CONTRACTION PATTERNS OF MAMMALIAN MYOCARDIUM: INFLUENCE OF STRONTIUM ON EXCITATION-CONTRACTION COUPLING

BY

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

Some effects of strontium on excitation-contraction coupling in canine ventricular trabeculae were studied mechanically and electrophysiologically. Strontium ion (Sr) is a divalent cation with a hydrated radius similar to that of Ca. Since Sr can replace Ca in most, but not all, steps in E-C coupling in mammalian myocardium, it was thought that the ion could help to decipher certain features of E-C coupling.

Replacing the bathing Ca with Sr (2.5 mM) greatly prolonged tha action potential duration, the contraction duration, and the time to onset of contraction while having a positive inotropic effect. At a stimulating frequency of 0.5 Hz, contractions with two phasic peaks (P1 and P2) were seen during the Ca/Sr transition period and could be maintained by adding low concentrations of Ca to the solution. The early component of contraction (P1) was affected by the Ca concentration and by drugs which alter sarcoplasmic reticular Ca handling (caffeine, isoproterenol, and ryanodine). The late contraction component (P2) was affected by the Sr and Ca concentrations as well as by treatments which influence either the slow inward current channel (Mn and Ni ions) or the Na concentration gradient during the action potential plateau (ouabain and tetrodotoxin). It was concluded that P1 was caused by either 1) Ca release from the sarcoplasmic reticulum by some unknown mechanism secondary to sarcolemmal depolarization, or 2) Ca-induced Ca release from the sarcoplasmic reticulum where Sr cannot replace Ca as the "trigger". P2 was concluded to result from direct

activation of the contractile apparatus by Sr of sarcolemmal origin, mainly from the slow inward current. A tonic phase of contraction (P3) which relaxed only when the membrane repolarized was evident when the stimulation frequency was low or after complete removal of extracellular Ca. P3 is thought to the contractile manifestation of an electrogenic Na-Ca exchange during the long action potential plateau.

The results showed that the action potential is prolonged in Sr solution by both increased slow inward current and a blocking action of Sr on outward currents carried by K.

Biphasic contractions were used to study the force-interval relationship. P1 was more frequency-dependent and behaved as the sarcoplasmic reticulum pool has been hypothesized to react in a number of models of E-C coupling. Contractions potentiated by alterations in the stimulation rate were characterized by an increased P1. P2 was most important for contractile activation of an extrasystole.

A model of mammalian ventricular E-C coupling was presented in which the sarcoplasmic reticulum contributes about 70% of the activator Ca during normal contractions at 0.5 Hz and a smaller percentage at lower frequencies. The remaining activator Ca is derived from the slow inward current and probably also from "reversed" Na-Ca exchange during the action potential plateau.

Dedicated to

my parents

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GENERAL INTRODUCTION

A. CARDIAC EXCITATION-CONTRACTION COUPLING

The aim of this section is to provide a very general background for the material presented in subsequent sections. Very good and detailed reviews of cardiac excitation-contraction (E-C) coupling have been published recently (Langer, 1973; Morad and Goldman, 1973; Fozzard, 1977; Langer, 1977; Chapman, 1979; Winegrad, 1979).

The requirement for Ca in muscle contraction was gradually worked out over a number of years by workers studying both cardiac and skeletal muscle (Ringer, 1883a; Locke and Rosenheim, 1907; Heilbrunn and Wiercinski, 1947; Sandow, 1952; Bozler, 1952). The term "excitation-contraction coupling" was coined by Sandow (1952) and was defined as "the entire sequence of events - excitation, inward acting link, and activation of contraction." Current ideas on each stage will be discussed separately.

1. EXCITATION

The cardiac action potential has a complex shape resulting from the interaction of a number of membrane currents. Initially, the membrane is maintained at a potential of about -80 mV primarily by a time-independent background K current, I_{K1} (Dudel et al., 1967; McAllister et al., 1975). This conductance channel is inward rectifying, meaning that the current gets smaller as the electrochemical gradient for K gets larger, which accounts for the fact that the membrane resistance increases when the cell is depolarized in the presence or

absence of Na (Deck and Trautwein, 1964; McAllister and Noble, 1966; Dudel et al., 1967). Since the resting membrane is not at the K equilibrium potential (about -115 mV), a background inward current carried mostly by Na is also present (Noble and Tsien, 1968). This is partly offset by a small outward current generated by an electrogenic Na pump (c.f. Weidmann, 1974).

The upstroke of the action potential (phase 0) is the result of an explosive increase in Na conductance leading to the fast inward current (Weidmann, 1974). The membrane potential then rapidly approaches the Na equilibrium potential (about +40 mV) and falls once again due to the very fast (1-2 msec) inactivation of the current (Beeler and Reuter, 1970a; New and Trautwein, 1972a). The channel can be inactivated by keeping the membrane depolarized to about -60 mV or less by voltage clamping (Beeler and Reuter, 1970b) or by increasing the extracellular K concentration (e.g. Pappano, 1970). Tetrodotoxin (TTX) is able to selectively block the channel (Hagiwara and Nakajima, 1965; Beeler and Reuter, 1970a).

Phase 1 of the action potential is caused by the very rapid inactivation of the fast inward current and the activation of a rapid, transient outward current, Iqr (Deck and Trautwein, 1964; McAllister et al., 1975). Although the latter current is affected by the chloride concentration (Dudel et al., 1967), recent evidence suggests that most or all of it is carried by K ions (Kenyon and Gibbons, 1979). Iqr is thought to be important in Purkinje fibres but not in cardiac muscle fibres (Trautwein, 1973) and is increased by elevated intracellular Ca concentration (Siegelbaum et al., 1977).

As previously mentioned, the membrane resistance is greater at the action potential plateau than at the resting potential, indicating that the currents flowing at that time are of smaller magnitude than when the tissue is at rest. The major inward current during the plateau is the slow inward current, Isi. This current is very sensitive to the external Ca concentration (Reuter, 1967; Beeler and Reuter, 1970b; New and Trautwein, 1972b), is unaffected by TTX (Hagiwara and Nakajima, 1975; Beeler and Reuter, 1970b), is increased by B adrenergic receptor stimulation (Reuter and Scholz, 1977b), and is blocked by Mn, Ni, La, and various verapamil-like Ca "antagonists" (Hagiwara and Takahashi, 1967; Ochi, 1970; Vassort and Rougier, 1972; Kohlhardt et al., 1973a; Bayer et al., 1975c; Fleckenstein, 1977). However, these blocking agents may also affect outward conductance channels (Kass and Tsien, 1975). The slow inward current is activated at a potential of between -45 and -35 mV, reaches a maximum intensity between -15 and 0 mV, and reverses at about +60 mV (Beeler and Reuter, 1970b; New and Trautwein, 1972a). The low reversal potential (compared to that expected for a pure Ca channel by the Nernst equation) is due to the channel being slightly permeable to both Na and K ions (Reuter and Scholz, 1977a). The magnitude of the current and the time constants for activation and inactivation are voltage-dependent (c.f. Weidmann, 1974; Reuter, 1979; Noble and Shimoni, 1981b). Increasing the intracellular Ca concentration actually increases the magnitude of the current but quickens inactivation (Isenberg, 1977a and b).

Final repolarization results from the time- and voltage-dependent inactivation of the slow inward current and activation of the outward

plateau current (McAllister et al., 1975). Based on their activation kinetics, the outward plateau current has been divided into a fast (I_{X1}) and a slow (I_{X2}) current (Noble and Tsien, 1969). In action potentials of normal duration, only I_{X1} is activated to a degree sufficient to induce final repolarization (McAllister et al., 1975; Vassalle, 1979). The time-independent outward current, I_{K1}, and the time-dependent outward current, I_{K2}, also influence repolarization and are enhanced by increasing the intracellular Ca concentration (Bassingthwaighte et al., 1976; Kass and Tsien, 1976; Isenberg, 1977a,c, and d). It is quite likely that a net outward current due to an electrogenic Na-Ca exchange at plateau potentials contributes to repolarization (Mullins, 1981). Finally, a TTX-sensitive, potential-dependent background current carried by Na significantly prolongs the action potential duration in Purkinje fibres but not in ventricular muscle (Attwell et al., 1979; Coraboeuf et al., 1979).

2. CONTRACTION

Activation of the contractile proteins is modified by Ca, Mg, ATP, and the degree of phosphorylation of regulatory components (Winegrad, 1979). For E-C coupling, the most important determinant is the regulation by Ca.

The contractile apparatus is composed of thick filaments, made up of the protein myosin, and thin filaments, made of actin, tropomyosin, and troponin. The "head" of the myosin molecule (the crossbridge) normally has a great affinity for the strand of actin polymer but tro-

pomyosin prevents their interaction in the relaxed muscle. The tropomyosin inhibition is regulated by troponin, a complex of three proteins called troponin I (TnI), troponin T (TnT), and troponin C (TnC). TnT holds the complex to tropomyosin while TnI binds to actin. TnC is the Ca-binding component which, when bound to Ca, causes the TnI to dissociate from actin, at least in skeletal muscle. The interaction of TnI and TnC caused by Ca is less clear in cardiac muscle (Ebashi et al., 1975). Binding of Ca releases the tropomyosin inhibition of the actin-myosin interaction and the muscle contracts, utilizing ATP for energy (c.f. Gergely, 1976; Winegrad, 1979). Thus, Ca binding to TnC is the ultimate switch for contraction.

There are three Ca binding sites on cardiac TnC (four on skeletal TnC), two of high affinity and one of low affinity (two of each type on skeletal TnC). The ATPase activity and tension development of myofibrillar preparations are closely correlated over a wide range of Ca concentrations with 50% of the maxima being reached at a Ca concentration of about 2 uM. Tension rises steeply over a small range of Ca concentrations: tension is about 10% of maximum at 0.8 uM Ca and about 90% of maximum at 5 uM (Solaro et al., 1974). This suggests that some form of cooperative binding of Ca occurs (Solaro et al., 1974) and that activation requires Ca to be bound to the low affinity site as well as to the high affinity sites (Winegrad, 1979). A similar importance of the low affinity sites on skeletal TnC has been shown (Bremer and Weber, 1972). A further discussion of the effects of Ca and Sr on the contractile proteins is included in the Mechanical Effects of Sr section.

3. THE COUPLING

Since the mechanical activity of heart muscle is preceeded by an increase in the intracellular Ca concentration (Allen and Blinks, 1978), the "inward acting link" mentioned by Sandow (1952) must be some mechanism which is able to transiently raise the level of Ca in the cell enough to produce tension. Activator Ca could originate from the extracellular space, the sarcolemma, or some intracellular store.

In skeletal muscle, it is well established that the lateral cisternae of the sarcoplasmic reticulum provides the activator Ca (Porter and Palade, 1957; Winegrad, 1968; Ebashi and Endo, 1968; Endo, 1977). Ca is released primarily from the lateral cisternae (Huxley and Taylor, 1958; Winegrad, 1965) where it may be bound to calsequestrin, a protein having a high capacity and low affinity for Ca (MacLennan and Wong, 1971). The mechanism of release is the subject of some controversy. In skinned muscle fibres (fibres in which the sarcolemma has been removed chemically (Winegrad, 1971) or mechanically (Fabiato and Fabiato, 1972) enabling the "intracellular" conditions to be altered), Ca can be released from the sarcoplasmic reticulum by increasing the Ca concentration (Ca-induced Ca release; Ford and Podolsky, 1970) or by depolarizing the membrane of the sarcoplasmic reticulum through a change from an external impermeant to a permeant anion (depolarization-induced Ca release; Endo and Nakajima, 1973). In tissues with intact sarcolemmae, the low resistance connection between the T-tubules and the sarcoplasmic reticulum may allow T-tubular depolarization to be transferred to the sarcoplasmic reticu-

lum (Trautwein et al., 1975;c.f.Ebashi, 1976). An excellent review of the release mechanisms has been provided by Endo (1977).

In cardiac muscle, the absolute requirement for extracellular Ca (e.g. Tritthart et al., 1973b) suggests that either Ca-induced Ca release is very important or that extracellular Ca is entering the cell during the action potential and is activating contraction directly. The cardiac sarcoplasmic reticulum can take up a large amount of Ca in a short enough time to account for the entire contraction (Solaro and Briggs, 1974; Levitsky et al., 1981) and is able to release it in response to membrane depolarization in intact tissue (Gibbons and Fozzard, 1971a) or by increasing the Ca concentration in skinned fibres (Fabiato and Fabiato, 1975; 1979). Furthermore, the close proximity of the lateral cisternae to the contractile proteins means that the short time to onset of contraction is in accord with the time required for diffusion of Ca from this source (Winegrad, 1979). A number of E-C coupling models have been presented which rely totally or in part on the release of Ca from the sarcoplasmic reticulum (Wood et al., 1969; Morad and Goldman, 1973; Kohlhardt et al., 1974; Allen et al., 1976; Fabiato and Fabiato, 1979). These models are able to account for features of cardiac contractions, such as the force-interval relationship (Koch-Weser and Blinks, 1963),which would be difficult to explain otherwise.

Acceptance of the sarcoplasmic reticulum as the sole source of activator Ca is not universal. It has been pointed out that the organelle is less abundant in cardiac than in skeletal muscle (Fawcett and McNutt, 1969) and that the loss of contractility when washing out Ca

corresponds temporally to the loss of Ca from a sarcolemma binding site (Bailey and Dresel, 1968; c.f. Langer, 1973). The action of lanthanum ion (La) on the sarcolemma has been used to illustrate the importance of the basement membrane-bound pool of Ca to contraction (Sanborn and Langer, 1970). The above arguments cannot rule out the possibility that Ca release from the sarcoplasmic reticulum depends upon the presence of the slow inward current. This has recently been recognized by workers who previously thought that virtually all the activator Ca was attributable to the sarcolemma (Langer, 1977; Philipson and Langer, 1979).

It is conceivable that activator Ca enters the myocardial cell during the action potential by way of the slow channel and there is some evidence in its favour. The threshold potential for generating the slow inward current is the same as for initiating contraction (Beeler and Reuter, 1970c; Gibbons and Fozzard, 1971b; New and Trautwein, 1972b) but the amount of Ca carrying the current has been estimated to be sufficient for only about 10% of a given contraction (Beeler and Reuter, 1970c; New and Trautwein, 1972a; Reuter, 1973, 1974). Furthermore, although the twitch tension follows the magnitude of the slow inward current after a frequency change in frog atrium (Noble and Shimoni, 1981a), the same relationship does not hold in mammalian myocardium possessing a well-developed sarcoplasmic reticulum (Beeler and Reuter, 1970c; Simurda et al., 1981). However, estimations of the absolute amount of Ca entering through the slow channel by integration of the inward current record are undoubtedly too low due to the concurrent activation of the outward membrane currents. When the transi-

ent outward current, Iqr, is virtually eliminated by replacing intracellular K with Cs, the slow inward current is both faster activating and much larger than control (Marban, 1981). At the present time, it appears that contractile activation by electrogenic transmembrane entry of Ca provides a minor contribution to a contraction but does influence subsequent contractions by increasing the total cellular Ca content (Fozzard and Gibbons, 1978).

Under constant current or voltage clamp conditions, tissues from both mammalian and amphibian hearts show a "phasic" and a "tonic" tension when the duration of depolarization is prolonged, as initially noted by Kavaler (1959). In frog ventricle, depolarizing clamps of more than 100 msec duration produce increasing amounts of tension with the time to peak tension being identical to the clamp duration. Tension is greater at higher depolarizations, even beyond the Ca equilibrium potential (Morad and Orkand, 1971). Frog atrial tissue behaves similarly except that an initial phasic response, due to Ca entry through the slow channel (Horackova and Vassort, 1976b), is greater than the tonic response at intermediate levels of depolarization (Vassort and Rougier, 1972).

Mammalian heart shows an initial phasic response with depolarizations as short as 5 msec (Morad and Trautwein, 1968). With increasing lengths of depolarization, the phasic tension reaches a maximum and is followed by a maintained tonic contraction that relaxes only upon repolarization (Morad and Trautwein, 1968; Wood et al., 1968; Beeler and Reuter, 1970c; Ochi and Trautwein, 1971; Braveny and Sumbera, 1971; Morad and Goldman, 1973). Since the tonic phase is a direct

function of membrane potential, even beyond the Ca equilibrium potential where Ca should flow passively out of the cell due to a reversed electrochemical gradient, it has been suggested that the influx of Ca is coupled to the efflux of another ion whose outward driving force is greater than that of Ca. The exchanging ion may be K (Morad and Goldman, 1973; Trautwein et al., 1975) or Na (Mullins, 1981). The existence of a Na-Ca exchange mechanism (Glitsch et al., 1970) which is electrogenic (i.e. exchanges 3 or more Na for one Ca; Reeves and Sutko, 1980; Coraboeuf et al., 1981; Mullins, 1981) makes this mechanism more probable (Horackova and Vassort, 1976a). The finding that the tonic phase is eliminated by replacing Na with Li (Vassort, 1973) and that the carrier is inhibited by Li (Reuter, 1973) also supports this view. Finally, toxins such as veratridine (Honerjager and Reiter, 1975) and scorpion toxin II (Coraboeuf et al., 1975) which increase Na conductance during the action potential plateau, thereby increasing the intracellular Na concentration, produce pronounced tonic phases.

Determining the contribution of the tonic phase to a normal contraction is difficult because of the early release of other Ca pools. The finding in dog ventricular trabeculae that the phasic tension relaxes completely before the tonic phase begins (Beeler and Reuter, 1970c) may indicate a very minor contribution. However, the frequency of clamping would influence the intracellular Na level and, at a very low frequency, a depolarization of 1 sec or more might be needed to raise the Na concentration high enough to stimulate sufficient "reverse" exchange. The sarcoplasmic reticulum may also be able to take

up much of the Ca entering the cell before it is able to activate the contractile proteins.

In conclusion, contractions of mammalian myocardium are the result of an increased intracellular Ca concentration. The Ca is probably derived mainly from the sarcoplasmic reticulum with smaller contributions being made by Ca from the slow inward current and from "reverse" Na-Ca exchange. Therefore, the increased intracellular Ca concentration accompanying contraction has at least 2 and probably 3 components with different time courses. Of paramount importance in this regard is the demonstration of two peaks of intracellular Ca increase during a contraction using the Ca-sensitive photoprotein aequorin (Wier, 1980).

B. STRONTIUM IN CARDIAC E-C COUPLING

1. MECHANICAL EFFECTS

Ringer and Sainsbury (1883) noted that the loss of contractility of frog ventricle in the absence of Ca could be prevented by adding strontium chloride to the bathing medium. They realized that the effect was specific for Ca and Sr and not divalent cations in general because "barium differs almost entirely from either calcium or strontium in its physiological action" (Ringer, 1883b). Thirty years later, Mines (1913) reported that, when Ca was replaced by Sr, the duration of contraction of frog ventricle was greatly increased, as was the duration of the electrical activity.

Interest in the effects of Sr on muscle, especially cardiac muscle, seemed to experience a thirty-five year lull. In 1951, Garb (1951) studied the effects of a number of monovalent and divalent cations, including Sr, on cat ventricular papillary muscles. When Sr was added to normal Ca-containing Locke's solution, he found that it produced a contractile response identical to that seen when an equivalent amount of Ca was added. In the presence of low concentrations of Ca (0.27 mM or less), however, addition of Sr resulted in an increased duration of contraction and a prolonged R-T interval of the electrogram. Although he did not comment on it, his records also show that the tension produced at a given Sr concentration was greater in the presence of 0.27 mM Ca than with 2.16 mM Ca. Clearly, the effects of Ca and Sr on contractions in mammalian ventricle were not strictly additive. This apparent antagonism between Ca and Sr on myocardial function was studied further by Thomas (1957) on frog ventricle. Cellular levels of K and Na were measured after perfusion of the ventricle with either Ca- or Sr-Ringer's solution. Three times as much K loss was found in Sr-perfused hearts than in Ca-perfused hearts. Intracellular Na was found to increase to the same extent as K was decreased. On initiating Sr-Ringer's perfusion, twitch tension initially declined but subsequently rose to greater than that seen in Ca-Ringer's solution. Adding Ca to the Sr-containing perfusate decreased tension. Furthermore, EDTA, a chelating agent which has a greater affinity for Ca than for Sr, greatly increased the rate of development of the positive inotropic effect of Sr (as did cardiac glycosides). A competition between Ca and Sr at the cell membrane level was seen as the

cause of these responses.

It was found that not all mammalian ventricular tissues behave in the same manner following replacement of Ca with Sr. Weyne (1966a) reported that Langendorff-perfused rat heart showed a negative inotropic effect and an increase in tone in Ca-free, Sr-containing solution while cat papillary muscle displayed a positive inotropic effect at stimulation frequencies up to 0.8 Hz. In the cat papillary muscle, time to peak tension was much more frequency-dependent and the rate of tension development was less at all frequencies studied (0.1 to 1.6 Hz) in the Sr medium. The ability of Sr to increase twitch tension by prolonging the duration of the active state while lowering the degree of activation was compared to the positive inotropic effect of cooling.

In 1967, de Hemptinne et al. (1967) examined the contractile effects on cat auricle of varying the proportion of Sr and Ca while keeping the total concentration of both constant and found a tension minimum with 20% Ca in the medium (at 0.1 Hz). They also showed that the prolonged contraction seen after Sr replacement of Ca in cat papillary muscle was associated with an increased action potential duration. Relaxation of the muscle always occurred during the rapid repolarization phase of the action potential. Their speculation that Sr is "less efficient than calcium in activating" the mechanism that triggers repolarization has since been demonstrated (see Membrane Effects section).

Brutsaert (1967) studied the temperature- and frequency-dependence of cat papillary muscle contractions following Ca replacement by Sr.

At 22°C, peak tension was seen near the end of each contraction while at 34°C the peak was reached early in the contraction and was followed by a plateau phase, as had been previously shown (de Hemptinne et al., 1967). The preparation still showed a force-frequency relationship but the curve relating tension to frequency was shifted in the direction of longer intervals. Beyond a rate of 15 to 18 stimuli per minute, tension did not increase while the contraction duration continued to shorten. The shift of the curve may not have been apparent, however, if Brutsaert (1967) had expressed the interval as the time between relaxation of one contraction and the onset of the next. Postextrasystolic potentiation was abolished but the extrasystole produced more tension than the previous steady state contraction when the basic driving rate produced submaximal tension. The author concluded that "the strontium ion connot substitute for calcium in regard to its property of adapting contractility from beat-to-beat, as encountered in postextrasystolic potentiation and paired stimulation potentiation." In light of the presumed role of the sarcoplasmic reticulum in these phenomena, this would indicate that Sr handling by that organelle is substantially different from Ca handling.

