-free KH solution at the point shown eliminated P2. When 2.5 mM Sr was added to the bath, P2 reappeared on the next contraction. Panel B: the tissue exhibited only P2 in SrKH ([Ca]o = 0. At the point indicated, 1.0 mM Ca was added to the bath, causing P1 to reappear after a delay of three contractions.

**FIGURE 5.** Effect of 0.4 mM EGTA on P1 (■) and P2 (●). The tissue was contracting biphasically with [Ca]o = 0.05 mM and [Sr]o = 2.5 mM. One second before contraction 1, 0.4 mM EGTA was added to the bath. Tension is expressed as a percentage of pre-EGTA steady state (SS) tension. Inset: change of contraction shape with time in 0.4 mM EGTA (with contraction number shown). Note the appearance of a plateau phase (P3) following two phasic components (P1 and P2). Vertical bar: 1 gram tension; horizontal bar: 1 second.
Drug Effects on Biphasic Contractions

The previous section showed that P1 is a Ca-dependent component, while P2 is Sr-dependent. In order to clarify further the origin of the pools of activator cation responsible for the two components of contraction, inhibitors and an activator of both the Ca channel and the sarcoplasmic reticulum were used.

If Sr ions entering the cell through the slow inward current are responsible for the development of P2, then blockade of the current should affect P2 tension more rapidly and to a greater extent than P1 tension. This was confirmed with manganese (Mn). Manganese ion has been shown to competitively block Ca entry through the sarcolemmal voltage-sensitive Ca channels (Vitek and Trautwein, 1971). In a muscle showing stable biphasic contractions, the addition of 0.25 mM Mn produced a slowly developing decline of P2 tension (Fig. 6) which reached a steady state within 3-5 minutes. Although P1 tension also decreased, neither the degree nor the rate of tension change was as great as that of P2 tension (Figs. 6 and 7). Although measurements of total cellular Ca have not been made, it is conceivable that the decline of P1 may be a consequence of a lowered Ca content of the sarcoplasmic reticulum secondary to the inhibition of the slow inward current. Alternatively, the decline of P1 may result from inhibition of the Ca influx responsible for Ca-induced Ca release (Fabiato and Fabiato, 1979). The inhibition of P2 was not due to shortening of the action potential, since Mn had very little effect on the action potential (Fig. 8A). Assuming that the only effect of the added Mn was to block the divalent cation channel in the membrane, we concluded that the tension developed in P2 was more intimately related to trans-membrane cation movement than was P1 tension.

The contribution of the sarcoplasmic reticulum to biphasic contractions was tested with two inhibitors. In KH solution, the addition of 1.0 mM caffeine produced an immediate followed by a slower increase in tension (Fig. 9). The pattern probably is related to two antagonistic actions of methylxanthines (Blinks et al., 1972). An immediate increase was seen in P1 but not P2 tension after the addition of 1.0 mM caffeine. The enhancement of P1 tension was transient and usually declined to less than the levels seen prior to the addition of caffeine within 2 minutes. After 1 minute or less in the presence of caffeine, the amplitude of P2 began to slowly rise, reaching steady state in 6-10 minutes (Fig. 9, bottom). At steady state, P1 was visible only as a slight hump on the contraction and, in some cases, was not discernible at all. The actual tension of P1 was not accurately reflected by the

![Figure 6. The effect of 0.25 mM manganese chloride on control and biphasic contractions. At the top is shown a typical tracing of the negative inotropic effect of Mn in KH solution. In the middle, 0.25 mM Mn was added to the bath (at arrow) in which a tissue was contracting biphasically in SrKH. At the bottom are fast chart speed records of contractions at the indicated times after addition of Mn.](image)