An extremely interesting series of experiments were carried out by Verdonck and Carmeliet (1971) on cow Purkinje fibres contracting in Ca- and Na-free, 10mM Sr Tyrode solution. They found that, in the absence of Na, relaxation did not accompany final repolarization of the sarcolemma. Furthermore, at the very low frequencies studied, frequency potentiation and rest potentiation were evident in a fast-rising phase of contraction, a phase which was eliminated by caf-

feine or ryanodine, agents presumed to affect Ca uptake and release, respectively, by the sarcoplasmic reticulum (Blayney et al., 1978; Sutko et al., 1979). Replacing up to 70 mM of choline with Na produced two effects: 1) the plateau of contraction was maintained at a higher level throughout a prolonged action potential and 2) relaxation accompanied repolarization, producing a large increase in the rate of relaxation. These observations bear a particular importance for both maintenance of the plateau phase and the mechanism of relaxation in Sr-containing media. Both phenomena may be explained on the basis of a Na-divalent cation exchange (Reuter and Seitz, 1968; Glitsch et al., 1970; for reviews, see Chapman, 1979; Mullins, 1981). Inward Sr movement across the sarcolemma, coupled to outward Na movement, during the prolonged action potential plateau could maintain the tension plateau while an exchange in the reverse direction could greatly speed relaxation. In this regard, Verdonck and Carmeliet (1971) cited a then unpublished observation of Van Kerkhove and Carmeliet (1972) that Sr efflux is increased by Na ions. The implications become important when the models of contraction are discussed.

Of particular relevance to the results presented in this thesis is a study by Braveny and Sumbera (1972). Using papillary muscles from rabbits, cats, and guinea pigs, they produced two-component contractions by replacing 90% of the Ca in Tyrode solution with Sr. The early peak of contraction was dependent on the presence of Ca. Increasing the frequency of stimulation produced an immediate increase in the late phase. The early phase increased after a delay of one contraction. They concluded that the early phase was caused by Ca re-

lease from the sarcoplasmic reticulum while the late phase was due to direct activation by a trans-sarcolemmal flux of Sr.

Blinks et al. (1972) also showed that, in the presence of a normal concentration of Ca, Sr prolonged contraction without delaying the latency to the onset of contraction. After Ca was removed, however, the onset was delayed, indicating the loss of some early component of contraction. A similar delay in onset was seen by the same authors after treating the tissue with high concentrations of caffeine in normal Ca-containing solution. Brutsaert and Claes (1974) also noted a delay in the onset of maximum unloaded shortening velocity in cat papillary muscle after Sr replacement of Ca and reached the same conclusion as Braveny and Sumbera (1972) with regards to the mechanism of production of the two contraction components. The similarity of the actions of Sr and caffeine on the early component further supported the contention that the early component of contraction was due to Ca release from the sarcoplasmic reticulum and that Sr was either not taken up or not released from that store. If this was true, then Sr should produce different effects on cardiac tissue in which the sarcoplasmic reticulum plays a minor role in contraction.

In contrast to its effect on mammalian ventricular tissue, replacement of Ca by Sr in amphibian ventricle prolongs contraction duration but not the time to onset or to peak tension. Bass et al. (1975) studied the action of Sr on isometric and isotonic contractions of toad ventricle. They found that the ion prolonged action potential and contraction durations without affecting the time to peak tension and only slightly increasing the time to onset of tension development

or shortening. The latter effect they ascribed to the lower sensitivity of troponin and actomyosin for Sr than for Ca (Ebashi et al., 1968). Henderson and Cattell (1976) compared the effects of Sr on cat papillary muscle with its effects on frog ventricle. In Ca-free, Sr-containing solution, both tissues showed a relatively fast "twitch" phase followed by a plateau. The plateau responded to Sr, adrenaline, temperature changes, La, Mn, and caffeine when the intervention was applied during a contraction. After emphasizing the differences between the "twitch" and plateau phases, the authors concluded that the twitch was due to release of Sr from an internal store (the sarcoplasmic reticulum) while the plateau was caused by direct activation of the contractile proteins by extracellular Sr flux during the prolonged action potential. This interpretation, however, is not consistent with their own data since: 1) the influence of internal stores of activator cation, especially the sarcoplasmic reticulum, in frog heart is probably very small (Anderson et al., 1976; Anderson et al., 1977), and 2) they acknowledge that the difference in response of cat and frog ventricle to caffeine was due to a greater development of the sarcoplasmic reticulum in cat heart (resulting in a delayed onset of contraction with caffeine) and yet their results showed that caffeine increased the twitch component in cat heart. Thus, while their speculated source of activator Sr for the plateau may be correct, another explanation for the twitch component must be found.

So far we have seen that Ca replacement with Sr produces the following effects on contractions in mammalian heart tissue:

1) an increased latency to onset of tension development or maximum

shortening velocity,

2) a slower rate of tension development at a given stimulation frequency, indicating a decreased intensity of the active state,

3) a prolonged time to peak tension and a positive inotropic effect in spite of the lower intensity of the active state, indicative of an increased duration of the active state (e.g. Sonnenblick, 1967),

4) a relatively rapid twitch phase followed by a plateau phase whose duration is directly related to the interval between contractions,

5) a relaxation phase clearly dependent on the membrane potential and on extracellular Na,

6) a significant alteration of the force-interval relationship, especially postextrasystolic potentiation and paired stimulation potentiation.

Amphibian hearts exhibit all except effects 1 and 6. If the differences between mammalian and amphibian hearts are attributed to the greater importance of the sarcoplasmic reticulum in the former preparation, then it is plain that cation stores in that organelle are required for the early onset of contraction. Similarly, the force-interval relationship is seen to be intimately associated with the sarcoplasmic reticulum.

The possibility that Sr is unable to directly activate the contractile proteins but instead causes the release of some tightly-bound intracellular store of Ca requires further discussion. Removal of tightly-bound Ca in Sr-containing media by EDTA has been shown to pro-

duce a faster increase in Sr-induced positive inotropy in frog ventricle (Thomas, 1957). In addition, toad ventricle is able to sustain a contracture of considerable magnitude in Ca-free Sr-Ringer's solution after treatment with EDTA (Nayler and Emery, 1962). Presumably, the tension produced by Sr after EDTA represented direct activation of the myofibrils. Frank (1962), however, found that Sr cannot restore KCl or caffeine contractures in frog toe muscle that had been depleted of Ca by repeated contractures in Ca-free, Co-containing solution. The same tissue yielded different results for Edwards et al. (1966). After removing most of the Ca from the toe muscle by repeated contractures in Mn solution, soaking the tissue in Sr solution increased the contracture area, indicating that "the effect of Sr on contractures is qualitatively similar to that of Ca, though weaker." However, they found that a thirty times higher Sr concentration was required for half maximal ATPase activity compared to Ca.

Ebashi et al. (1968) determined the sensitivity of a reconstituted contractile system for Ca and Sr. They found that the cow heart system was activated by Sr but that a concentration three times greater than of Ca was needed. In contrast, they noted that rabbit fast skeletal muscle contractile systems needed 20 times more Sr than Ca. Different sensitivities to Ca and Sr have recently been shown to exist not only between cardiac and skeletal fibres but also between different types of skeletal muscle. In skinned fibres, slow twitch skeletal muscle had the same sensitivity to Ca and Sr while fast twitch muscle had a much greater sensitivity for Ca (Hoar and Kerrick, 1979). Skinned frog skeletal muscle, however, produces about the same maximum

tension with either Sr or Ca, though (Donaldson and Derrick, 1975; Moisescu and Thieleczek, 1979).

Finally, Kerrick and coworkers have demonstrated the ability of Sr to cause tension generation in functionally skinned cardiac tissue from a number of species. The sensitivity of the contractile apparatus to Sr was seen to be the same as the sensitivity to Ca in fibres from rat (Donaldson et al., 1978), chicken (Kerrick et al., 1979), rabbit, and cow hearts (Kerrick et al., 1980). In the study on rat fibres, however, Donaldson et al. (1978) concluded that Sr and Ca sensitivity were the same based on the concentration of each ion needed for half maximal tension development (pCa 5.06 and pSr 5.10 at a Mg concentration of 1.0 mM). Since they found that the "maximum tension with Sr^{2+} activation was always greater than with Ca^{2+} activation," it follows that, for a given concentration of either ion, Sr resulted in greater tension development than Ca. If sensitivity were defined as the ability of the ion to induce tension development then the sensitivity of rat ventricular fibres was, in fact, greater for Sr than for Ca. In any case, the salient point is that Sr can directly and efficiently activate the contractile proteins without having to release an internal store of Ca ions.

The possibility that the sarcoplasmic reticulum can take up Sr and release it in response to sarcolemmal depolarization is important for interpretation of the results presented in this thesis. Edwards et al. (1966) observed that isolated sarcoplasmic reticulum from frog toe muscle was much more sensitive to Ca than to Sr in that the Ca concentration needed for half maximal filling was 20 nM while the same

degree of loading required a Sr concentration of 300 nM. The authors noted that this means that the reticulum would be unable to lower the sarcoplasmic Sr concentration to as small a level as it lowers the Ca concentration. That Sr is taken up by the sarcoplasmic reticulum of frog heart has been clearly shown by Winegrad (1973) and by Kawata and Hatae (1977). In his electron micrographs, Winegrad (1973) found electron densities in the organelle following a KCl contracture in Ca-free, Sr-Ringer's solution. Similar densities in the sarcoplasmic reticulum of vascular smooth muscle spontaneously contracting in Sr solution have been seen by Somlyo and Somlyo (1971). Both groups suggested the use of Sr ion as a marker for the sarcoplasmic reticulum. Kawata and Hatae (1977) have identified the electron densities in frog ventricle as Sr by X-ray microanalysis. Quantitatively, rabbit skeletal muscle sarcoplasmic reticulum vesicles transport Sr faster than Ca, using one ATP per Sr transported, and may store 10-20 times more Sr than Ca (Mermier and Hasselbach, 1976).

The ability of the sarcoplasmic reticulum to release bound Sr following membrane depolarization is less clear. Although Henderson and Cattell (1976) attributed the twitch phase of contraction in cat papillary muscle to Sr release from the sarcoplasmic reticulum, caffeine actually increased that phase. More convincing evidence of release was given by Verdonck and Carmeliet (1971). As previously mentioned, caffeine and ryanodine decreased the fast-rising phase of contraction in Na-free, Ca-free Sr-Tyrode solution. However, since the stimulating frequency was extremely low (0.5 to 1.0 per min) and the Na-free, 10 mM Sr conditions favoured very high intracellular Sr concentra-

tions, the significance of this release mechanism under more "physiological" conditions is questionable. Certainly the kinetics and energetics of uptake of Ca and Sr by fragmented sarcoplasmic reticulum differ in major respects (Mermier and Hasselbach, 1976) and so a difference in release of the two ions would not be unexpected.

It has recently been demonstrated by Moisescu and Thieleczek (1978) that the sarcoplasmic reticulum is able to release Sr in skinned frog skeletal muscle fibres. When the preparation was loaded with Sr and then transferred to an "activating" solution having the same concentration of Sr but also containing 10 mM caffeine, a phasic tension response was seen. Their finding that about 25 times more Sr than Ca was required to produce the same force, when coupled with the previous finding of about the same ratio of activities on the contractile proteins (Ebashi et al., 1968), indicates that approximately the same amount of Sr as Ca can be released from the sarcoplasmic reticulum by caffeine. Moreover, by loading the reticulum with Sr and then washing in a "relaxing solution", a phasic tension response was obtained by re-immersing the tissue in the loading solution (pSr 4.5). This showed that, in frog skeletal muscle, Sr-induced Sr release from the sarcoplasmic reticulum was possible. It had previously been shown that Sr-induced Ca release from skinned frog skeletal muscle is possible but that 50 times more Sr than Ca was required to see the induction (Endo et al., 1970). If the reticulum of cardiac muscle were able to release the same amount of Sr as Ca then the twitch tension in Sr solution would be expected to be the same or greater than in Ca solution since the sensitivity of the contractile proteins is about the

same for both ions. The positive inotropic effect of equimolar replacement of Ca with Sr could be explained by this mechanism but the relative insensitivity of contractions in Sr solutions to agents affecting cation release from the sarcoplasmic reticulum (caffeine and ryanodine, Verdonck and Carmeliet, 1971) indicates that the sarcoplasmic reticulum is a minor source of activator Sr. Furthermore, due to the fairly substantial differences between skeletal and cardiac muscle sarcoplasmic reticulum (Van Winkle and Entman, 1979), extension of the results of Moisescu and Thieleczek (1978) in skeletal muscle to cardiac muscle is not entirely safe. No similar studies on cardiac muscle have been done as yet but the above discussion points to a lack of involvement of the sarcoplasmic reticulum in initiating Sr-mediated contractions in this tissue.

2. MEMBRANE EFFECTS

The striking effects of Ca replacement by Sr on the electrical characteristics of heart muscle were first observed by Mines (1913). The frog ventricle used in his studies showed a pronounced prolongation of "electrical activity" when Sr was added to a Ca-free perfusate. Like the mechanical effects, the electrical changes induced by Sr in excitable tissue that were first observed in the early 1900's seemed to be forgotten until the 1950's. Garb (1951) merely mentioned that the R-T interval of cat papillary muscle was longer in the presence of Sr, especially if the Ca concentration was first lowered.

It was not until 1957 that any real effort was made in determining

the membrane effects of Sr in muscle. As mentioned in the preceeding section, Thomas (1957) found an increased loss of cellular K and gain of cellular Na during perfusion of frog ventricle with Ca-free Sr Ringer's solution. The addition of only 0.2 mM Ca to a perfusate containing 1.0 mM Sr prevented the monovalent ion flux changes, indicating that Sr cound not substitute for Ca in this important membrane effect. In the same year, Niedergerke and Harris (1957) published a report of possibly greater interest. The authors were studying Ca fluxes in frog ventricular strips during the positive inotropy of low extracellular Na concentration but, for uptake experiments, used radiolabelled Sr rather than Ca as the tracer due to the the more penetrating radiation given off by the former isotope. Significantly, they justified the use of Sr as a measure of Ca uptake by noting that "tracer loss from the tissue which had first been treated with either tracer had a similar time course." The results showed that Sr uptake was greatly increased when the tissue was bathed in low Na solution. This was the first demonstration of the ability of Sr to replace Ca in the Na-Ca exchange mechanism, a fact that was used in subsequent studies (Niedergerke, 1957). It was speculated at this time that Na and Ca were competing for a membrane binding site.

The inability of Sr to substitute for Ca in maintaining the resting membrane potential was demonstrated by Nayler and Emery (1962). They found that the resting potential of toad ventricle varied with the Ca concentration, being about -95, -70, and -45 mV at Ca concentrations of 1.3, 0.05, and 0 mM, respectively. Addition of 4 mM EDTA to the Ca-free perfusate presumably removed tightly-bound Ca from the mem-

brane and produced a further 10 mV depolarization. When Ca was returned to the perfusate the membrane repolarized but when Sr was added in concentrations of from 0.05 to 2.6 mM the resting membrane potential did not repolarize and a contracture ensued.

Weyne (1966) studied the membrane effects of Sr on a large variety of ventricular and atrial preparations from frogs, cats, rats, and rabbits. He noted that after substitution of Ca by Sr the rate of spontaneously-beating hearts was decreased, the durations of both the absolute and relative refractory periods were increased, and that conduction velocity was reduced. In addition, the rate constant of radiolabelled K efflux from cat papillary muscles stimulated at 1 Hz was found to be 27% greater in Sr-containing solution than in equimolar Ca-containing solution. In fact, the K efflux rate in the presence of Sr was not different from that seen in the absence of both Ca and Sr. The author hypothesized that the effect of Sr on K efflux was the causative factor in producing prolonged action potentials and a positive inotropic effect.

The first tracings of a cardiac action potential under the influence of Sr were not the preliminary figures of de Hemptinne et al. (1967), as later stated by Bass et al. (1975), but were contained in an interesting study by Niedergerke and Orkand (1966). These researchers found that, in frog heart, increasing Sr concentrations in low Ca perfusate resulted in about the same increase in action potential overshoot as Ca without changing the maximum rate of rise of the action potential. Furthermore, the action potential duration was increased three or more times even though the solution also contained

0.2 mM Ca. The paper by de Hemptinne et al. (1967) made the important observation that the time course of contraction was very closely linked to the time course of the action potential and that Sr is not as good an activator of repolarization as is Ca.

At this point it would be instructive to examine some of the effects of Sr on membranes of other excitable tissues pulished in the mid- to late-1960s. Hagiwara and Takahashi (1967) produced Ca-dependent action potential-like spikes in barnacle skeletal muscle and looked at the electrical effects of a number of ions. As previously reported by Hagiwara and Naga (1964), the spikes could be maintained in Ca-free, 50 mM Sr solution but repolarization was greatly prolonged. When 10 mM Ca was added to the perfusing solution, the maximum rate of depolarization was reduced and the duration of the spike, though still longer than control, was shortened. This was interpreted as showing a competition between Sr and Ca for membrane binding sites. Ca was thought to have a greater affinity than Sr for the site but other ions (notably La, Co, Mn, and Ni, in decreasing order) had greater affinities than either Ca or Sr. Two years later, Baker et al. (1969) mentioned that, at least qualitatively, Sr could replace Ca in Na-Ca exchange in squid axon and support Na efflux into Li-substituted seawater. Shortly afterward, Katz and Miledi (1969) showed that a tetrodotoxin-insensitive current in squid stellate ganglion was sensitive to the external Ca concentration. Sr ion was able to substitute for Ca in generating the current, thought to be important for excitation-secretion coupling.

In 1970, interest in the properties of the slow inward current was

increasing rapidly. Pappano (1970) conducted a series of experiments on guinea pig atria partially depolarized by 22 mM KCl. Under these conditions, the fast inward current, carried by Na and sensitive to tetrodotoxin, was inactivated and the tissue was inexcitable. Stimulation of β adrenergic receptors, which increases the amplitude of the slow inward current (Reuter and Scholz, 1977b), was able to restore Ca-dependent action potentials. Increasing the Ca concentration in the bathing solution in the absence of an adrenergic agonist was also able to restore excitability. More importantly for this discussion, 4 mM Sr was able to restore action potentials that were insensitive to tetrodotoxin but were blocked by Mn ion, demonstrating that Sr could enter the cell through the voltage-dependent slow channel as the slow inward current. This was supported by the findings of Verdonck and Carmeliet (1971), as was discussed in the previous section. Briefly, they noted that Sr could restore excitability in cow Purkinje fibres bathed in Na-free solution.

In a short but interesting communication, Van Kerkhove and Carmeliet (1971) showed that the efflux of radioactive Sr from cow Purkinje fibres that had been loaded with the tracer depended on the extracellular concentrations of both Na and Sr. Furthermore, the rate coefficient of efflux was increased three-fold when Sr was added to the efflux solution. Thus, by 1971, Sr had been shown to cross the sarcolemma by the slow inward current (see above), by Na-Sr exchange, and by Sr-Sr exchange.

Two papers were published in 1971 in which Sr action potentials were extensively studied (Vereecke and Carmeliet, 1971a and b). The

experiments were carried out on cow Purkinje fibres bathed in Na-free, Ca-free 10 mM Sr Tyrode solution and stimulated at the low rate of 1 per min. In the first paper, Vereecke and Carmeliet (1971a) showed that the upstroke velocity (dV/dt) and the overshoot of the prolonged Sr action potential both increased with increasing Sr concentrations (although dV/dt reached a plateau at about 20 mM Sr). This indicated that the upstroke current was carried by Sr ions. The membrane resistance during the plateau of the action potential was lower than at the resting potential, similar to the situation in Na-free Ca solution (H Reuter, 1965 quoted by Reuter, 1973). This meant to Vereecke and Carmeliet that Sr conductance remained high throughout the long action potential. To support this idea, they found that under voltage clamp conditions the inward current flowed for the entire duration of the clamp (up to 1.3 sec). The lack of effect of tetrodotoxin and the ability of adrenaline to increase both the overshoot and dV/dt provided further proof that Sr was entering the cell by way of the slow channel normally used by Ca.

The second paper by Vereecke and Cameliet (1971b) noted that as little as 0.5 mM Ca added to Na-free 10 mM Sr solution resulted in a marked depression of dV/dt, overshoot, and duration of the action potential. This blocking action of Ca is in accord with the current channel having a greater affinity for Ca, calculated as 35 times that for Sr,but a greater mobility of Sr (a concept discussed in greater detail by Hagiwara and Byerly, 1981). In this context, Vereeke and Carmeliet (1971b) also noted that Mn and Mg could block the Sr conductance with Mg requiring much larger (25 mM) concentrations than Mn

(compare with Hagiwara and Takahashi, 1967; see above).

The use of voltage clamp techniques came "in vogue" in the early 1970s and it was only a matter of time until the method was used to study in depth the effects of Sr on the slow inward current. Two reports from Kohlhardt's laboratory were published in 1973, both using a double sucrose gap to measure the current in cat trabeculae and papillary muscles in the presence of Sr. In the first report (Kohlhardt et al., 1973a), 5.4 mM Sr added to Tyrode solution containing 2.2 mM Ca increased the maximum slow inward current by about 85 % without changing the rates of activation and inactivation of the current. They stated that similar changes were seen when the Ca concentration was reduced to 0.55 mM prior to adding Sr, that the threshold potential for activation of the current was shifted about 5 mV in the hyperpolarizing direction, and that the reversal potential was "markedly shifted to higher values." Based on the ability of Sr to increase the magnitude of the slow inward current and to overcome its blockade by D-600, a methoxy derivative of verapamil, the authors concluded that Sr used the same channel as Ca to enter the cell.

The next paper from the same laboratory extended their observations on the interaction of Sr with the slow inward current (Kohlhardt et al., 1973b) and made some useful observations. In experiments in which Sr replaced all the Ca in the bathing solution (2.2 mM), the duration of an action potential was increased by only 60-75%. In contrast, Weyne (1966) found that the absolute refractory period, an indirect measure of action potential duration, was 186% greater than control after the equimolar replacement of 1.8 mM Ca with Sr, also in

cat papillary muscle. Similar results were obtained by Niedergerke and Orkand (1966) in frog ventricle even in the presence of 0.2 mM Ca. The authors, however, did not mention the frequency of stimulation (see below). Regardless, Kohlhardt et al. (1973b) did find that the magnitude of the slow inward current was unchanged by complete replacement of Ca by Sr. For unexplained reasons, most of the remainder of the study compared the effects of 2.2 mM Ca to the effects of 4.2 mM Sr with the result being that the current-voltage curves are difficult to interpret. Nevertheless, they observed that the kinetics of current activation were the same but that the time course of inactivation was much longer in Ca-free, Sr containing solution (compare with Kohlhardt et al., 1973a with low Ca concentrations plus Sr). This fact is important in interpreting the results presented in this thesis. The authors in both studies (Kohlhardt et al., 1973a and b) concluded that Sr could replace Ca as the charge carrier through the slow inward current channel with equal effectiveness. They also rejected the possibility of any competition between Ca and Sr for the carrier: they considered the ions to be additive on the magnitude and either functional (Ca) or non-functional (Sr) in altering the inactivation kinetics of the current. The data shown demonstrates competition quite effectively. In the current-voltage curves shown by Kohlhardt et al. (1973b), changing the perfusate from 2.2 mM Ca, Sr-free solution to Ca-free, 4.2 mM Sr solution increased the maximum slow inward current by 90%. Thus a 91% increase in divalent cation concentration resulted in a 90% increase in current. In the curves shown by Kohlhardt et al. (1973a), however, changing the perfusate

from 2.2 mM Ca, Sr-free solution to 0.55 mM Ca, 5.4 mM Sr solution augmented the current by only 30%. In this case, a 170% increase in divalent cation concentration (they had shown that Ca and Sr produce the same current at the same concentrations; see above) yielded a mere 30% change in current. Clearly, the presence of 0.55 mM Ca had competitively inhibited the expected large rise in Sr-mediated slow inward current. In any case, the noteworthy points of these studies are that Sr can replace Ca as the major charge carrier of the slow inward current and that in its presence the rate of inactivation of the current is slowed, as previously shown by Sassine and Bernard (1972) in frog ventricle. Since intracellular Ca hastens inactivation of the slow channel (Isenberg, 1977b), it is possible that Sr is unable to replace Ca in this action, resulting in a prolonged inward current.

The applicability of the voltage clamp technique for measuring Sr fluxes across the sarcolemmae of multicellular preparations is probably more doubtful than is its use for measuring Ca fluxes. A number of cautious (Beeler and Reuter, 1970a; Morad and Goldman, 1973; Trautwein, 1973; Beeler and McGuigan, 1978) and even condemning (Johnson and Lieberman, 1971) discussions of the inadequacy of voltage control in multicellular preparations have been published. In Na-free Sr solution the results may be particularly error prone since: 1) the membrane capacitance in Na-free, Ca-free 10 mM Sr solution has been shown to be 3.77 times that in normal Tyrode solution (Carmeliet and Willems, 1971) and 2) guinea pig papillary muscles loaded with Sr in Na-free, Ca-free 20 mM Sr Tyrode have an internal resistance 10 times greater than preparations in normal Tyrode solution due to electrical

uncoupling of the cells (Ochi, 1977). Therefore, estimations of the magnitude of the Sr-carried slow inward current, as well as the analysis of drug effects on the current, are probably best made by measuring the maximum dV/dt in Sr solution after blocking the fast Na current with tetrodotoxin or high K concentration. However, careful use of the voltage clamp technique has provided a wealth of information about the various membrane currents.

Recent voltage clamp experiments have shown the ability of Sr to enter the myocardial cell by carrying the slow inward current. Reuter and Scholz (1977a) mentioned that, at equal extracellular concentrations, the slow inward current carried by divalent cations follows the order Ba > Sr > Ca >> Mg (D. Potreau, 1975 and H. Reuter and B.G. Katzung, unpublished; both quoted by Reuter and Scholz, 1977a). Noble and Shimoni (1981a) also showed that the magnitude of the slow inward current in frog atrium was greater after replacing Ca with Sr. The latter authors also noted the greatly slowed inactivation time in Sr solution as had previously been shown by Kohlhardt et al. (1973b). Stimulating the atria at 1 Hz after a 90 sec rest resulted in a slow inward current staircase in both Ca and Sr solution but the staircase magnitude with Sr was 200% greater than with Ca. Since Kohlhardt et al. (1973b) did not mention the stimulating frequency at which they had found no potentiation of the slow inward current with Sr, it is possible that the driving rate was too slow to enable the increase to be seen.

The discussion so far has shown that Sr can replace Ca in Na-Ca exchange, Ca-Ca exchange, and in the slow inward current. A competition

exists between Ca and Sr for membrane binding sites with Ca being more tightly-bound. The competition extends to the slow inward current (see above) and to Na-divalent cation exchange (Wakabayashi and Goshima, 1981). On the other hand, there appears to be certain membrane binding sites crucial for the maintenance of the resting potential where Sr is ineffectual. Finally, Sr seems to alter the inactivation kinetics but not the activation kinetics of the slow inward current.

Little has been said thus far about the mechanisms underlying the prolongation of the action potential. The action potential plateau is known to depend upon the balance of a number of inward and outward currents (Weidmann, 1974; McAllister et al., 1975; Vassalle, 1979). Since in normal Ca-containing solution the plateau has a complex shape, an almost limitless number of combinations of voltage- and time-dependent currents could explain the shape. When all or most of the Ca in the bathing medium has been replaced by Sr, the plateau is flat and repolarizes slowly until a potential (unstudied as yet) is reached where the membrane rapidly repolarizes to the resting level. The genesis of a flat plateau surely could be explained by only a few combinations of currents. Since the same shape is seen in the absence (Vereeke and Carmeliet, 1971a) as in the presence (cat, Henderson and Cattell, 1976; frog, Kawata and Hatae, 1977) of extracellular Na, any background Na current may be disregarded. The only remaining inwardly-directed current is the slow inward current. If one extends the observations of Vereeke and Carmeliet (1971a) in Na-free solution to the action potentials seen in normal Na solution then the slow inward current flows throughout the plateau. In that case, the gradual,

linear repolarization must be due to either: 1) a slightly larger, constant magnitude outward current if the slow inward current continues at a constant intensity during the plateau, or 2) a balance of inward and outward currents with the same time constants for decay, or 3) an outward current whose magnitude is directly determined by the amount of divalent cation entering the cell as the inward current. Alternatively, the slow inward current may disappear relatively early in the plateau. The outward current would then have to be a small, voltage-independent current. Noble and Tsien (1969) have shown that the X1 plateau K current has a constant magnitude between about -40 and +60 mV and thus could explain the flat plateau if the other outward conductance channels were blocked. In any event, there must be alterations in outward conductance whether or not the slow inward current flows throughout the plateau. It is fairly well established that intracellular Ca stimulates outward K currents, including the transient outward current, Iqr (Kass and Tsien, 1976; Siegelbaum et al., 1977), the time-independent inward rectifier, I_{K1} (Bassingthwaighte et al., 1976; Isenberg, 1977a,c, and d), and the time-dependent pacemaker K current, I_{K2} (Isenberg, 1977a,c, and d). However, Ca may not affect the outward plateau currents, I_{X1} and I_{X2} (Kass and Tsien, 1976). Although the effects of Sr on outward currents of cardiac muscle have not been studied, it has been shown that Sr blocks inward rectification in skeletal muscle (Standen and Stanfield, 1978).

In summary, Sr increases the duration and prolongs the inactivation of inward current while possibly decreasing the outward K currents. The overall effect is a long action potential duration.

STATEMENT OF THE PROBLEM

Excitation-contraction coupling in mammalian myocardium is more complicated than in skeletal muscle, requiring extracellular Ca pools in addition to intracellular pools. The extracellular Ca may be merely a trigger for the release of Ca from intracellular stores or may actually participate in contractile activation. A number of models of excitation-contraction coupling have been published, each based on valid observations and arguments but reaching different conclusions on the origin of activator Ca. The prime obstacle in choosing the correct model is arguably the inability to see tension production caused by each of the Ca pools under a variety of conditions.

Separation of the contractile effects of divalent cations derived from different pools is difficult due to the fast and smooth time course of contraction in normal Ca-containing solution and the non-specificity of most blocking agents. If each component could be examined in isolation, the effects of inotropic interventions such as the force-interval relationship, drugs, and ions would be better understood. Further, drugs could be classified according to their actions on each determinant of contraction.

Replacement of Ca with Sr, as pointed out in the General Introduction, increases the magnitude of the slow inward current, prolongs the action potential to generate a maintained, "tonic" tension, and eliminates the sarcoplasmic reticulum as a significant source of activator cation. Since Sr can activate the contractile proteins as well as Ca in cardiac muscle, the ion should be useful in accentuating contrac-

tile activation caused by extracellular or sarcolemmal cation, thereby providing further insight into the mechanism of contraction under normal or altered inotropic conditions.

The objectives of these studies may be summarized as follows:

1) to determine the mechanical and electrophysiological effects of Sr in mammalian ventricle,
2) to ascertain the origin of each contraction component,
3) to examine the effects of a variety of inotropic interventions, both physiological and pharmacological, on each component, and
4) to construct a model of cardiac excitation-contraction coupling based on the results.

GENERAL METHODS

GENERAL METHODS

Each section of the results contains a short description of the methods used in that particular study. This section contains a detailed coverage of all the methods used in all sections.

A. PREPARATION OF THE TISSUES

Dogs of either sex were obtained from the local dog pound and were usually used within 5 days of arrival at the animal holding quarters. The animals used were generally mongrels weighing between 3 and 12 kg of undetermined age. Only these small- to medium-sized dogs were used since: 1) the smaller heart size made dissection easier, 2) we found by experience that smaller hearts tended to have more numerous thin trabeculae than larger hearts, and 3) tissues obtained from smaller dogs produced biphasic contractions (see section I) more readily and reproducibly than tissues from larger dogs.

The small dogs were anesthetized with pentobarbital given in one large bolus dose intraperitoneally. Medium-sized animals were given an intravenous injection of pentobarbital in a paw vein. Following loss of consciousness, the heart was rapidly removed through a left thoracotomy by cutting all connecting structures. Fifty ml of pre-oxygenated, ice-cold Krebs-Henseleit solution was used to flush the coronary circulation by way of the aorta. The heart was then placed in ice-cold, pre-oxygenated Krebs-Henseleit solution and quickly transported from the animal quarters to the laboratory.

The solution containing the heart was immediately bubbled with 95% O_2, 5% CO_2. All subsequent dissections were carried out in cold, aerated Krebs-Henseleit solution, pH 7.4. The heart was gently agitated to remove excess blood and transferred to the primary, deep dissection tray. The atria and aorta were quickly removed. In order to facilitate adequate oxygenation of the endocardial surface, the free walls of both the right and left ventricles were cut from the apex to the base. When inspection of the inside wall of the ventricle showed a suitable tissue, that section of the wall was cut free and placed in the secondary, shallow dissection tray.

Tissues were selected according to the following priority: 1) free-running (i.e. not attached to the ventricular wall except at the ends) trabeculae carneae of less than 1 mm diameter and 4 mm or more in length, 2) free-running papillary muscles with the same dimension constraints as above, 3) thin trabeculae attached to the wall over up to 50% of their length.

The priority system of muscle selection produced the thinnest preparations with the least amount of tissue damage. Most hearts contained at least one type 1 tissue, usually in the right ventricle close to the valves or the interventricular septum.

Type 1 and 2 tissues were tied at each end with 4.0 silk thread which was subsequently tied into a loop. The preparation was cut from the wall and kept in bubbled Krebs-Henseleit solution until being transferred to the tissue bath. Type 3 tissues were gently dissected free from the wall over the length that was attached and then prepared in the same manner as the other tissues.

B. MECHANICAL EXPERIMENTS

1. THE TISSUE BATHS

Glass tissue baths with volumes of either 5 ml or 25 ml were used (fig. 1a). A water jacket, through which a constant flow of warm water was pumped, enclosed the tissue compartment and maintained the temperature at 37 ± 0.2° C. The stimulating electrodes were made of two small blocks of acrylic plastic held together by a length of 12 guage stainless steel wire threaded through holes drilled in the centre of the blocks. One end of the wire was bent to form a hook for securing one end of the tissue with the help of a thread loop. The other end of the wire was bent around the lip of the tissue bath in order to immobilize the electrode. Two platinum punctate electrode wires (about 1 mm long) protruded from the lower plastic block and made contact with the tissue.

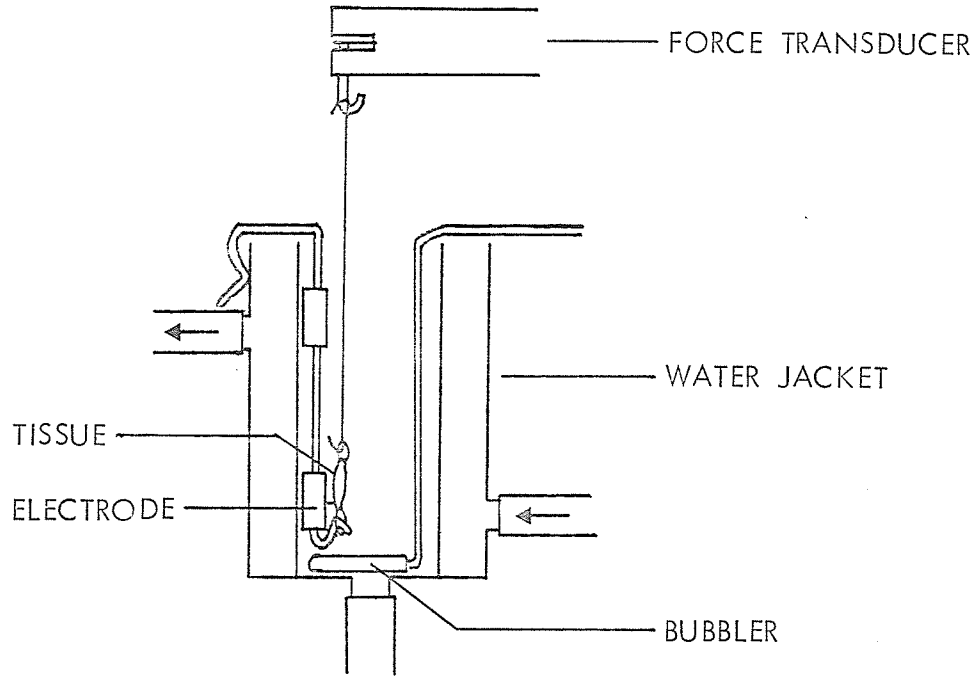
The Krebs-Henseleit solution in the bath was vigorously bubbled with 95% O_2, 5% CO_2through a length of sintered tube. A drain at the bottom of the tissue compartment was attached to a length of rubber hose leading to a sink. The rubber hose was opened and closed, within 2 cm of the bath, by a clamp.

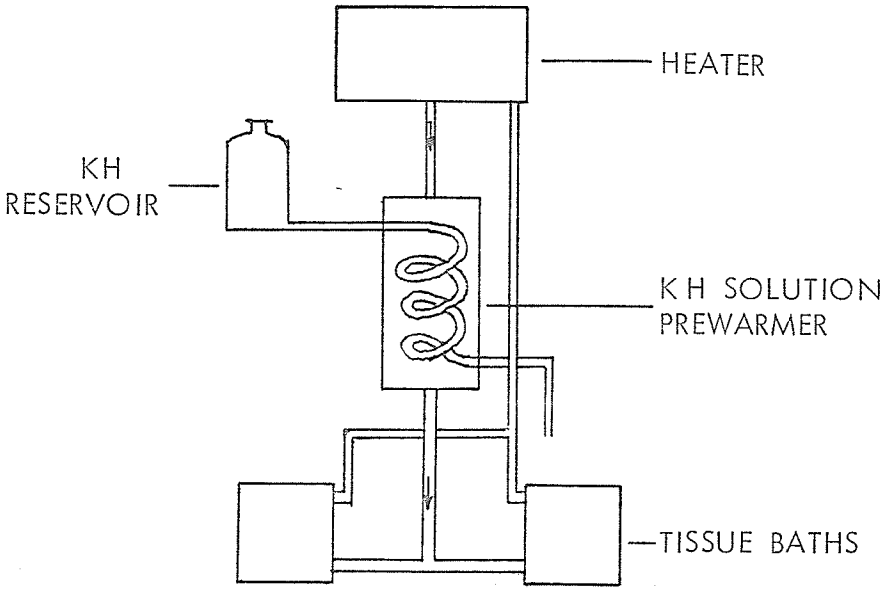
2. THE FLUID SYSTEM

A constant-temperature water pump (MGW Lauda model B1, Brinkmann Instruments) pumped distilled water through a glass heat exchanger to

FIGURE 1

Experimental setup for mechanical studies. A. The tissue bath design. B. The heating and perfusing solution arrangement.


FORCE TRANSDUCER
WATER JACKET
TISSUE
ELECTRODE
BUBBLER


HEATER
KH
RESERVOIR
KH SOLUTION
PREWARMER
TISSUE BATHS

the tissue baths (fig. 1b). The two tissue baths were arranged in parallel and the water leaving them was returned to the water pump. This arrangement kept the temperature of the solution in the tissue compartments to within $0.2^{o}C$ of each other. The heat exchanger was used to warm Krebs-Henseleit solution before adding it to the tissue compartments. The solution, bubbled with 95% O_2, 5% CO_2in a reservoir, flowed out of the heat exchanger through a silastic tube.

3. TISSUE STIMULATION

Electrical stimulation in most experiments was provided by a Pulsar 6i digital stimulator connected to a Pulsar ICR digital stimulus parameter incrementor (Frederick Haer Co.). The incrementor was adjusted to give trains of stimuli of a chosen number of pulses followed by pauses of increasing or decreasing durations. Thus, one train of stimuli was followed by another train after a variable interval ranging from a fraction to a multiple of the regular interval. In some experiments, the stimulator used was a Grass SD 5 (Grass Instruments Co.). These experiments were used only for constant-frequency studies since the stimulator was unable to instantly change its rate.

4. TENSION MEASUREMENT

Muscles were attached to the base of the stimulating electrode and to a Grass FT 03C isometric force transducer (Grass Instruments Co., Quincy, Mass). The latter attachment was made either with thread or

through a thin stainless steel wire. The transducer was held in place by a rack and pinion device that allowed raising or lowering of the transducer accurately to change the resting tension on the tissue. The transducer output was connected directly to a Grass polygraph pen recorder incorporating a Grass model 5P1 low-level D.C. preamplifier and a Grass model 5E D.C. driver amplifier (Grass Instrument Co.).

Once the tissues were in place in the tissue baths, stimulation was begun for an equilibration period of 1 hour at 0.5 Hz. The resting tension was increased during the running-in period until the muscle developed maximum twitch tension, according to the Frank-Starling Law. In addition, the solution in the baths was replaced at 15 to 20 minute intervals to maintain an adequate glucose supply to the muscle and to prevent the buildup of metabolites.

C. ELECTROPHYSIOLOGY EXPERIMENTS

1. THE BATH, STIMULATION, AND TENSION MEASUREMENT

Electrophysiology experiments were conducted in a metal-shielded room in which the bath setup was surrounded by a Faraday cage. All electrical equipment in the room was grounded.

The bath, made of acrylic, had a volume of 40 ml (fig. 1b). A silicon rubber block was fixed to the base of the bath, enabling the silk thread loop at one end of the muscle to be pinned in place. Two small aerators were attached to the base of the bath to continuously

bubble the solution with 95% O_2, 5% CO_2. The entire bath was immersed in a large acrylic box containing distilled water and a glass heat exchanger. The temperature of the water was 37 ± 0.5° C, controlled by a Narashige DC temperature controller with a heating coil in the water bath. Krebs-Henseleit solution was preoxygenated in a reservoir and entered the base of the bath after passing through the heat exchanger. The level of solution in the bath was kept constant (at 40 ml) by a small suction hose, operated by running water.

After one end of the tissue had been pinned to the rubber block on the base of the bath, the silk thread loop at the other end was fixed to a hook extended from a Grass model FT 03C force transducer (Grass Instruments Co.). The transducer was mounted on a 3-directional micromanipulator, allowing its position to be easily adjusted according to the requirements of the muscle. The output was connected to a strain guage preamplifier having variable gain and a built-in adjustable low-pass filter. Its output was connected in parallel to a Hewlett-Packard model 3960 4-channel instrumentation tape recorder, used for storing the events for later computer analysis (see Data Analysis section), a Hewlett-Packard model 141B oscilloscope, for fast sweep speed viewing and for obtaining photographs, and finally to a Telequipment model D52 oscilloscope, for slow sweep speed monitoring of tension and membrane potential. A Gould Brush model 440 pen recorder was also connected to all the output channels of the Tandberg tape recorder and provided permanent records of events for later data analysis. The oscilloscope screen was photographed with a Tektronix Polaroid oscilloscope camera (model C-12). The output of the strain

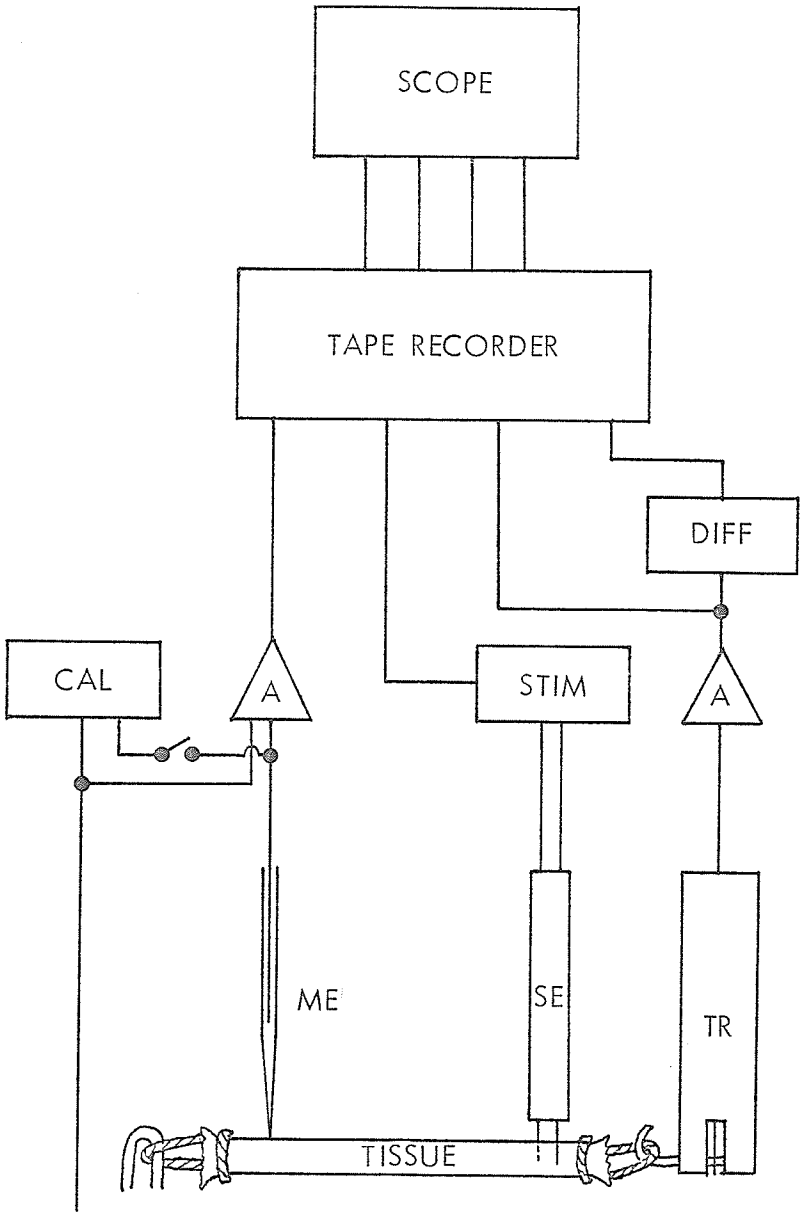


FIGURE 2

Electrical connections in the electrophysiology apparatus. The following abbreviations are used: A, amplifier; CAL, voltage calibrator; DIFF, tension differentiator; ME, microelectrode; SE, stimulating electrode; STIM, stimulator; TR, transducer.

guage preamplifier was also fed to an electronic differentiator when necessary for measuring the rate of tension change (dF/dt).

2. MEASUREMENT OF MEMBRANE POTENTIAL

Microelectrodes were made from glass capillary tubes (1.5 mm diameter; Frederick Haer Co.), containing an extruded glass fibre to facilitate filling, with a model 700C vertical pipette puller (David Kopf Instruments). The microelectrode tips were back-filled with 3M KCl by capillary action and the remaining length of the tubing was filled with a 30 guage hypodermic needle attached to a 1 ml syringe. A chlorided silver wire was eased into the barrel of the microelectrode and was held in place by a drop of a melted mixture of 25% dental wax and 75% hard paraffin.

The microelectrode holder incorporated a high-impedence head stage connected to a Neuroprobe amplifier (model 1600; Transidyne General Corp.). The output was connected to both oscilloscopes and the Hewlett-Packard tape recorder. The microelectrode holder was held in a Brinkmann model MM 33 M motorized micromanipulator (Brinkmann Instruments) allowing precise placement of the microelectrodes. The microelectrode preamplifier measured the potential difference between the microelectrodes, which typically had tip resistances of between 7 and 35 megohms, and an indifferent electrode consisting of a chlorided silver wire fixed at one end of the bath. The tissue was held between the tension transducer and a pin and was illuminated with a model ME1-2 fibre optic microelectrode light source (Narishige Instrument).