![Figure 7. Left: steady state changes in tension of control (KH), the early component (P1), and the late component of contraction (P2) produced by 0.25 mM Mn. KH: n = 7. P1 and P2: n = 8. Right: change in tension at steady state produced by 0.1 µM isoproterenol. KH: n = 9. P1 and P2: n = 7.](image)
FIGURE 8. Panel A: the effect of Mn on action potentials and contractions. The muscle was contracting biphasically in SrKH with [Ca]₀ = 0.1 mM (upper tension trace). The addition of 0.25 mM Mn (middle tension trace) or 0.5 mM Mn had more of a negative inotropic effect on P2 than on P1 while producing no change in the action potential. Panel B: the effect of 10 μM ryanodine on a muscle contracting biphasically with [Ca]₀ = 0.1 mM. The ryanodine contraction (higher baseline) showed no P1, whereas the accompanying action potential had a slightly higher plateau than control.

hump, since no attempt was made to subtract the early rising portion of the P2 contraction from it. The observed tension changes of P1 and P2 are shown in Figure 10.

The second agent used was ryanodine, an alkaloid which is thought to disrupt the connection between the T-tubules and the lateral cisternae of the sarcoplasmic reticulum (Penefsky, 1974a, 1974b). Ryanodine, at a concentration of 10 μM, reduced peak tension to about 30% of the pre-drug level in KH solution (Fig. 11, top). The inhibition began within 1 minute of addition of the drug and reached a steady state in 5-10 minutes. The effect of ryanodine on biphasic contractions was similar to the action of caffeine except that there was no initial increase of P1. The slowly developing inhibition of P1 was more profound than that caused by caffeine. Within 10 minutes, ryanodine appeared to eliminate P1 completely while moderately increasing P2 (Fig. 11 bottom; Fig. 10). Moreover, these contractile changes occurred without any appreciable change of the action potential (Fig. 8B).

These results suggested that P1 was due to activation of the myofibrils by Ca released from the sarcoplasmic reticulum and that P2 was caused by Sr which entered the cells through the voltage-sensitive divalent cation channel during the prolonged action potential. Stimulation of β-receptors is known to increase both the amount of Ca sequestered and released by the sarcoplasmic reticulum and the Ca current during the action potential (Tsien, 1977). Hence, isoproterenol was expected to increase the magnitude of both P1 and P2. As shown in Figure 12 (top), the addition of 0.1 μM isoproterenol to a trabecula in KH solution produced an immediate rise followed by a slowly developing rise in tension. In a trabecula contracting biphasically, the same concentration of the drug caused an immediate increase of P1 tension and a smaller, delayed increase of P2 tension. In the few preparations where there was no separation of the two phases of contraction in the presence of Sr (as mentioned in the first two paragraphs under Results), isoproterenol was able to separate P1 and P2 by preferentially potentiating P1 and, especially, by increasing the rate of relaxation of P1. The isoproterenol-induced increase of P2 was significantly less than the increase of P1 (Fig. 12, bottom; Fig. 10). The true degree of potentiation of P2 by isoproterenol may have been obscured, however, since the drug consistently shortened both the action potential and contraction durations. As previously mentioned, the onset of P2 relaxation is dependent on membrane repolarization.

Discussion

The present results confirm the findings of Braveny and Šumbera (1972) that biphasic contractions of mammalian ventricular tissue can be maintained by replacing most of the Ca in the bathing solution with Sr. If the presumed sites of drug action are correct, the results indicate that two morphologically distinct pools of activator cation are involved and that biphasic contractions are not due merely to the partial replacement of Ca by Sr in intracellular stores (Henderson and Cattell, 1976). This conclusion is supported by the finding that various blocking agents are able to preferentially change one component of rested state contractions (Beresewicz and Reuter, 1977) and of biphasic contractions produced by noradrenaline at low temperatures (Bogdanov et al., 1979).