Stimulation was provided by Pulsar 6 and 6b digital stimulators (Frederick Haer Co.) connected together so that extrasystoles could be produced. They were wired to movable, platinum punctate electrodes which were placed against one end of the tissue.

Trabeculae were equilibrated for one hour by stimulating at 0.5 Hz at 10% above threshold voltage. At the same time, the resting tension was increased until the tension produced approached the maximum. The microelectrode was lowered until it touched the tissue (as shown by a slight deflection of the voltage tracing on the oscilloscope). At this point, moving it down with the micromanipulator, sometimes accompanied by slight "taps" on the table, usually put the tip of the microelectrode into a cell. The resting membrane potential of most preparations was in the -70 to -80 mV range, indicating that measurements were being made from healthy cells.

D. SOLUTIONS, DRUGS, AND IONS

Normal Krebs-Henseleit solution was made from concentrated stock solutions and distilled, deionized water. The composition (in mM) was: NaCl 118.0; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.4; $NaHCO_3$ 26.2; glucose 11.1. Strontium Krebs-Henseleit solution was identical except for the equimolar replacement of $CaCl_2$ with $SrCl_2$. When bubbled with 95% O_2, 5% CO_2, the pH of all solutions was approximately 7.4.

Drugs and chemicals used were: $CaCl_2$ (Fisher), caffeine (Sigma), ethylene glycol-bis(aminoethyl ether)-N,N'-tetraacetic acid (EGTA,

Eastman and Sigma), isoproterenol (Sigma), $MnCl_2$(Fisher), $NiCl_2$ (Fisher), ouabain (Sigma), ryanodine (courtesy of Dr. J.L. Sutko), $SrCl_2$ (Fisher), tetraethylammonium chloride (TEA, Fisher), and tetrodotoxin (TTX, Sigma). Stock solutions were prepared with distilled, deionized water for all agents. In most experiments where isoproterenol was used, 10-20 uM ascorbic acid was added to the bath before adding the drug to prevent oxidation. The ascorbic acid had no obvious effects on the tissue.

E. DATA ANALYSIS AND STATISTICS

1. MECHANICAL EXPERIMENTS

Two computer programs, SAMANA and SAMAN2, both written in BASIC, were used to analyze these experiments (see Appendix for program listings). Both were used in conjunction with a Hipad digitizer (Houston Instruments) capable of measuring to within 0.1 mm. Points were entered at the bottom and top of each contraction on the paper chart record. The tension of each "test" contraction (i.e. after a drug or frequency change) was measured and converted to tension and percent of control tension. Control tension was defined as the tension of the contraction immediately preceeding the test contraction or, in the case of a drug effect, the tension of the contraction just prior to addition of the drug. SAMANA was used for normal contractions in Krebs-Henseleit solution and force-interval relationship data. SAMAN2

was used to analyze biphasic contractions after the addition of a drug or ion. Both programs stored the results on file for future reference.

2. ELECTROPHYSIOLOGICAL EXPERIMENTS

The Hewlett-Packard oscilloscope was fitted with a mount for a Polaroid camera, enabling photographs to be taken during an experiment or at a later date, by playing taped events back. In addition, the taped events were played back onto the pen recorder at a fast chart speed for analysis by computer. Since we were not concerned with the upstroke of the action potential, the limited frequency response of the pen recorder (-3 dB at 100 Hz) was not a major concern.

Charts showed stimuli, action potentials, dF/dt, and tension. Using the Hipad digitizer, the PDP-11/34 computer (Digital Equipment Corp.), and the BASIC program EPHYSIOL (see Appendix), points were collected in rapid succession by tracing each event with the Hipad cursor. The program, for action potentials, calculated maximum voltage deflection, times to 50%, 90%, and 95% repolarization, and the area under the action potential curve. Action potential duration was defined as the time to 95% repolarization. For the dF/dt and tension traces, the following parameters were calculated: (dF/dt)max, (-dF/dt)max, the times to both (from the stimulus), peak tension, time to peak tension, latency, contraction duration, and area

3. STATISTICS

An effort was made to plan all experiments such that data points could could be paired or blocked for analysis. In these cases, a paired Student's t-test was performed where there was only one comparison to be made or a block analysis of variance (ANOVA) followed by Duncan's multiple range test where multiple comparisons were required (Steel and Torrie, 1960). In any situation where it was determined that the variances were not homogeneous, a t'-test or logarithmic transformation was performed for single or multiple comparisons, respectively.

A standard Student's t-test or completely random design ANOVA was used whenever the number of replications between groups was unequal or whenever the control values and treatment values were obtained in different preparations. Graphs and tables show the means ± SEM with the number of replications being given in the legends. For all statistical tests, the following abbreviations will be used: * $P<.05$; ** $P<.01$; *** $P<.001$.

F. ABBREVIATIONS

For the sake of clarity, the number of abbreviations used in this thesis have been kept to a minimum. The following abbreviations have been employed:

KH Krebs-Henseleit solution (see Solutions section, above),

SrKH Strontium Krebs-Henseleit solution (see Solutions section, above),

P1 The early, phasic component of contraction in SrKH (see sec-

tion I),

P2 The late, phasic component of contraction in SrKH (see section I),

P3 The tonic component of contraction seen after P1 and P2 in SrKH (see section I).

In addition, the chemical abbreviations calcium (Ca), strontium (Sr), sodium (Na), potassium (K), manganese (Mn), nickel (Ni), and chloride (Cl), used extensively, always refer to the common ionic forms. Drug abbreviations (e.g. TTX, EGTA) are described in part D of the General Methods.

I. MECHANISM OF BIPHASIC CONTRACTIONS IN STRONTIUM-TREATED VENTRICULAR MUSCLE

MECHANISM OF BIPHASIC CONTRACTIONS IN STRONTIUM-TREATED VENTRICULAR MUSCLE

A. INTRODUCTION

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, following a 10 to 15 minute stimulation-free period, in the absence (Allen et al., 1976) or in the presence of an adrenergic agonist (Berescewicz and Reuter, 1977; Seibel et al., 1978), in low-temperature, constant frequency contractions under the influence of noradrenaline (Bogdanov et al., 1979), and following 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). 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.

At least four 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; Berescewicz 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, fairly high frequency (0.5 Hz) stimulation while enabling a quantitative comparison of the effects of adrenergic agonists on each component to be made. The results are discussed in relation to the other models of two component contractions.

B. METHODS

The methods used in this section have been described in detail in the General Methods with the following additions. The stimulation frequency was maintained at 0.5 Hz since preliminary experiments had indicated that this was the fastest rate at which a reproducible separation between the two components was seen. Similar results were seen at lower frequencies but it was considered advisable to keep the rate as close to physiological as possible in order to characterize the biphasic contraction phenomon and extend the results to "normal" contractions.

C. RESULTS

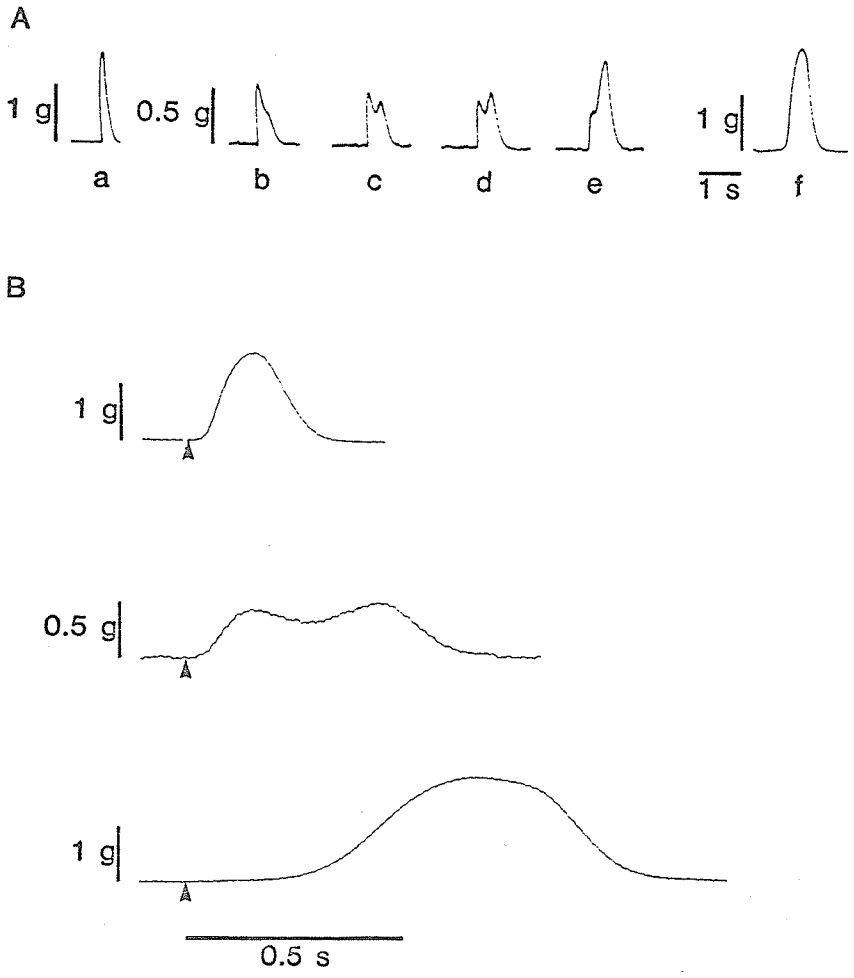
1. EFFECTS OF COMPLETE REPLACEMENT OF Ca BY Sr

Trabeculae were equilibrated in KH solution before Ca replacement by stimulating at 0.5 Hz for one hour. Replacing the bathing medium with SrKH produced a series of changes in the shape of the contractions (fig. 3). Peak tension decreased and the contraction duration increased within 2 min in most tissues. A hump appeared during relaxation and became progressively larger until there was a partial relaxation separating two peaks of contraction. After 10-20 minutes in SrKH, the two components, designated phase 1 (P1) and phase 2 (P2), had approximately equal tensions. In preparations from 15% of dogs, however, P1 and P2 were not separated by the partial relaxation seen in fig. 3(d) 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 after stimulation. The tension developed was greater than in KH solution within 60 min of washing with SrKH, as previously shown in cat papillary muscle (Weyne, 1966a; Brutsaert, 1967; Henderson and Cattell, 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. While the steady state contractions in SrKH without Ca had no discernable P1, after a rest period of 1 to 2 min 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 per min showed a small fast-rising phase that was eliminated by caffeine or ryanodine.

FIGURE 3

Contraction patterns during Ca washout in Sr-containing 0 mM Ca Krebs-Henseleit solution (SrKH). A) Slow chart speed recordings of contractions with increasing time in SrKH. (a) Control (KH) contraction; (b)-(e) Contractions in SrKH at approximately 5 minute intervals beginning 5 minutes after addition of SrKH; (f) Contraction after 60 minutes in SrKH. B) Fast chart speed recordings corresponding to figure 3a, contractions a,c, and f. Arrows indicate the time of stimulation of the tissue.

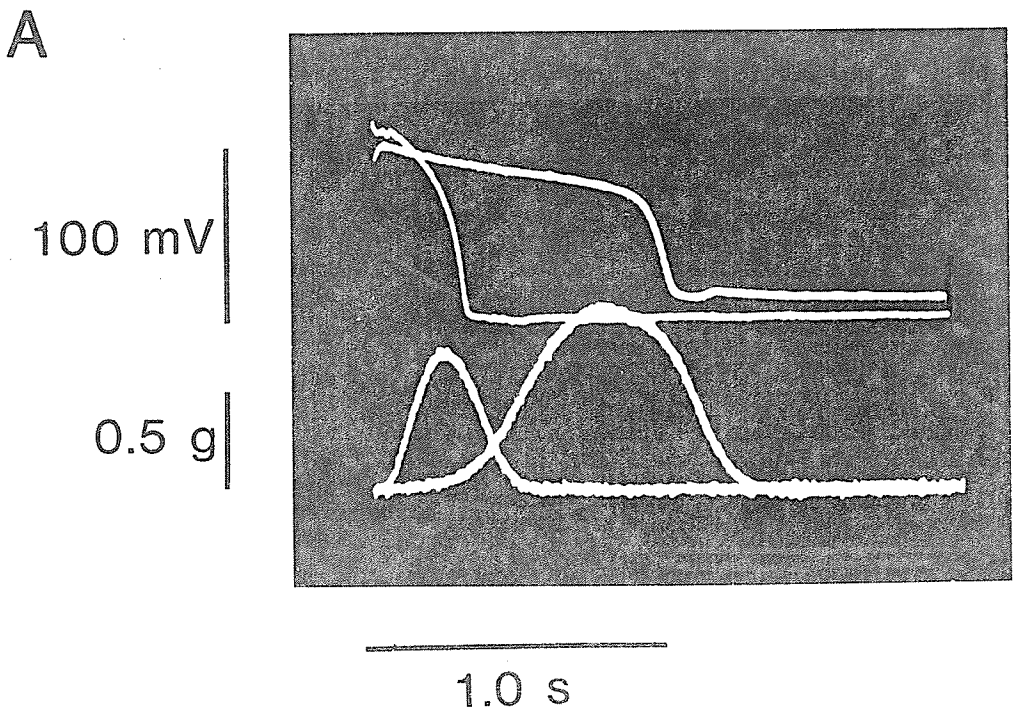

A
1 g
0.5 g
1 g
a
b
c
d
e
1 s
f
B
1 g
0.5 g
1 g
0.5 s

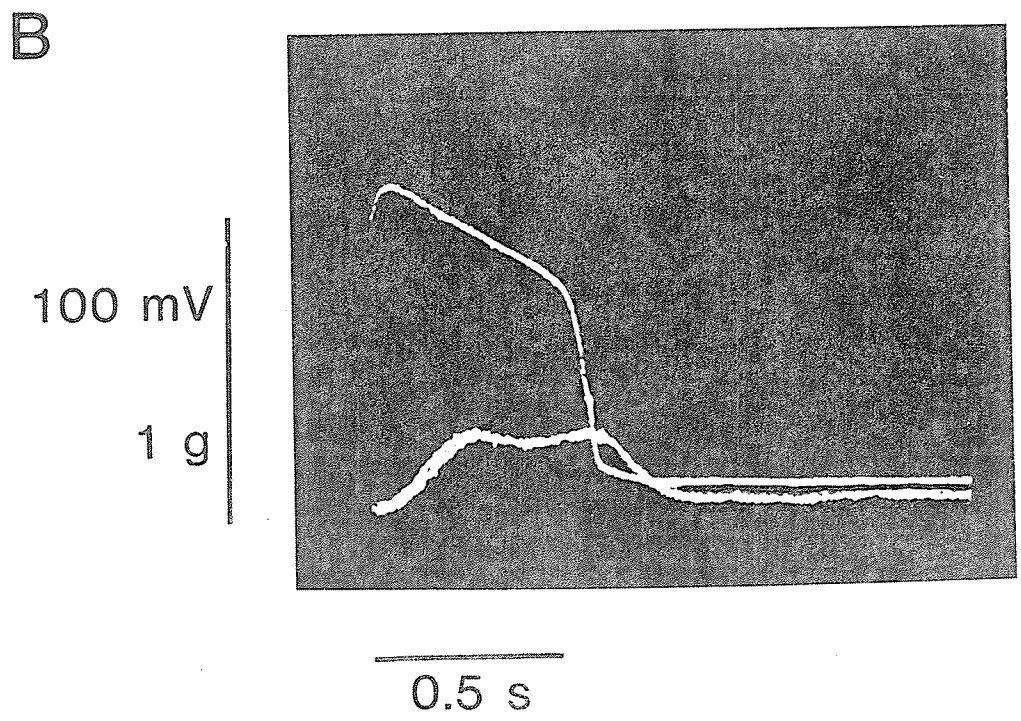
As mentioned above, the tissues were in SrKH for many minutes before P1 was the same height as P2 on the chart. In one experiment, the trabecula was equilibrated in KH solution with a stimulation frequency of 1.0 Hz. At this stimulation rate in this tissue, P1 and P2 had equal amplitudes after 440 sec in SrKH with each component producing 38% of the tension the tissue developed in KH solution. The tissue was then re-equilibrated in KH solution but at a frequency of 0.2 Hz. When the bathing solution was replaced with SrKH at this rate of stimulation, P1 was equal to P2 after 400 sec with each component having about 25% of the tension seen in KH solution. Therefore, it seems that the production of biphasic contractions is more time-dependent than rate-dependent. It is interesting to note that a linear plot of P1 and P2 tensions in this experiment yielded an exponential decline of P1 and a linear increase of P2 at both stimulation frequencies.

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 fig. 3a was complete within 3 min after EGTA treatment. This observation was used in a number of experiments to fascilitate the production of biphasic contractions, particularly in the relatively uncommon experiments where repeated washing with SrKH did not result in a progressive decline of P1. These facts suggest that the loss of P1 is intimately dependent on Ca bound to a high affinity site whose ability to lose Ca may be different between dogs. Furthermore, Sr is unable to substitute for Ca at the site. As pointed out later, the site is probably intracellular, most likely the sarcoplasmic reti-

FIGURE 4

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


A
100 mV
0.5 g
1.0 s


B
100 mV
1 g
0.5 s

culum.

During Ca replacement by Sr the action potential configuration also changed appreciably (fig. 4). The most prominent effect was on the duration, which increased from 255 ± 9 ms (n = 6) in KH solution to 2 s or more in some muscles. In addition, as seen in fig. 4, phase 1 repolarization disappeared and phase 2 became long and flat. Final repolarization to the resting level occured 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 even after complete repolarization (fig. 4b).

2) MAINTENENCE AND CHARACTERISTICS OF BIPHASIC CONTRACTIONS

After incubation in SrKH, when the contraction consisted of 2 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, and abrupt phase 3 repolarizations (fig. 4b and fig. 5). As was the case with the P2 contractions seen

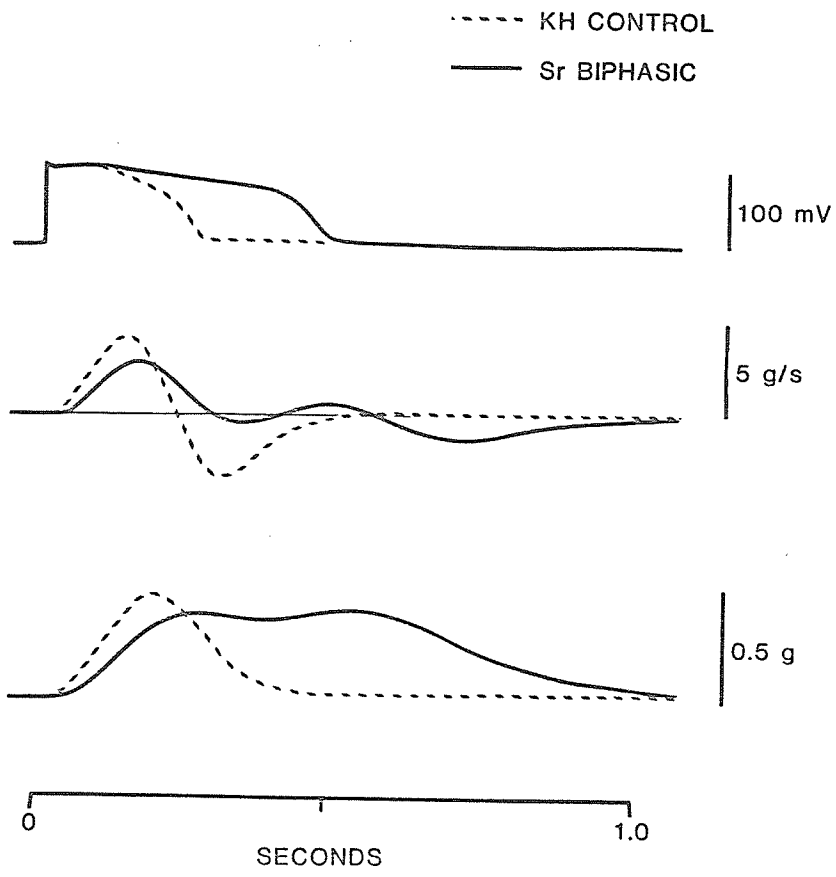


FIGURE 5

Action potentials, rates of contraction (dF/dt), and contractions in Krebs-Henseleit solution (KH control) and following 95% replacement of Ca with Sr (Sr biphasic).

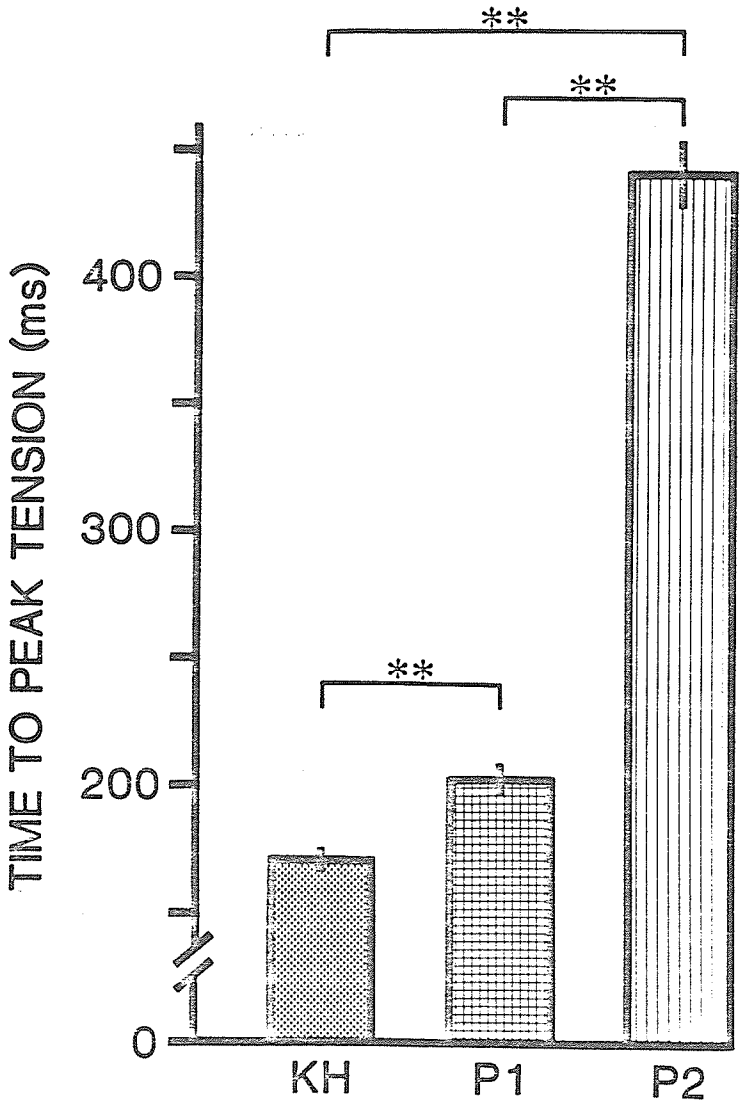
TABLE 1

Some characteristics of action potentials and contractions under control and biphasic conditions. Significance was tested using a paired 2-sided t-test (means + SEM).

	KH		SrKH		
CONTRACTION DURATION	353 ± 12	(28)	706 ± 30	(28)	***
ACTION POTENTIAL DURATION	255 ± 9	(6)	609 ± 43	(6)	***
TIME TO 50% REPOLARIZATION	219 ± 11	(6)	549 ± 33	(6)	***

FIGURE 6

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.


**
**
**
TIME TO PEAK TENSION (ms)
400
300
200
0
KH
P1
P2

in the absence of Ca (fig. 4a), relaxation usually began near the end of phase 3 repolarization. As shown in fig. 4b, 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 showed the same temporal relationship between the action potential and contraction.

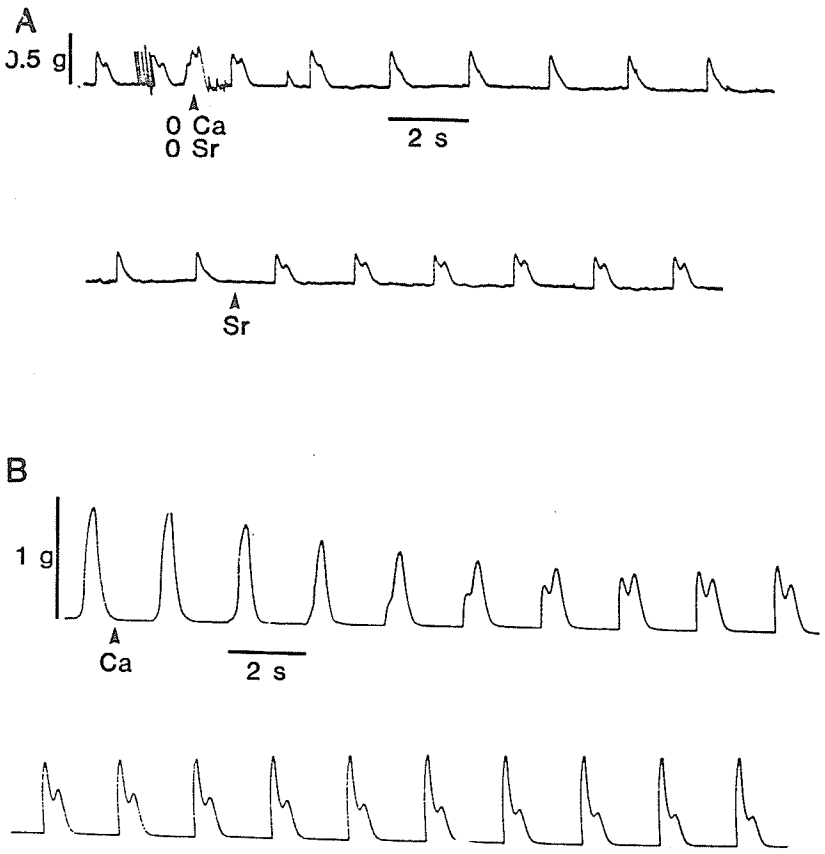
Figure 6 illustrates the time to peak tension of contractions in KH solution and of P1 and P2 in 28 preparations. The time to peak P1 was found to be significantly greater than the time to peak in KH (203 ms and 171 ms respectively). Time to peak P2 (441 ms) was significantly greater than either KH contractions or P1.

3) EFFECTS OF Ca AND Sr ON BIPHASIC CONTRACTIONS

The dependence of P2 on extracellular Sr is shown in fig. 7a. In a trabecula which was bathed in SrKH in the absence of any Ca and was contracting biphasically, P2 was decreased in the second contraction following a wash with 0 Ca, 0 Sr KH solution and eliminated within 5 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

FIGURE 7

Dependence of P1 and P2 on Ca and Sr. A) After the contractions of the muscle had progressed to the biphasic stage in SrKH (as in fig. 3a), 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. B) The tissue was contracting with 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 3 contractions.


A
0.5 g
0 Ca
0 Sr
2 s
Sr
B
1 g
Ca
2 s

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 3 contractions (5 sec). Furthermore, the added Sr did not affect P1. Therefore, it was concluded that P1 is due to a tightly-bound pool of activator cation while P2 is due to the presence of Sr in the extracellular space.

In fig. 7b the effects of adding 1.0 mM Ca to the bath in which a muscle was showing only a P2 contraction in 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 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 the opposite effect of Ca addition (fig. 8). 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). Upon adding 0.4 mM EGTA to a preparation contracting biphasically with 0.1 mM Ca and 2.5 mM Sr, P2 rapidly increased without an

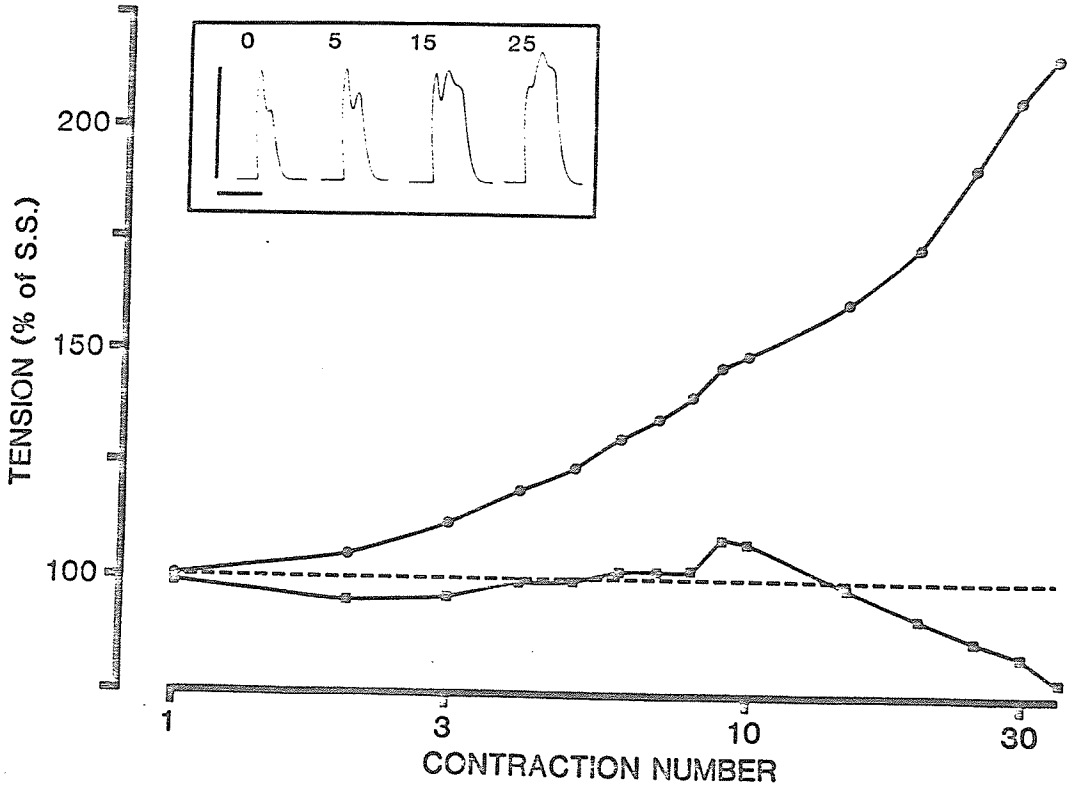


FIGURE 8

Effect of 0.4 mM EGTA on P1 (■——■) and P2 (●——●). The tissue was contracting biphasically with [Ca] = 0.05 mM and [Sr] = 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 (S.S.) 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.

accompanying change in P1 tension. With time, however, P1 declined until it was no longer detectable. The difference in the timing of the P1 and P2 tension changes supports our contention that P1 requires intracellular Ca while P2 requires extracellular Sr.

4) DRUG EFFECTS ON BIPHASIC CONTRACTIONS

The previous section showed that P1 is a Ca-dependent component while P2 is Sr-dependent but allowed no conclusions to be drawn regarding the sites of the activator pools of Ca and Sr. In order to study this problem, we used inhibitors and an activator of both the Ca channel and the sarcoplasmic reticulum.

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. 9) which reached a steady state within 3 to 5 minutes. Although P1 tension also decreased, neither the degree nor the rate of tension change was as great as that of P2 tension (fig. 9; fig. 10). 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

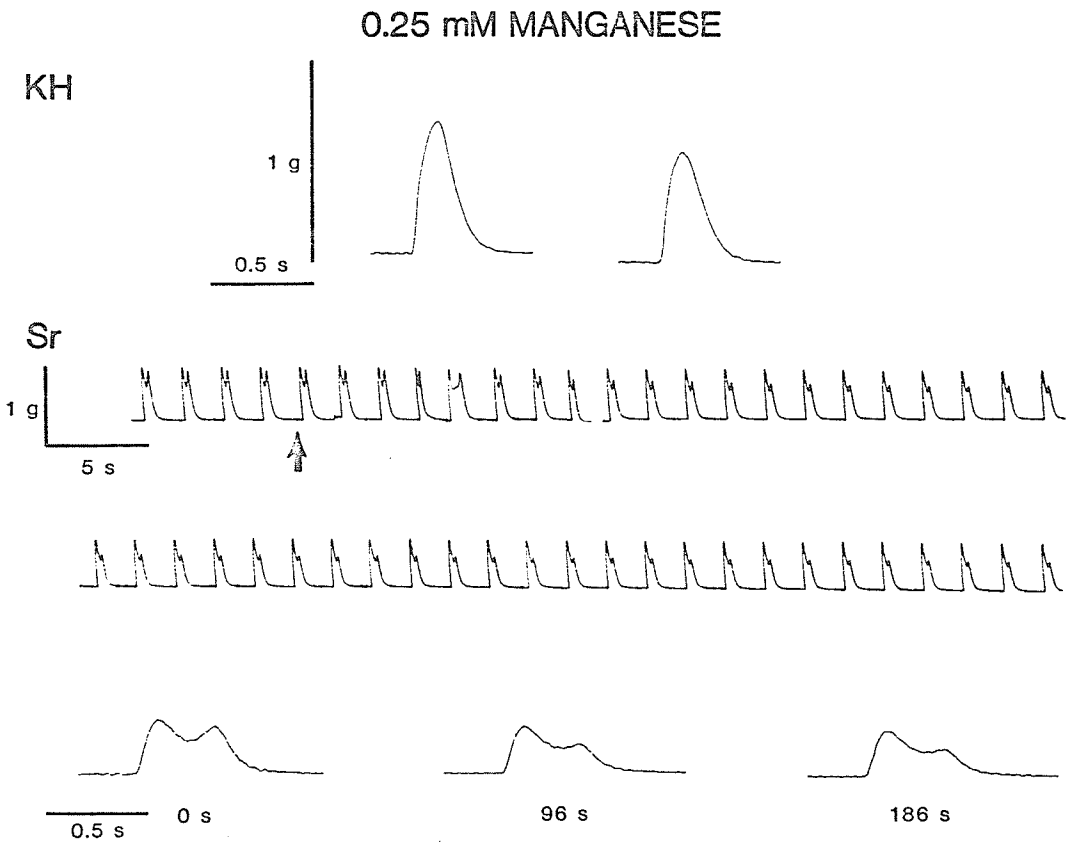


FIGURE 9

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.

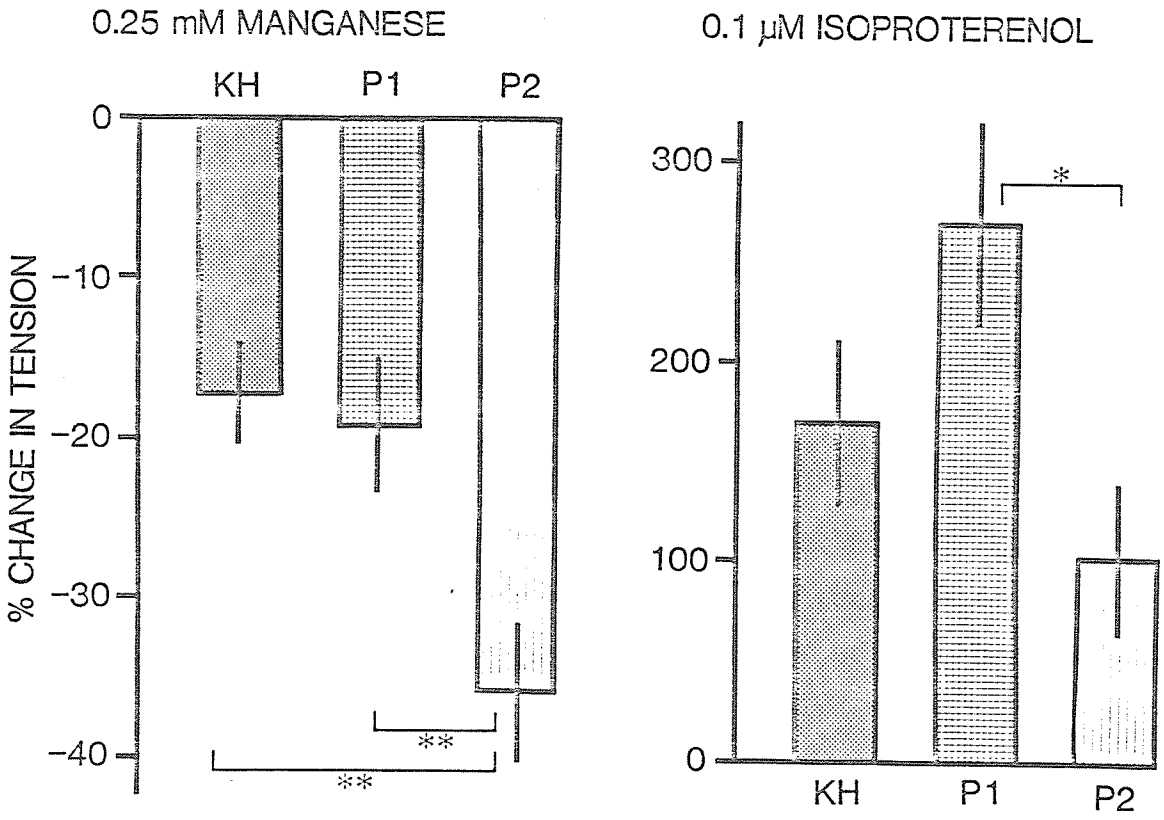


FIGURE 10

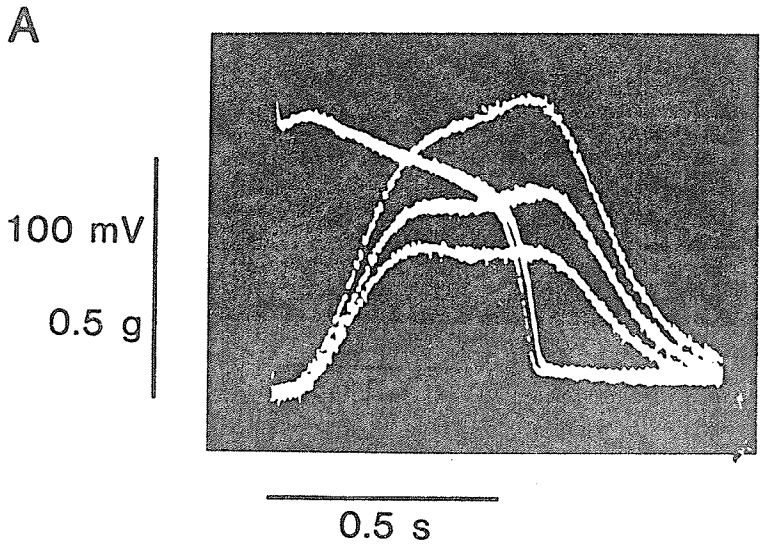
Left: Steady-state changes in tension of control (KH), the early component (P1) and the late component of contraction (P2) produced by 0.25 mM Mn. KH: n = 7. P1 and P2: n = 8. Right: Steady-state changes in tension produced by 0.1 uM isoproterenol. KH: n = 9. P1 and P2: n = 7.

current. Furthermore, the inhibition of P2 was not due to shortening of the action potential since Mn had very little effect on the action potential under biphasic conditions (fig. 11a). In this regard, it is interesting to note that the long action potentials accompanying two component contractions of mammalian heart in the presence of scorpion toxin II are not shortened by 4 mM Mn (Coraboeuf et al., 1975). 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 Sr movement than was P1 tension. Since Mn also blocks Na-Ca exchange (Mullins, 1981), this assumption was not entirely correct but, at the low concentration of Mn used, the conclusion that the slow inward current supplied divalent cation for contraction was considered probable (also, see section II).

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. 12). The rapid increase was probably due to sarcoplasmic reticulum release of Ca while the slower rise in tension may be due to an increased Ca current mediated by an increase in cellular cAMP (Blinks et al., 1972). An immediate increase was seen in P1 but not P2 tension after 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 to 10 minutes (fig. 12 bottom). At steady

FIGURE 11

A) The effect of Mn on action potentials and contractions. The muscle was contracting biphasically in SrKH with $[Ca]_o$ = 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. B) The effect of 10 uM ryanodine on a muscle contracting biphasically with $[Ca]_o$ = 0.1 mM. The ryanodine contraction (higher baseline) showed no P1 while the accompanying action potential had a slightly higher plateau than control.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0.5 g
0.5 s
B
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0.5 g
0.5 s

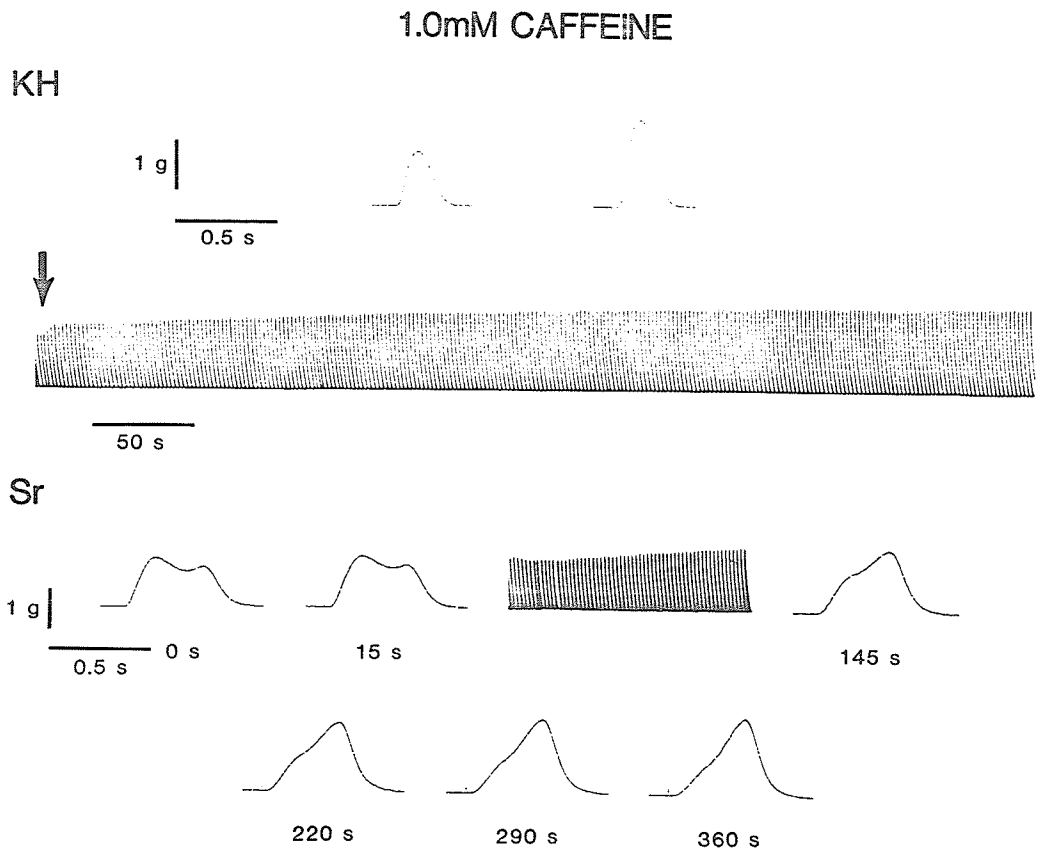


FIGURE 12

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.

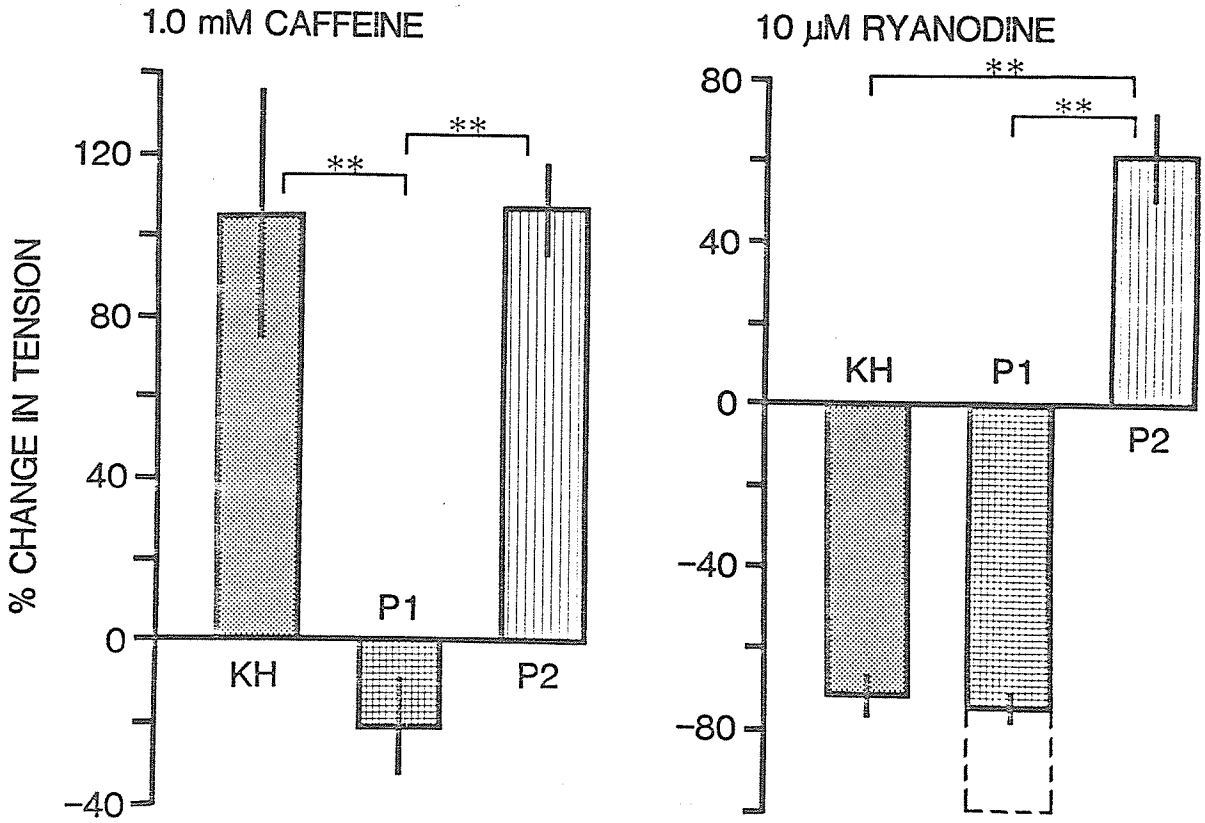
state P1 was visible only as a slight hump on the contraction and, in some cases, was not discernable at all. The actual tension of P1 was not accurately reflected by the hump since no attempt was made to subtract the early rising portion of the P2 contraction from it. The observed tension changes of P1 and P2 are shown in fig. 13.