Site of Cation Causing P1

The results have shown that P1 is dependent on the presence of a small amount of extracellular Ca and that Sr is ineffective in replacing Ca in this capacity (Figs. 1 and 4). A number of explanations could fit these data. First, P1 may be derived from slow inward current cation with Sr having a lesser ability of enter-
ing the cell by this means with each action potential than Ca. Voltage clamp studies have shown, however, that the magnitude and duration of the slow inward current (and, presumably, the amount of divalent cation entering the cell) is the same or increased after replacing Ca with Sr (Kohlhardt et al., 1973a, 1973b; Noble and Shimoni, 1981). Alternatively, one might propose that Pi results from Na-Ca exchange and that Sr is incapable of exchanging with Na. It has previously been demonstrated, though, that Sr ion can replace Ca effectively to produce a Na-Sr exchange (Van Kerkhove and Carmeliet, 1971). The last major possibility is that Sr cannot replace Ca at some stage of activator divalent cation release from the sarcoplasmic reticulum. Based on the Ca-induced Ca release mechanism of excitation-contraction coupling (Fabiato and Fabiato, 1979), the loss of P1 in Ca-free, Sr-containing media could result from Sr being an ineffective trigger for Sr (or Ca) release or from an inability of the sarcoplasmic reticulum to release suffi-

cient cation to initiate contraction. The cardiac muscle sarcoplasmic reticulum can take up Sr (Winegrad, 1973) and may actually store 10 to 20 times more Sr than Ca (rabbit skeletal muscle; Mermier and Hasselbach, 1976). Although Kawata and Hatae (1977) have shown electron micrographic deposits of strontium in toad ventricular sarcoplasmic reticulum, the quantitative aspects of this situation in mammalian cardiac muscle is not clearly known, and the Sr stores may well be small. Such a possibility has to be present to explain the absence of P1 in the presence of Sr alone if the depolarization-induced Ca release rather than the Ca-induced Ca release hypothesis of E-C coupling is invoked. It has been shown that Sr can trigger Ca release in skinned frog skeletal muscle, but that 50 times more Sr than Ca is needed to get the same release (Endo et al., 1970). Sr-induced Sr release has also been seen at a pSr of 4.5 in frog skeletal muscle (Moicescu and Thieleczek, 1978). Unfortunately, the authors did not attempt to measure release with lower

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**Figure 9.** Top: the effect of 1.0 mM caffeine on control contractions showing the time course of tension change after addition of caffeine (at arrow) and fast chart speed records of control and caffeine contractions. Bottom: the effect of 1.0 mM caffeine on biphasic contractions at indicated times after caffeine addition.

**Figure 10.** Left: steady state tension changes produced by 1.0 mM caffeine on control (n = 6) and biphasic (n = 6) contractions. In certain cases, P1 was not discernible after caffeine. The P1 tension in those instances was estimated by measuring the tension present at the time-of-peak tension of P1 found in pre-caffeine contractions. The actual decrease of P1 was therefore probably larger than indicated. Right: steady state tension changes caused by 10 μM ryanodine on control (n = 6) and biphasic (n = 5) contractions. P1 was not observable after ryanodine in any of the biphasically contracting muscles (dashed line). When tension was measured at the expected time of peak P1, the indicated tension change was seen.
Sr concentrations. In summary, it is possible that P1 is dependent on Ca-induced divalent cation release. Whether the released activator cation is Ca or Sr is unknown. Ca-induced Sr release has not been demonstrated but, since Sr can release either Sr or Ca (in skeletal muscle) and Ca can release Ca (Fabiato and Fabiato, 1979), it is reasonable to assume that Ca can release Sr. The ions have practically identical abilities in activating the myofibrils of cardiac muscle [but not skeletal muscle (Donaldson et al., 1978; Kerrick et al., 1980)] so that the time course of P1 cannot distinguish between the ions.

The observed effects of caffeine and ryanodine are supportive of the interpretation that P1 is the result of sarcoplasmic reticulum release of activator cation. The concentration of caffeine used in this study (1 mM) significantly reduces both the rate of Ca accumulation and the net steady state amount of Ca accumulated by fragmented sarcoplasmic reticulum isolated from rabbit heart (Blayney et al., 1978). The

![Image](http://circres.ahajournals.org/)

**Figure 11.** Time course of ryanodine action on control (top) and on biphasic (bottom) contractions. Ryanodine (10 μM) was added at the arrow in the control and at 1 second in the Sr biphasic sequence.