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 and b) and to inhibit release of Ca from the sarcoplasmic reticulum (Sutko et al., 1979; Jones et al., 1979). Ryanodine, at a concentration of 10 uM, reduced peak tension to about 30% of the pre-drug level in KH solution (fig. 14 top). The inhibition began within one minute of addition of the drug and reached a steady state in 5 to 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 completely eliminate P1 while moderately increasing P2 (fig. 14 bottom; fig. 13). Moreover, these contractile changes occured without any appreciable change of the action potential (fig. 11b).

These results suggested that P1 was due to activation of the myofibrils by Ca released by 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 cur-

FIGURE 13

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 uM 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.


1.0 mM CAFFEINE
% CHANGE IN TENSION
120
80
40
0
-40
**
**
KH
P1
P2
10 µM RYANODINE
80
40
0
-40
-80
**
**
KH
P1
P2

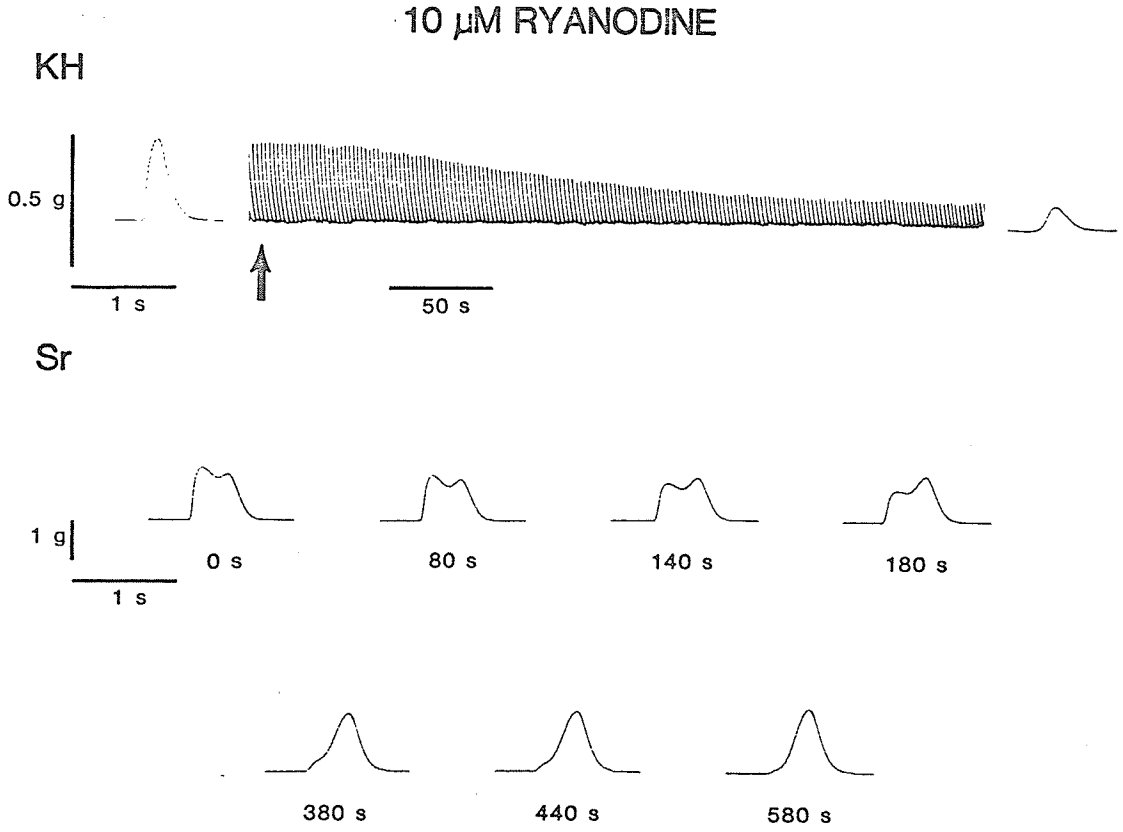


FIGURE 14

Time course of ryanodine action on control (top) and on biphasic (bottom) contractions. Ryanodine (10 uM) was added at the arrow in the control and at 1 s in the Sr biphasic sequence.

rent during the action potential. Hence, isoproterenol was expected to increase the magnitude of both P1 and P2. As shown in fig. 15 (top), the addition of 0.1 uM 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 part 1 of this section), isoproterenol was able to separate P1 and P2 by preferentially incrementing 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. 15, bottom; fig. 10), an effect possibly related to the shortening action on the action potential and contraction durations. Very similar results were obtained with dibutyryl cAMP (1 - 4 mM) but the effects took much longer to develop.

D. DISCUSSION

The present results confirm the findings of Braveny and Sumbera (1972) that biphasic contractions of mammalian ventricular tissue can be maintained by replacing most of the Ca in the bathing solution with Sr. Also in accord with Braveny and Sumbera (1972), we interpret the results as showing that two morphologically distinct pools of activator cation are involved and that biphasic contractions are not merely due to the partial replacement of Ca by Sr in intracellular stores

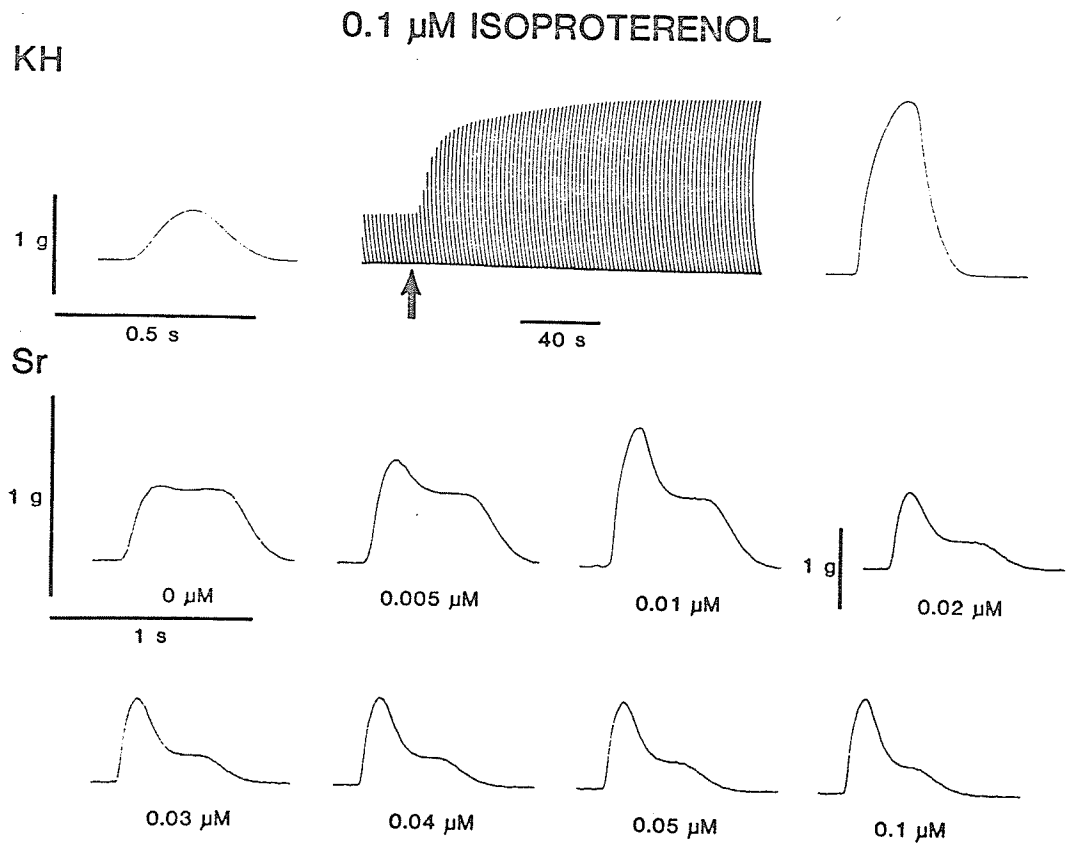


FIGURE 15

Top: Effect of 0.1 uM isoproterenol (at arrow) on control (KH) contractions. Bottom: Dose-response relationship of isoproterenol on biphasic contractions showing the predominent effect on P1.

(Henderson and Cattell, 1976). Our conclusion is supported by the finding that various blocking agents are able to preferentially change one component of the contraction in Sr-induced biphasic contractions (present results), in rested-state contractions (Beresewicz and Reuter, 1977), and in biphasic contractions produced by noradrenaline at low temperatures (Bogdanov et al., 1979).

Site of cation causing P1

The data presented in this report strongly sugggest that P1 is caused by Ca release from a tightly-bound intracellular pool, probably the sarcoplasmic reticulum. That Ca and not Sr is responsible is shown by the progressive loss of P1 when Sr was substituted for Ca and the reappearance of P1 when Ca was added back (figs. 1 and 5). The length of time required to eliminate P1 in SrKH (20-40 min) indicates that the Ca is tightly-bound. Finally, the sarcoplasmic reticulum is the probable site of the pool since P1 is eliminated or greatly decreased by ryanodine and caffeine.

This interpretation of the origin of P1 is supported by a number of investigators using a variety of methods for separating two components of contraction (Braveny and Sumbera, 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 Sumbera, 1972;

Beresewicz and Reuter, 1977; Seibel et al., 1978; Bogdanov et al., 1979; unpublished observations), a pattern attributed to the sarcoplasmic reticulum. Furthermore, the early component can be reduced or abolished by theophylline (Beresewicz and Reuter, 1977) or by caffeine (Bogdanov et al., 1979).

Direct activation by divalent cations entering the cell through the slow inward current as a means of explaining the early component (Coraboeuf, 1974) seems unlikely for a number of reasons. The time to peak tension of a clamp-induced contraction in normal Tyrode's solution is not related to the intensity or duration of the calcium current or to the membrane potential (Beeler and Reuter, 1970a and b). If the slow inward current were the cause of the early component of contraction then an increase in the duration of the current by clamping at a higher potential would be expected to prolong the time to peak. Furthermore, the time to peak tension of the early component is independent of the action potential or voltage clamp duration, except at very short durations (Morad and Trautwein, 1968; Braveny and Sumbera, 1970; Ochi and Trautwein, 1971; Coraboeuf et al., 1975). Finally, verapamil (Bogdanov et al., 1979) and manganese ion (this study), slow inward current blocking agents, have a relatively small effect on the early component of contraction.

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 a number of preparations, in fact, 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, three components may actually be involved with the second "phasic" component (P2) being masked by a large "tonic" component (P3) under voltage clamp conditions. The late component or "tonic" phase would then be a composite of our P2 and P3 components with P2 being much smaller in Sr-free solution.

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 (1971) demonstrated that Sr could greatly prolong the current in cow Purkinje fibres 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 fibres after Sr replacement of Ca (Kohlhardt et al., 1973 a and b). Since Sr is equipotent to Ca in activating the 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.

Seibel et al. (1978) discarded the possibility that the late component of contraction could be caused by direct activation by extracellular cation on the basis that the slow inward current is inactivated well before the onset of the late component. However, it has been shown that intracellular Ca levels, as indicated by aequorin luminescence, reach a maximum and start to decline to baseline values before the onset of contraction (Wier, 1980). Considering that the distance Ca entering the cell through the slow inward current must diffuse to reach the contractile proteins must be greater than the distance travelled by sarcoplasmic reticulum Ca and that there is normally a considerable delay between an increase of intracellular Ca and the onset of contraction, it can be seen that the late component probably is caused by extracellular Ca.

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 Na-Ca exchange (Horackova and Vassort, 1976; Mullins, 1981), two processes which would be capable of driving Ca into the cell as the electrochemical gradient for K or Na increased.

Our model of myocardial contractions predict that, in contractions

elicited in normal, Ca-containing media, approximately 70% of the total tension is attributable to sarcoplasmic reticulum release of Ca, as shown by the loss of about 70% of tension in the presence of ryanodine (Penefsky, 1974a; present results). Due to its relatively rapid time course, this component would also determine the maximum rate of tension development. The remaining 30% of tension is due to the total of the P2 and P3 components and would add to the duration of contraction.

On the basis of its ability to distinguish between cation released from the sarcoplasmic reticulum and that entering through voltage-sensitive cation channels, this experimental model should prove useful for probing the mechanism of action of cardioactive drugs. An added benefit of this model is the ease with which it can be produced and the "normal" driving rates at which the actions can be studies.

II. COMPARATIVE EFFECTS OF MANGANESE AND NICKEL ON THE DELAYED COMPONENT OF CONTRACTION

COMPARATIVE EFFECTS OF MANGANESE AND NICKEL ON THE DELAYED COMPONENT OF CONTRACTION

A. INTRODUCTION

In part I of the Results, identification of P2 as direct activation of the contractile apparatus by Sr entering the cell through the slow channel depended primarily on the action of Mn ion. Voltage clamp studies have shown that Mn blocks the slow inward current in frog myocardium (Vassort and Rougier, 1972), mammalian myocardium (Ochi, 1970; Kohlhardt et al., 1973a), and Purkinje fibres (Vitek and Trautwein, 1971; Kass and Tsien, 1975). Furthermore, the slow response (a Ca-mediated action potential in depolarized cells) is blocked by Mn (Pappano, 1970; Kass and Tsien, 1975). Unfortunately, the ion is not a specific slow channel blocker since it can carry some of the slow inward current, at least at high concentrations (Ochi, 1970). Low concentrations of Mn can actually produce a positive inotropic effect after entering the cell (Vierling and Reiter, 1979), compete with Ca for sarcoplasmic reticulum uptake sites (Chiesi and Inesi, 1981), and modify adenylcyclase activity (Wiemer et al., 1978). Most importantly for this discussion, Mn can block Na-Ca exchange (Roulet et al., 1979; Mullins, 1981).

To confirm the site of origin of P2 cation suggested by Mn, it was desirable to test another blocker of the slow inward current. Nickel ion (Ni) has been demonstrated to block the slow channel in mammalian ventricle (Kohlhardt et al., 1973a; 1979) but is less potent than Mn

in barnacle muscle (Hagiwara and Takahashi, 1967). A series of experiments were conducted using Ni as the slow inward current blocker.

B. METHODS

The methods have been described in the Mechanical Experiments section of the General Methods. The stimulation frequency was 0.5 Hz.

C. RESULTS

Addition of Ni, as the chloride salt, to KH solution bathing the trabeculae resulted in a dose-dependent negative inotropic effect (fig. 16). Twitch tension declined more slowly than in Mn-containing solution. Significantly, Ni was able to produce a greater depression of tension development than was Mn in the concentration range studied (0.25 to 1.0 mM; fig. 16). Although the same negative inotropic effect was seen with 0.25 mM of either ion, increasing concentrations of Mn produced a relatively small change in tension compared to Ni. At a Ni concentration of 1 mM, twitch tension was 45% of control ($n = 3$), virtually identical to the decrease found by Bass (1976) in calf and sheep ventricular preparations with the same amount of Ni.

The results obtained by adding Ni to trabeculae contracting biphasically in SrKH were somewhat unexpected. At a Ni concentration of 0.25 mM, P1 and P2 were reduced by equal amounts. The negative inotropic effect on both phases of contraction was greater than on control contractions, although not significantly so (fig. 17a). This is

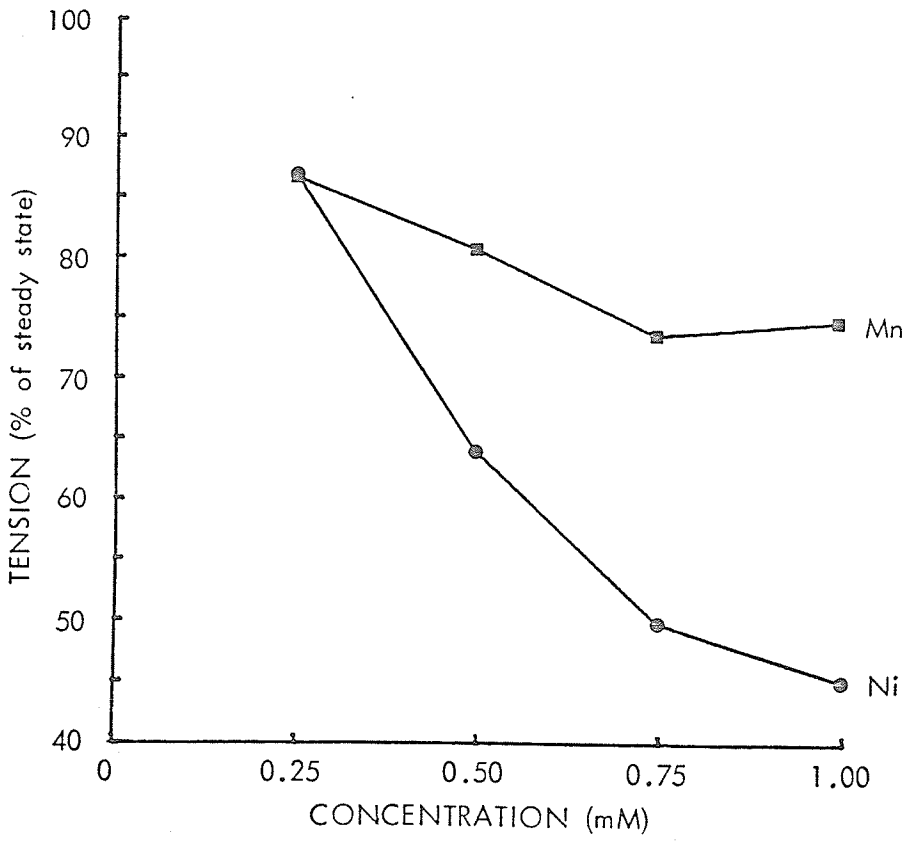


FIGURE 16

Dose-response relationship of Mn and Ni in KH solution. Steady state refers to the tension produced before adding the ions. Each point represents the mean of tissues from 3 dogs. The negative inotropic effect of Mn and Ni was identical at 0.25 mM but not at higher concentrations (see text).

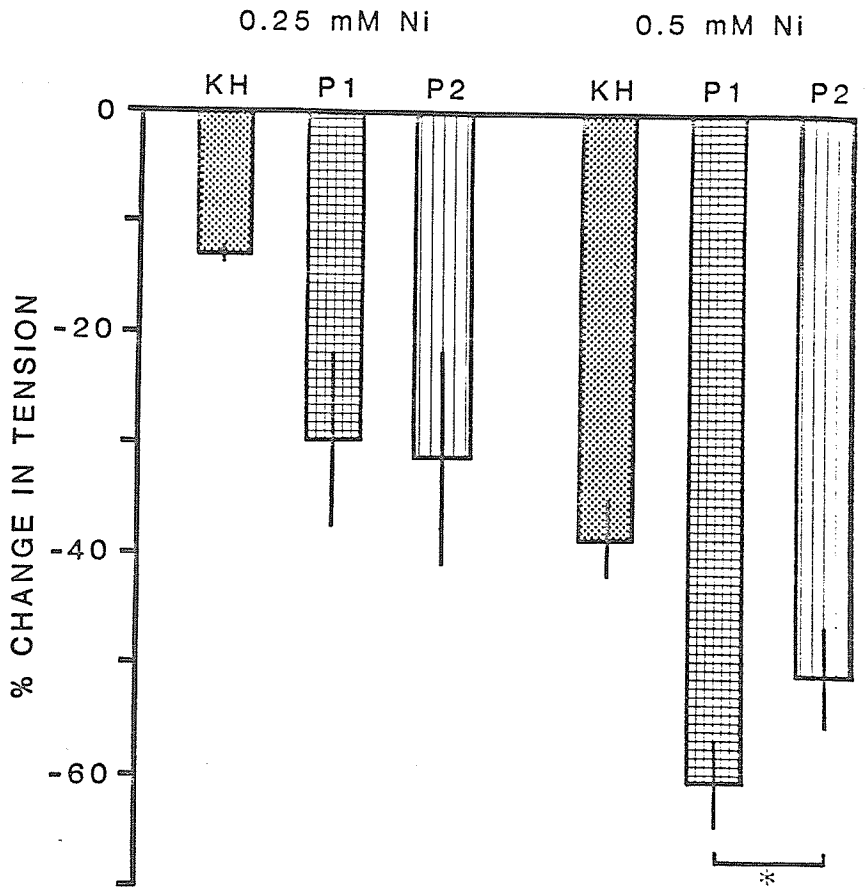


FIGURE 17

Dose-dependent depression of P1 and P2 by Ni. Both P1 and P2 were decreased significantly more than contractions in KH solution at both concentrations of Ni (as determined by ANOVA and Duncan's test). Most importantly, P1 was depressed significantly more than P2 at a NI concentration of 0.5 mM (2-sided paired t-test). For KH, n = 4 while for P1 and P2, n = 6.

consistent with the findings that Ni inhibition is of a competitive nature, varying with the inverse of the Ca concentration (Kohlhardt et al., 1979), and that the relative order of affinity, at least for the slow channel, is Mn > Ni > Ca > Sr (Hagiwara and Byerly, 1981). Therefore, under conditions where the Ca concentration is low and the slow inward current is carried predominantly by Sr, Ni should have a relatively greater blocking effect. The same reasoning applies to Mn inhibition.

When the Ni concentration was increased to 0.5 mM, P1 was reduced to a greater extent than P2 ($P < 0.05$; fig. 17b). This is in direct contrast to the results found with 0.25 mM Mn (section I, figs. 9 and 10) where P2 was decreased significantly more than P1. Of course, at least part of the decline of P1 may be explained as a reduction sarcoplasmic reticulum Ca caused by lower refilling of the pool due to a smaller electrogenic Ca influx in the presence of Ni.

D. DISCUSSION

The results have shown that at 0.25 mM, Mn and Ni are equipotent in inhibiting contraction in KH solution but at higher concentrations Ni is more effective. Since the blocking effect of Mn is greater than that of Ni on the slow inward current of barnacle muscle (Hagiwara and Takahashi, 1967; Hagiwara and Byerly, 1981) and about the same as Ni on the current of cat ventricular trabeculae (Kohlhardt et al., 1973a), the larger effect of Ni on contraction indicates that another, possibly intracellular, action of Ni exists. This is supported by the

Sr-induced biphasic contraction experiments in which a significant reduction of P1 was seen. It was previously shown (section I) that P1 is due to intracellular release of Ca, most likely from the sarcoplasmic reticulum. Therefore, it is suggested that Ni directly lowers the amount of Ca released from that pool. This effect of Ni is presumably additive to the decrease in the pool size expected as a consequence of blocking Ca entry through the slow channel.

Although there is a paucity of data published on the effects of Ni, some evidence for an action of the ion on the sarcoplasmic reticulum does exist. Frank (1962) noted that replacing Ca with Ni in the solution bathing frog toe muscle resulted in the same or increased contracture tension in response to KCl depolarization. Thus, Ni is able to release Ca from the sarcoplasmic reticulum, at least in skeletal muscle. A more significant finding is that the microsomal Ni content of kitten hearts perfused with Ni-containing KH solution is more than 65% of the Ca content and that the washout kinetics of Ca and Ni in that tissue are very similar (Ong, 1972; Ong and Bailey, 1973).

The primary aim of the Ni experiments was to confirm the origin of P2 cation by using a slow channel blocker other than Mn. The two possibilities considered most likely for the origin of P2 cation are the slow inward current and Na-Sr exchange, both involving the translocation of extracellular Sr. The problem is that all Ca channel blockers, inorganic or organic, also block Na-Ca exchange (Mullins, 1981). However, Chapman and Ellis (1977) showed that, at equivalent concentrations of Ni or Mn, Ni has a greater effect on the early, "phasic" component of Na-withdrawal contractures in frog heart. Since such

contractures are a result of Na-Ca exchange (Chapman and Ellis, 1977; Chapman, 1979), it follows that Ni is a more potent blocker of the mechanism than is Mn. Consequently, if P2 tension is a result of Na-Sr exchange, one might expect Ni to produce a greater P2 depression than the same concentration of Mn. This was not seen (compare fig. 10 to fig. 17a). The late, "tonic" phase of the Na-withdrawal contracture, also a manifestation of Na-Ca exchange in frog heart, is actually increased at Ni concentrations up to 8 mM (Chapman and Ellis, 1977). Again, P2 was not increased by Ni (fig. 17a and b). These results do not conclusively eliminate the chance that P2 is caused by Na-Sr exchange but they certainly do not support it. On the other hand, the actions of Ni and Mn are both consistent with P2 being the result of an enhanced slow inward current carried by Sr.

III. CONTRIBUTION OF Na-Sr EXCHANGE TO THE LATE CONTRACTION COMPONENT: EFFECTS OF OUABAIN AND TETRODOTOXIN

CONTRIBUTION OF Na-Sr EXCHANGE TO THE LATE CONTRACTION COMPONENT: EFFECTS OF OUABAIN AND TETRODOTOXIN

A. INTRODUCTION

The previous sections showed that at least part of the P2 component of contraction is caused by cation entering the cell by way of the slow inward current. An additional contribution to P2 could hypothetically be made by the same process which produces P3, the "tonic" phase of contraction. As discussed in the General Introduction and in the preceeding sections, the tonic component is probably the result of "reverse" electrogenic Na-divalent cation exchange which increases the intracellular Ca (or Sr) concentration at the expense of intracellular Na.

The magnitude of Na-Ca exchange is a function of the intracellular and extracellular concentrations of Na and Ca (e.g. Chapman, 1979; Mullins, 1981). In the case of direct contractile activation secondary to Na-Sr exchange, it seems reasonable to assume that the magnitude of the "reverse" exchange is related to the concentration gradients of Na and Sr at the time of the action potential plateau. Changing the gradient of either ion would thus be expected to change the size of the P3 component and also of P2, if P2 has a significant amount of Na-Sr exchange contributing to tension or if P2 and P3 temporally overlap. Henderson and Cattell (1976) showed that increasing the extracellular Sr concentration in the solution bathing cat papillary muscles augmented both the P2 and P3 components. However, P2 should

be increased under these conditions even if it is caused solely by the slow inward current (Reuter, 1967; Beeler and Reuter, 1970b; Reuter and Scholz, 1977a). Lowering extracellular Na not only increases divalent cation entry by Na-divalent cation exchange (Reuter and Seitz, 1968; c.f. Chapman, 1979) but also decreases the slow inward current (Reuter and Scholz, 1977 but see Reuter, 1967). Therefore, alteration of extracellular Na or Sr concentrations would provide only ambiguous results concerning the influence of Na-Sr exchange on P2.

Changing the intracellular Na concentration affects the magnitude of Na-Ca exchange (Glitsch et al., 1970) and might be expected to have more effect on the exchange than on slow inward current divalent cation. It is well-established that Na-K ATPase inhibition by cardiac glycosides increases the intracellular Na and Ca concentrations (Langer, 1973; Langer, 1977; Sheu, 1981). Tetrodotoxin (TTX), on the other hand, abolishes much of the slow entry of Na into cardiac cells during the action potential plateau (Attwell et al., 1979; Coraboeuf et al., 1979). Ouabain and TTX should therefore exert opposite effects on Sr entry by way of Na-Sr exchange during the action potential plateau. The following experiments tested the effects of ouabain and TTX on biphasic contractions and, in particular, on P2.

B. METHODS

Tissues were prepared as described in the General Methods section. In both the ouabain and the TTX experiments, the basic stimulation rate was 0.5 Hz.

C. RESULTS

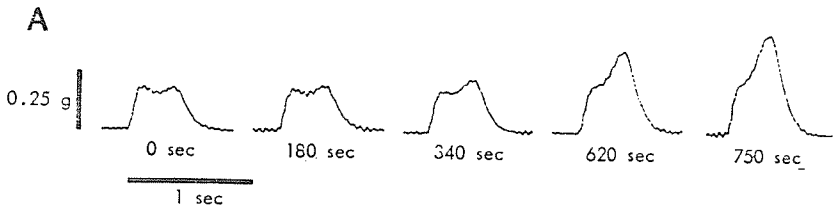
Ouabain, in concentrations from 0.1 to 1.0 uM, was tested on 8 tissues contracting biphasically in SrKH. In contrast to the immediate positive inotropic response produced by isoproterenol, maximum tension increased very slowly after ouabain addition. The most important observation was that the positive inotropic effect was due almost entirely to an increased P2 (fig. 18a). P2 increased linearly during the entire 10 min exposure to ouabain (fig. 18b and c). Furthermore, the change in the contraction pattern was unaffected by the presence of 10 uM sotalol, indicating that ouabain-induced catecholamine release was unimportant in contributing to the inotropy (fig. 18c).

In addition to increasing P2, ouabain caused a progressive loss of the transient relaxation between P1 and P2 in 4 experiments. No conclusions regarding the effects of ouabain on P1 and P2 could be drawn from these tissues since it was impossible to distinguish one component from the other. The peak of these contractions was generally rounded and prolonged, similar to that seen in some tissues in the absence of ouabain (see section I).

In 3 experiments using TTX in concentrations from 5 uM to 20 uM, P2 tension declined in a dose-dependent manner while the efffect on P1 was variable. Figure 19a shows 0.5 Hz contractions with 0, 5, and 10 uM TTX. Although contraction duration increased only very slightly, the separation between P1 and P2 was more pronounced after TTX. In this experiment P1 was depressed somewhat. A clearer effect of TTX on P2 is shown in fig. 19b. A marked dose-dependent inhibition of P2

FIGURE 18

Effects of ouabain on biphasic contractions. A. Changes in the contraction waveform with time in the presence of 0.5 uM ouabain. B. Positive inotropic effect of 0.1 uM ouabain on a trabecula without sotalol in the solution. C. Positive inotropic effect of 0.5 uM ouabain in the presence of 10 uM sotalol. In both B and C, the x axis is the exposure time to ouabain. Comparing B and C shows that endogenous catecholamine release has little effect on the relative positive inotropic effect of ouabain on P1 and P2.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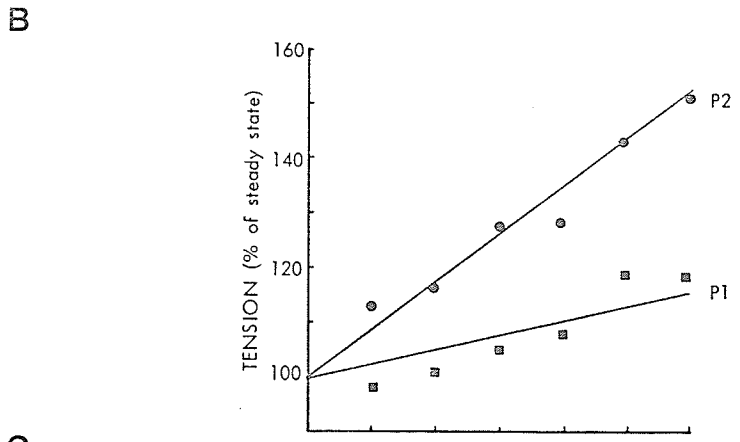
0.25 g
0 sec
180 sec
340 sec
620 sec
750 sec
1 sec


B
160
140
120
100
TENSION (% of steady state)
P2
P1

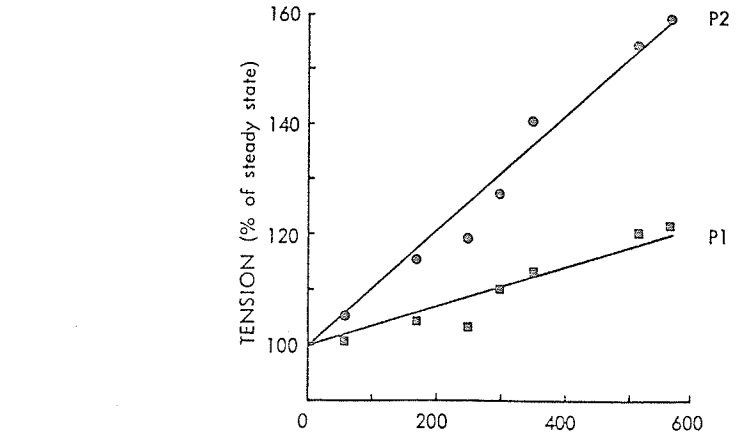
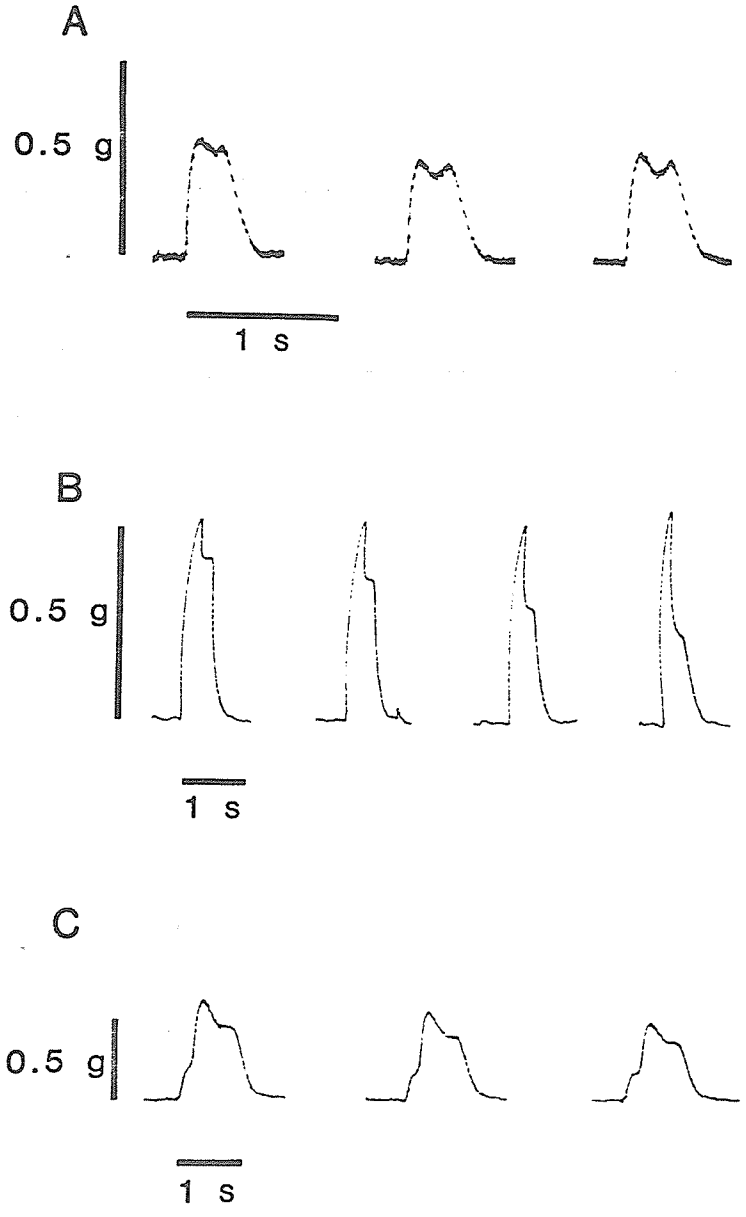

C
160
140
120
100
TENSION (% of steady state)
P2
P1
0
200
400
600

FIGURE 19

Reduction of P2 by tetrodotoxin (TTX). A. Biphasic contractions with 0, 5, and 10 uM TTX showing an increased separation between P1 and P2. The stimulating frequency was 0.5 Hz. B. Effect of 0, 5, 10, and 20 uM TTX on P2 at a stimulating frequency of 0.5 Hz. C. Effect of 0, 5, and 10 uM TTX on a tissue contracting at 0.2 Hz and showing a pronounced P3 component. TTX increased the separation of P1 and P2 and depressed P2 to a greater extent than P3 (see text for explanation).


A
0.5 g
1 s
B
0.5 g
1 s
C
0.5 g
1 s

was accompanied by a minor increase of P1 at 20 uM TTX. The contractions illustrated in fig. 19c were elicited at a frequency of 0.2 Hz. P3 was very prominent at this driving rate (discussed further in section V). P1 in the contractions is seen as a "hump" on the rising portion, P2 is the large, phasic tension, and P3 is the plateau preceeding final relaxation. Increasing concentrations of TTX under these conditions resulted in better separation between P1 and P2, as in fig. 19b, and a dose-dependent depression of P2 and P3. It is interesting to note that the inhibition of P2 was greater than that of P3.

D. DISCUSSION

The results with ouabain and TTX suggest that some fraction of P2 tension is generated by divalent cation derived from an exchange with Na. In addition, the very small effect of either agent on P1 indicates that: 1) Na-Sr exchange contributes very little to P1 tension, and 2) neither ouabain nor TTX has a significant effect on sarcoplasmic reticulum Ca release.

The loss of separation between P1 and P2 frequently seen in the presence of ouabain could be explained either as an inhibition of sarcoplasmic reticulum uptake of Ca or as the mechanical manifestation of a quicker onset of Na-Sr exchange. Since TTX increased separation of P1 and P2, the latter mechanism seems more likely. Published reports also show that cardiac glycosides have no effect on sarcoplasmic reticulum Ca uptake (c.f. Lee and Klaus, 1971).

It is possible that the increased P2 produced by ouabain is the result of an increased slow inward current flow of Sr. However, voltage clamp data have shown that digitalis glycosides do not affect the slow inward current in cat ventricle (McDonald et al., 1975) or in frog ventricle (Greenspan and Morad, 1975).

Coraboeuf et al. (1979) found that TTX shortens the action potential duration to a far greater extent in Purkinje fibres than in ventricular muscle and suggested that the TTX-sensitive plateau Na conductance contributes very little to ventricular action potentials. One might then expect that, in the canine ventricular trabeculae used in this study, TTX would result in only a very small decrease in intracellular Na during the action potential plateau. However, since Purkinje fibres have a much longer action potential duration than ventricular muscle (Coraboeuf et al., 1979), it is possible that the Sr-induced prolongation of the action potential is able to increase the TTX-sensitive plateau current. The latter current probably does not contribute significantly to the duration of the action potential under these conditions, as evidenced by the lack of shortening of the contraction duration (recall that contraction duration mirrors action potential duration; see section I). Since TTX produced a greater depression of P2 than P3 (fig. 19c), it might by hypothesized that TTX-sensitive Na influx continues for only a part of the long action potential plateau. The increased Na concentration in the vicinity of the sarcolemma would then be able to trigger a TTX-sensitive increase in Na-Sr exchange.

In conclusion, the results have shown that P2 of biphasic contrac-

tions in Sr-containing solution is due not only to Sr derived from the slow inward current but also to the overlap of P3 with P2. Alterations in either the slow inward current or in Na-Sr exchange will then yield a change in P2.

IV. ON THE MECHANISM OF PROLONGED ACTION POTENTIALS IN Sr-CONTAINING SOLUTION

ON THE MECHANISM OF PROLONGED ACTION POTENTIALS IN Sr-CONTAINING SOLUTION

A. INTRODUCTION

Tetraethylammonium chloride (TEA) is a chemical which has been shown to block K conductance channels in nerve and skeletal muscle (Armstrong, 1975) and in cardiac Purkinje fibres (Haldimann, 1963; Kenyon and Gibbons, 1979; Reder et al., 1981). In adult Purkinje fibres, TEA reduces phase 1 repolarization by a blocking action on Iqr while final phase 3 repolarization is delayed, probably due to an action on the plateau K current, I_{X1}. Adult canine Purkinje fibres treated with 20 mM TEA have an action potential duration 18% greater than control (Reder et al., 1981).

It was expected that if the Sr-induced prolongation of the action potential was caused only by an increased duration of slow inward current without a change in outward conductances then TEA should be able to further delay repolarization. If, on the other hand, Sr was blocking K conductance channels, a variable effect on the duration would be expected.

B. METHODS

The General Methods section describes the electrophysiological techniques used. No osmotic adjustments were made to the solutions.

C. RESULTS

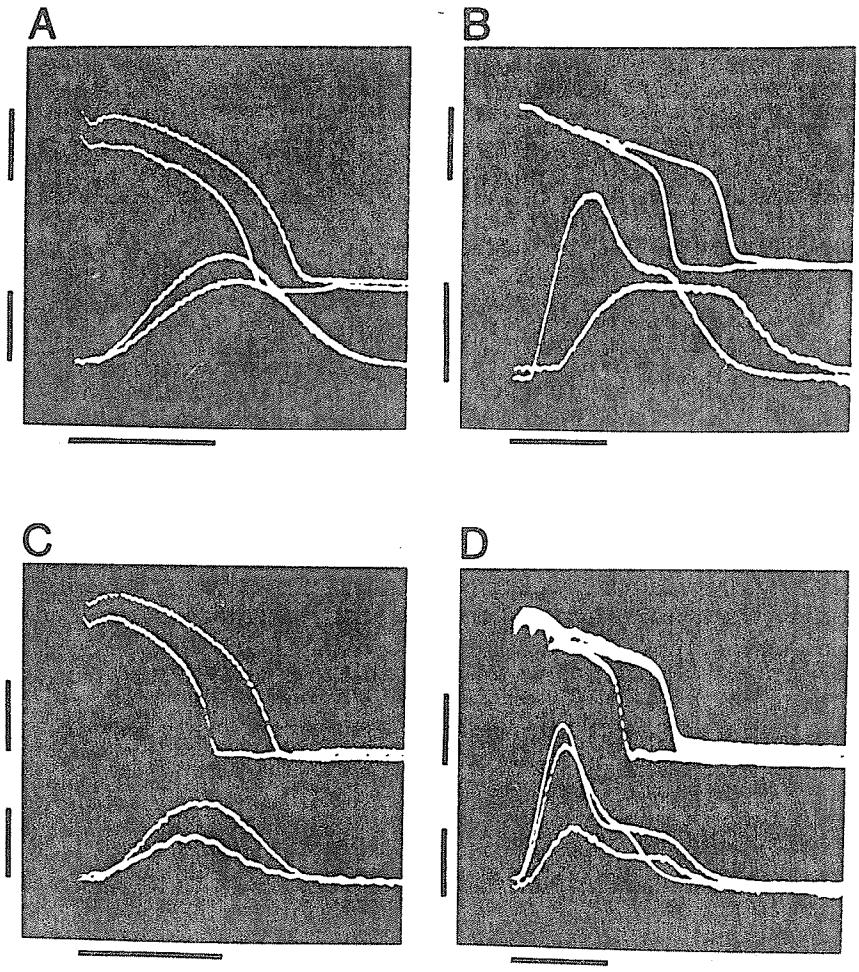
Three electrophysiology experiments were carried out on canine ventricular trabeculae using 20 mM TEA. In KH solution, the drug caused the following membrane effects: 1) a slight diastolic depolarization, 2) an increased overshoot of the action potential, 3) a markedly higher plateau potential, and 4) a pronounced increase in the action potential duration (fig. 20a). These changes were not related to TEA-induced catecholamine release since similar changes were seen in the presence of 1 uM sotalol, a B adrenergic blocker (fig. 20c).

Concurrent with the action potential changes, peak tension in KH solution was increased dramatically in the absence or presence of sotalol (fig. 20a and c). The time to peak tension was increased in the experiment where sotalol was included but not in the experiment without sotalol. The lack of change of this parameter in the latter experiment may be related to the release of endogenous catecholamines by TEA.

When the tissues were contracting biphasically in SrKH, the addition of 20 mM TEA markedly shortened the action potential duration, contrary to its effects in KH solution. In fact, TEA produced the reverse of all 4 electrical effects seen in KH solution (fig. 20b and d). Similar changes were seen in the absence (fig. 20b) or presence (fig. 20d) of 1 uM sotalol. The accompanying contraction had a greatly increased P1 component and a smaller increase in the P2 component. The large tension response made it difficult to avoid mechanical noise on the action potential tracing (fig. 20d). As expected

FIGURE 20

Effects of tetraethylammonium chloride (TEA) on action potentials and contractions. A and C were taken from two different preparations contracting at 0.5 Hz in KH solution while B and D are from the same tissues contracting biphasically in SrKH. A and C: 20 mM TEA increased the plateau height and duration of the action potentials and had a positive inotropic effect in KH solution. B and D: 20 mM TEA shortened the action potential duration while slightly lowering the plateau height. The positive inotropic effect was seen mainly in P1. In D, the initial effect of TEA was to increase P1 without affecting the action potential (middle tension trace). With time, the action potential duration was markedly shortened. The "noise" on the action potential plateau is an artifact of the extremely strong P1 contractions in the presence of TEA. In all cases, the voltage calibration is 50 mV. Tension calibration: A, 1 g (control) and 2 g (TEA); B, 0.5 g (control) and 1 g (TEA); C, 1 g (control) and 2 g (TEA); D, 0.5 g (control) and 1 g (TEA). Time calibration: A and C, 250 msec; B and D, 500 msec.


A
B
C
D

for the more brief action potential, the contraction duration was also reduced. One interesting observation is that TEA shortened the time to onset of contraction, especially in the experiment without sotalol. It might be speculated that TEA had sensitized the sarcoplasmic reticulum to release its Ca store faster.

Since no osmotic adjustments were made to the solutions, it was possible that the observed effects of 20 mM TEA chloride (40 meq total osmotic increase) were strictly due to the hypertonic solution. In one mechanical experiment, raising the osmolarity with 40 mM sucrose had little effect except a decrease in P1 tension in the presence of 0.1 uM isoproterenol. It has also been shown that hypertonic sucrose solutions (2 times isotonic) result in a dramatic shortening of the action potential, loss of the plateau, and a slight hyperpolarization in cat and guinea pig ventricle (Hermsmeyer et al., 1972). Tension declined concurrently. Thus, if the TEA effects were osmotic in nature one would expect changes in the opposite direction to that seen in KH solution. Interestingly, Hermsmeyer et al. (1972) noted that the electrical and mechanical effects of hypertonicity were reversed by 5 mM Sr.

D. DISCUSSION

The response of the trabeculae to TEA in KH solution points to a blockade of K conductance channels, as previously shown in other preparations (Haldimann, 1963; Armstrong, 1975; Kenyon and Gibbons, 1979; Reder et al., 1981). The increased plateau potential is then

due to an inward current that is less masked by K-mediated outward current while repolarization is delayed by blockade of the repolarizing K current. Reder et al. (1981) found the same action potential effects of TEA in dog Purkinje fibres except that the overshoot was unchanged after the tissues were perfused with 20 mM TEA for 5-10 min. Kenyon and Gibbons (1979), using the same concentration of TEA on sheep Purkinje fibres, noticed an increased overshoot but only after 2.5 hours of perfusion. Furthermore, they commented that "the first effects of TEA were seen within 15 min, and a full effect developed over several hours." The changes seen in fig. 20a and c were after about 5 to 10 min in TEA-containing solution, indicating that the response to TEA is different in Purkinje fibres from different species as well as in conducting and contracting tissue from the same species.