**Figure 12.** Top: effect of 0.1 mM isoproterenol (at arrow) on control (KH) contractions. Bottom: dose-response relationship of isoproterenol on biphasic contractions showing the predominant effect on P1.
marked reduction of P1 by caffeine may therefore be related to this action of caffeine rather than the membrane effects of the drug which tend to increase contractility (Blinks et al., 1972; Kavaler et al., 1978).

Since caffeine has multiple actions, it was considered desirable to test the effects of another inhibitor of divalent cation release from the sarcoplasmic reticulum.

Electron micrographs of mammalian ventricle treated with ryanodine show a disruption of the connection between the terminal cisternae and the T-tubules (Penefsky, 1974b). The result is a pronounced negative inotropic effect in preparations containing well-developed sarcoplasmic reticula. The alkaloid has no effect on frog ventricle (which has little sarcoplasmic reticulum) and actually increases tension development by 40% in toad ventricle (Ciofalo, 1973). Since both caffeine and ryanodine ultimately reduce the amount of activator cation released by the sarcoplasmic reticulum and both agents inhibit P1, the data are highly suggestive that the sarcoplasmic reticulum is a likely site of origin of P1 activator cation.

This interpretation of the origin of P1 is supported by a number of investigators who used a variety of methods for separating two components of contraction (Braveny and Šumbera, 1970; Ochi and Trautwein, 1970; Allen et al., 1976; Henderson and Cattell, 1976; Beresewicz and Reuter, 1977; Bogdanov et al., 1979). Increasing the stimulation rate of the tissue has been shown to progressively increase the early component after a delay of one contraction, usually reaching a steady state in six to eight contractions (Braveny and Šumbera, 1972; Beresewicz and Reuter, 1977; Seibel et al., 1978; Bogdanov et al., 1979; King, 1982). This pattern of tension changes is usually attributed to the sarcoplasmic reticulum. Furthermore, the early component of contraction produced by other means can be reduced or abolished by theophylline (Beresewicz and Reuter, 1977) or by caffeine (Bogdanov et al., 1979). The slow inward current-blocking agents, verapamil (Bogdanov et al., 1979) and Mn ion (present results), have a relatively small effect on the early component.

The precise mechanism of Ca release from cardiac sarcoplasmic reticulum is not clear. In skeletal muscle, the bulk of the evidence speaks against Ca-induced Ca release as the physiological mediator of contraction (Endo, 1977). It is easier to show Ca-induced Ca release in cardiac muscle (Endo and Kitazawa, 1978), and in rat ventricle it has been proposed as a physiological mechanism of Ca release (Fabiato and Fabiato, 1977). Certain results of the present study support the role of Ca-induced Ca release. The degrees of inhibition by Mn (0.25 mm) of P1 tension and the tension generated in normal KH solution were similar but about half of the inhibition of P2 tension. Furthermore, caffeine, which is known to transiently increase Ca-induced Ca release, increased P1 tension before finally inhibiting it. However, the data on the effects of EGTA do not support Ca-induced Ca release. EGTA, which chelates Ca much more than Sr, increased P2 amplitude (controlled by Sr as discussed later) within two to three contractions, whereas P1 was inhibited only after about 20 contractions. Therefore, either the Ca pool which competes with Sr at the sarcolemma is different from the pool involved in Ca-induced Ca release from the sarcoplasmic reticulum, or the relatively slow attenuation of P1 by EGTA is only apparent due to an increasing rising phase of P2 superimposed on a possibly decreasing P1. Obviously, more experiments are needed to resolve this question.

Site of Cation Causing P2

The relationship between P2 seen in our experiments, the late component described in ventricular preparations under the influence of epinephrine (Beresewicz and Reuter, 1977) or norepinephrine (Seibel et al., 1978; Bogdanov et al., 1979) or in rested state contractions without the addition of a drug (Allen et al., 1976), and the “tonic” component seen during prolonged voltage clamp (Morad and Goldman, 1973) is not clear. In our experiments, P2 seems to be due to Sr entry through the divalent cation channel since it is blocked by Mn, rapidly disappears when Sr is withdrawn, and is inhibited by the addition of Ca. In fact, in a number of preparations, P3 (a “tonic” phase) was present when both P1 and P2 were also present, especially at stimulation frequencies lower than 0.5 Hz and immediately after the addition of small concentrations of EGTA. Therefore, two delayed components may actually be involved with the delayed "phasic" component (P2) being masked by a large "tonic" component (P3) under voltage clamp conditions. The late component or "tonic" phase seen during prolonged voltage clamp produced by other investigators (Morad and Trautwein, 1968; Braveny and Šumbera, 1970) would then be a composite of our P2 and P3 components.

This hypothesis requires that the slow inward current be much greater in Sr-containing solution than in normal, Ca-containing solution in order for a large, phasic P2 to appear. Vereecke and Carmeliet (1971a, 1971b) demonstrated that Sr could greatly prolong the current in cow Purkinje fibers and that the amplitude of the current is also increased (Reuter and Scholz, 1977). It was subsequently shown that cat trabeculae and papillary muscles produce a similar response to cow Purkinje fibers after Sr replacement of Ca (Kohlhardt et al., 1973a, 1973b). In addition, the sarcoplasmic reticulum probably acts as a less effective sink for Sr than for Ca because a concentration of Sr fifteen times greater than of Ca is needed for half maximal filling of skeletal muscle sarcoplasmic reticulum vesicles (Edwards et al., 1986). Since Sr is equipotent to Ca in activating the cardiac contractile apparatus (Kerrick et al., 1980), it is very possible that P2 is due to direct activation secondary to an increase in the magnitude and duration of the slow inward current.

The present results do not allow any conclusions to be drawn regarding the site of origin of P3 cation, but
it has been established that the “tonic” phase of contraction is not a consequence of the slow inward current, since tension rises with increasing degrees of depolarization up to potentials well beyond the Ca equilibrium potential (Morad and Goldman, 1973). This has prompted the suggestions that the “tonic” phase is a result of K-Ca exchange (Morad and Goldman, 1973) or electricogenic Na-Ca exchange (Horackova and Vassort, 1976), two processes which would be capable of driving Ca into the cell as the membrane potential becomes more positive. With the present results, it is not possible to exclude the possibility that both P2 and P3 are consequences of such an exchange mechanism. Cardiac glycosides, which increase the intracellular Na concentration (Sheu, 1981), preferentially increase P2 (King, 1982), indicating that at least part of the P2 contractile response may be derived from Na-Sr exchange. Direct verification of this awaits further experimentation.

It is interesting to note that the effect of isoproterenol on P1 exceeded its effect on P2. One of the major effects of β-adrenergic stimulation is on the slow inward current, although an effect on sarcoplasmic reticular uptake of Ca is also well known (Tsien, 1977). It is difficult to assess quantitatively the relative magnitude of the two effects from these studies because the true magnitude of the effect on P2 was probably attenuated by a shortening of the action potential duration. However, the temporal relationship of increasing P1 and P2 seems to indicate that the action on the sarcoplasmic reticum precedes the effect on the slow inward current.

At the risk of placing too much faith in the specificity of ryanodine, a model may be constructed where perhaps up to 30% of ventricular contractile activation in the dog is caused by a ryanodine-resistant influx of activator cation (P2) at a stimulation frequency of 0.5 Hz. The remaining 70% of tension would then be attributable to a release mechanism dependent on the connection between the t-tubules and the sarcoplasmic reticulum (P1 contraction). At stimulation frequencies greater than 0.5 Hz, the fractional contribution from the sarcoplasmic reticum may increase.

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