Since TEA produced the opposite action potential effects on tissues in SrKH solution, it must be concluded that either Sr is a more complete blocker of K conductance than is TEA or that TEA blocks the slow inward current. In this regard, Inomata and Kao (1979) showed that replacing Ca with Sr in a solution in which Na was replaced by TEA produced a further increase in the action potential duration of guinea pig taenia coli. Competition between Sr and TEA for the same regulatory site on the K channels, with TEA being a partial agonist, would account for the observations. Furthermore, the pronounced positive inotropic effect of the drug on P1 points to an additional effect on the sarcoplasmic reticulum. It also is of interest to note that a positive inotropic effect was seen after TEA in SrKH in spite of the shortening of the action potential duration and slight reduction of

the plateau height. Although the increase in P2 was small, the phenomenon is still remarkable since the duration of the action potential (which regulates P2) was actually decreased. Virtually all positive inotropic agents either increase the plateau potential or the action potential duration.

It is concluded that at least part of the action potential prolonging effect of Sr is due to an inhibition of outward K conductance.

V. STUDIES ON THE FORCE-INTERVAL RELATIONSHIP USING BIPHASIC CONTRACTIONS

V. STUDIES ON THE FORCE-INTERVAL RELATIONSHIP USING BIPHASIC CONTRACTIONS

A. INTRODUCTION

Mammalian ventricular myocardium responds to alterations in the rate or pattern of stimulation in a complex manner. Increasing the frequency of stimulation results in an increase in developed tension over a species-dependent frequency range. Depending on the initial driving rate, the first contraction at the higher frequency may be less than, equal to, or greater than the preceeding contraction (Koch-Weser and Blinks, 1963). Reducing the driving rate of the tissue from a high frequency produces an initial potentiation which declines to a new steady state after eight to ten contractions while no potentiation is observed when the initial driving rate is also low (e.g. 0.05 Hz). Introduction of an extrasystole also produces characteristic tension changes in both the extrasystolic and post-extrasystolic contractions, again depending on the basic frequency of stimulation. Finally, interrupting the stimuli for up to 2 minutes results in a rest-potentiation in ventricular preparations from some species (cat, c.f. Koch-Weser and Blinks, 1963; dog, Endoh and Iijima, 1981) but not in others (rabbit, Edman and Johannsson, 1976).

A model of excitation-contraction coupling which would account for the force-interval relationship involves a recycling of activator Ca from an uptake site on the sarcoplasmic reticulum to a release site

during the interval between contractions (e.g. Wood et al., 1969; Morad and Goldman, 1973; Allen et al., 1976; Edman and Johannsson, 1976). The size of the release pool of Ca at the moment of onset of contraction is thought to be a function of time since the last release of Ca and the total cellular Ca content. The latter determinant is altered by the magnitude of Ca influx during the preceeding action potentials. An excellent review has been provided by Fozzard (1977).

The contribution of the Ca influx across the sarcolemma during an action potential to the total contractile response is the subject of some controversy, however, with estimates ranging from 0% to 50%. It would therefore be desirable to measure the changes in the contributions of sarcoplasmic reticular and extracellular cation to contraction during short-term contractility alterations induced by invoking the force-interval relationship.

Recently, we have produced evidence that Sr-induced biphasic contractions occur as a result of sarcoplasmic reticulum release of Ca followed by trans-sarcolemmal Sr (and, presumably, some Ca) movement leading to contractile activation (King and Bose, 1981). The present study was undertaken in order to determine if biphasic contractions are able to provide some insight into the cation pools involved in the force-interval relationship.

B. METHODS

The protocol for preparing the tissues and the equipment used have been described in detail in the General Methods section. Constant

frequency stimulation was performed at 1.0, 0.5, 0.2, and 0.1 Hz under conditions necessary for producing biphasic contractions (see Results part 1). The basic driving rate of 0.5 Hz was chosen since that is the highest frequency at which the two components of contraction were separated in most preparations (see section I).

For introduction of an extrasystole, a train of 10 stimuli was followed by a variable test pulse interval with the test pulse being the first contraction of the next train. The stimulator was programmed to increment the test pulse interval by 0.2 sec after each train. Thus, the first train of 10 contractions was separated from the second train by 0.2 sec (10% of the basic interval), the second train was separated from the third train by 0.4 sec (20% of the basic interval) and so on until the test pulse interval was 2.0 sec. The result is a constant interval between the test pulse (the extrasystole) and the post-extrasystolic contraction. Due to the long duration of the action potential accompanying a biphasic contraction, the test pulse did not produce a response in the tissues until the test pulse interval was at least 0.6 sec in most cases.

To produce rest periods of varying durations, the stimulator was programmed to give a train of 40 contractions at 0.5 Hz separated by a test pulse interval incremented between 2 and 17 sec in steps of 1 sec. The first contraction of each train will be termed the post-rest contraction. A train duration of 40 was chosen since peak tension had recovered to steady state levels well before that time.

C. RESULTS

1) PRODUCTION OF BIPHASIC CONTRACTIONS

Biphasic contractions were produced by changing the bathing solution from KH solution to SrKH solution. Within 20 minutes, most preparations contracted such that an initial, early component of contraction, designated P1, was followed by a late component, P2, which reached peak tension about 400 msec after stimulation (see section I). The biphasic contractions were maintained with P1 having about the same tension as P2 by the addition of between 0.05 and 0.2 mM Ca. Changes in the stimulation rate or pattern were studied after the contractions became stable.

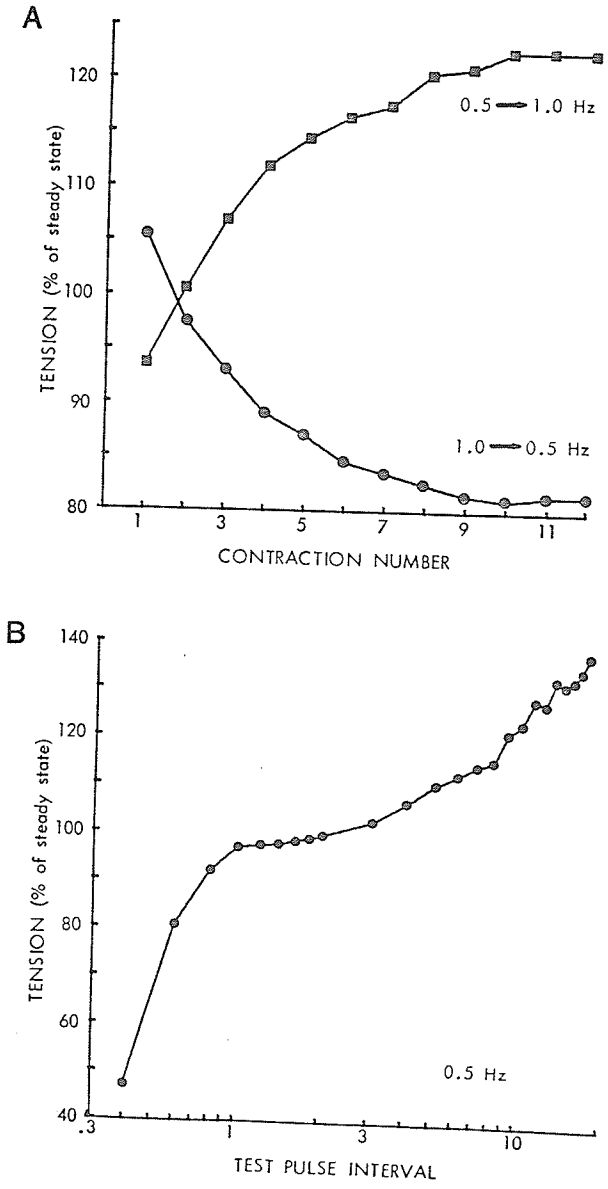
2) EFFECT OF FREQUENCY CHANGES

In KH solution, increasing the driving rate of the tissue resulted in increased tension production in the range from 0.1 to 1.0 Hz (fig. 21). The characteristic Bowditch staircase, where tension of the first contraction at a higher frequency is smaller than at the lower rate and is followed by a rise in tension up to a new, higher steady state, was seen in this preparation.

Reducing the stimulation frequency led to an increase in peak tension of the first contraction at the lower frequency, analogous to rest potentiation. The following contractions developed decreasing amounts of tension until a new steady state was approached after about 10 contractions. The immediate effects of frequency changes in either direction were less pronounced when both driving rates were low

FIGURE 21

Short-term force-interval relationship in canine ventricular trabeculae. A. Effect of increasing stimulation frequency from 0.5 to 1.0 Hz (squares) and decreasing frequency from 1.0 to 0.5 Hz (circles). In each case, steady state refers to the tension before the frequency change. Note that a new steady state is achieved within 10 contractions. B. Effect of changes in the interval between two contractions at a basic driving rate of 0.5 Hz. For test pulse intervals less than 2 sec the tension refers to an extrasystole (n = 19) while at test pulse intervals greater than 2 sec tension refers to a post-rest contraction (n = 5). The tension at 0.5 Hz is steady state.


A
120
110
100
90
80
TENSION (% of steady state)
0.5 → 1.0 Hz
1.0 → 0.5 Hz
1
3
5
7
9
11
CONTRACTION NUMBER
B
140
120
100
80
60
40
TENSION (% of steady state)
0.5 Hz
.3
1
3
10
TEST PULSE INTERVAL

(Koch-Weser and Blinks, 1963).

Accompanying the greater twitch tension development with increased driving rate was a decreased action potential duration and a slightly depolarized resting membrane potential. In addition, the plateau of the action potential was at a lower potential than at the lower frequency, as has been shown previously (Allen, 1977; Attwell et al., 1981).

After inducing biphasic contractions in a tissue, increasing the stimulation rate from 0.5 to 1.0 Hz caused P1 to rise to a new steady state after 8 contractions (fig. 22a). In 6 of 8 experiments, a delay of one contraction was seen before tension increased while in the other two experiments P1 tension in the first contraction at 1.0 Hz was 110% and 245% of the tension seen at 0.5 Hz. An increase in P2 was seen in the first contraction at 1.0 Hz in 7 of 8 experiments. Tension of the late component began to decline after the second contraction at the higher frequency due to shortening of the action potential and contraction durations (figs. 23a and 24). A similar sequence was seen when the frequency was changed from 0.2 to 0.5 Hz (fig. 22c) or from 0.1 to 0.5 Hz (fig. 22d).

Steady state contractions at 1.0 Hz were usually too short to enable a clear separation of the two components to be seen. In 4 experiments, though, both P1 and P2 were discernable at 1.0 Hz. A change of frequency of stimulation to 0.5 Hz resulted in decreased P1 tension of the first contraction at the new driving rate in 2 preparations and a 50% increase in one. As was the case with increasing frequency, P1 tension reached steady state in about eight contractions while P2 re-

FIGURE 22

Amplitude of P1 and P2 during frequency changes. A. The P1 tension is delayed by one contraction when changing from 0.5 to 1.0 Hz. B. Decay of P1 during change from 1.0 to 0.5 Hz. C. The first contraction is the 0.2 Hz steady state and the following contractions are at 0.5 Hz. The last contraction is the 0.5 Hz steady state. Note the similar pattern as in (A). D. The first contraction, at 0.1 Hz steady state, shows the prolonged "tonic" phase (P3) evident at low stimulation rates. The following contractions are at 0.5 Hz and eventually lead to the last contraction (0.5 Hz steady state). The prolonged P3 of the first contraction at 0.5 Hz may be due to a delayed inactivation of the mechanism for its production.

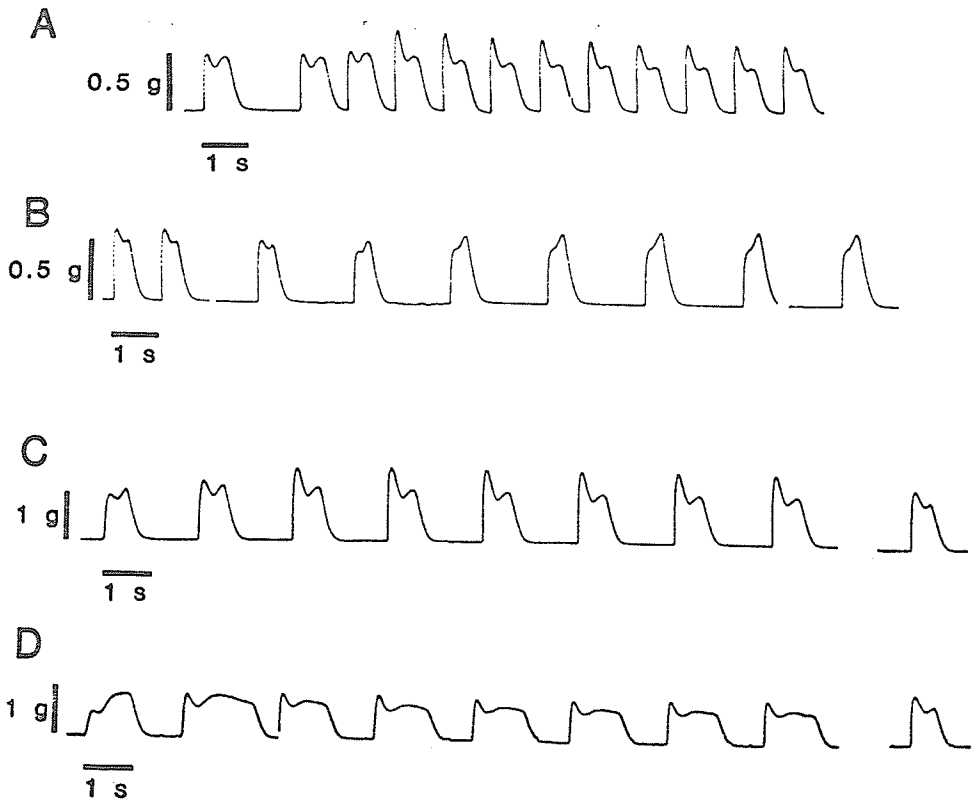
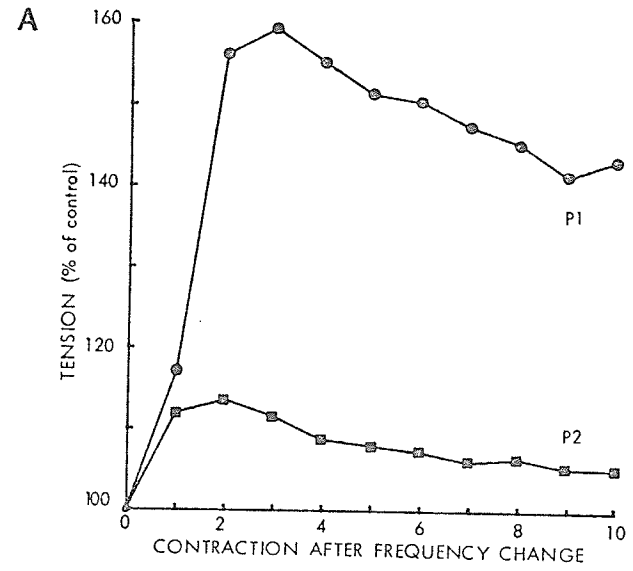
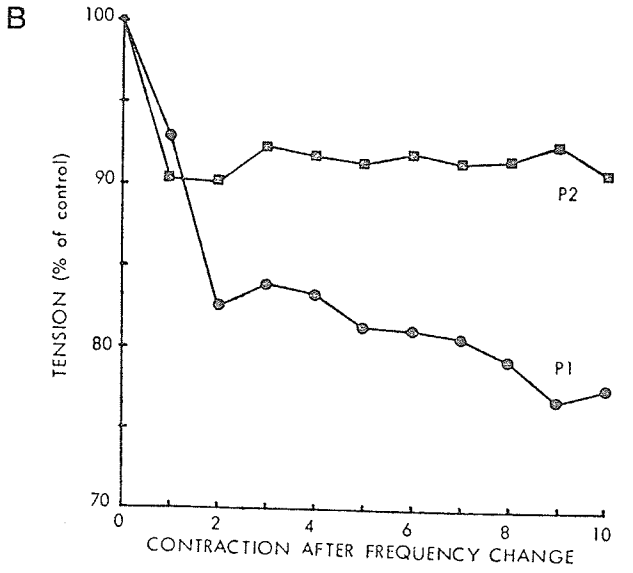

A
0.5 g
1 s
B
0.5 g
1 s
C
1 g
1 s
D
1 g
1 s

FIGURE 23

Time course of P1 and P2 phases of contraction during frequency changes. A. 0.5 to 1.0 Hz (n = 8). P1 of contraction 1 is higher than in most experiments due to one experiment in which the value was 245%. The mean of the other 7 experiments was 93.6 ± 6.7% for contraction 1. B. 1.0 to 0.5 Hz (n = 4). Note that P1 is more frequency-dependent than P2.


A
160
140
120
100
TENSION (% of control)
P1
P2
0
2
4
6
8
10
CONTRACTION AFTER FREQUENCY CHANGE


B
100
90
80
70
TENSION (% of control)
P2
P1
0
2
4
6
8
10
CONTRACTION AFTER FREQUENCY CHANGE

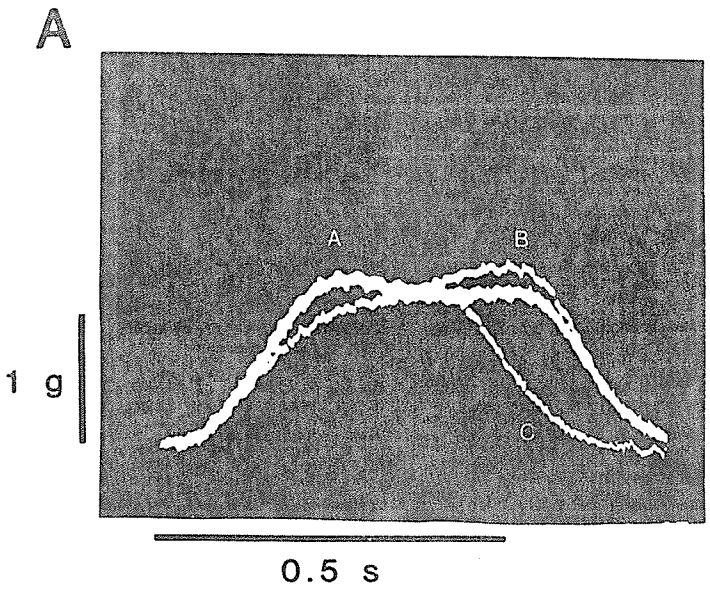
quired only two to three contractions to reach steady state (figs. 22b and 23b).

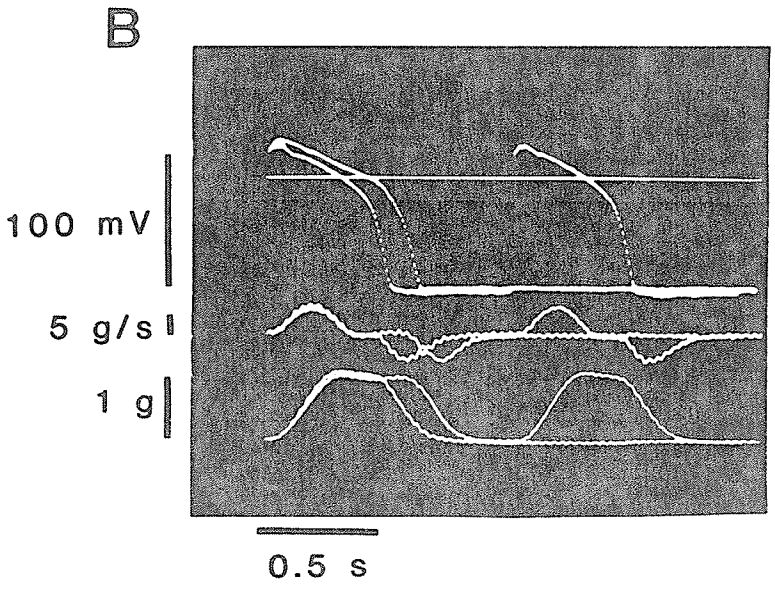
The tension developed by the early component was more influenced by frequency of stimulation than was the late component at all the rates studied (1.0, 0.5, 0.2, and 0.1 Hz). An increase in the driving rate tended to increase P1 while a decrease in the rate decreased P1 without greatly affecting peak P2 tension. It appears likely that the tendency of the Sr-mediated late component to develop more tension at a higher driving rate (Weyne, 1966; Brutsaert, 1967; Henderson and Cattell, 1976) is balanced by a shortening of the time available for development of the tension due to a decreased action potential duration. This is illustrated in fig. 22d. At a driving rate of 0.1 Hz, P1 is followed by P2 and a plateau phase of contraction (P3). P3 is a general feature of contractions in SrKH driven at less than 0.2 Hz and is accompanied by a very long action potential. At all rates, relaxation followed the rapid repolarization phase of the action potential. Thus, at 0.1 Hz, P2 was able to develop fully whereas at 0.5 Hz (fig. 22d, right) P2 tension depended on the duration of the action potential.

One interesting observation on the frequency dependency of biphasic contractions concerns the degree of separation of P1 and P2. In 10-15% of preparations, two distinct components of contraction were not seen at 0.5 Hz. Rather, the peak of a contraction was either rounded or flat but consistently prolonged. The partial relaxation between P1 and P2 was often produced in these muscles by stimulating at 0.2 Hz. The effect is similar to the loss of separation seen in

FIGURE 24

Contraction and action potential alterations with increasing frequency. A. (a) is 0.5 Hz steady state contraction, (b) is the first contraction at 1.0 Hz, and (c) is 1.0 Hz steady state. B. Action potentials (top panel), rate of contraction (middle panel), and contractions (lower panel) at 0.5 Hz steady state (long APs and contractions) and at 1.0 Hz steady state.


A
A
B
C
1 g
0.5 s


B
100 mV
5 g/s
1 g
0.5 s

the majority of tissues at 1.0 Hz.

3) POST-EXTRASYSTOLIC POTENTIATION

A reduction in the interval between two contractions in a train of steady state contractions at 0.5 Hz in KH solution resulted in post-extrasystolic potentiation (PESP). In trabeculae from 18 dogs, the extrasystole or test pulse produced tension less than or equal to the steady state level at all test pulse intervals less than 2 sec. Typically, the extrasystole had recovered to the steady state tension after a test pulse interval of 1.0 to 1.2 sec. Assuming that the time required for recovery is a measure of the rate at which the sarcoplasmic reticulum recycles Ca, the speed of recycling in dog trabeculae is somewhat slower than in rabbit ventricle (800 msec, Edman and Johannsson, 1976).

The post-extrasystolic contraction was potentiated when the test pulse interval was less than 1.2 sec. The degree of potentiation was inversely proportional to the test pulse interval, as has been repeatedly demonstrated (cf. Koch-Weser and Blinks, 1963).

When tissues were contracting biphasically in SrKH at 0.5 Hz, the P2 component of an extrasystole was always greater than the steady state P2 tension but the P1 component showed a marked degree of variability, as demonstrated in fig. 25 for a test pulse interval of 0.8 sec. Although the response to an extrasystole in KH solution was the same in the three tissues, P1 of the extrasystole could be less than, equal to, or greater than P1 of the preceeding steady state contrac-

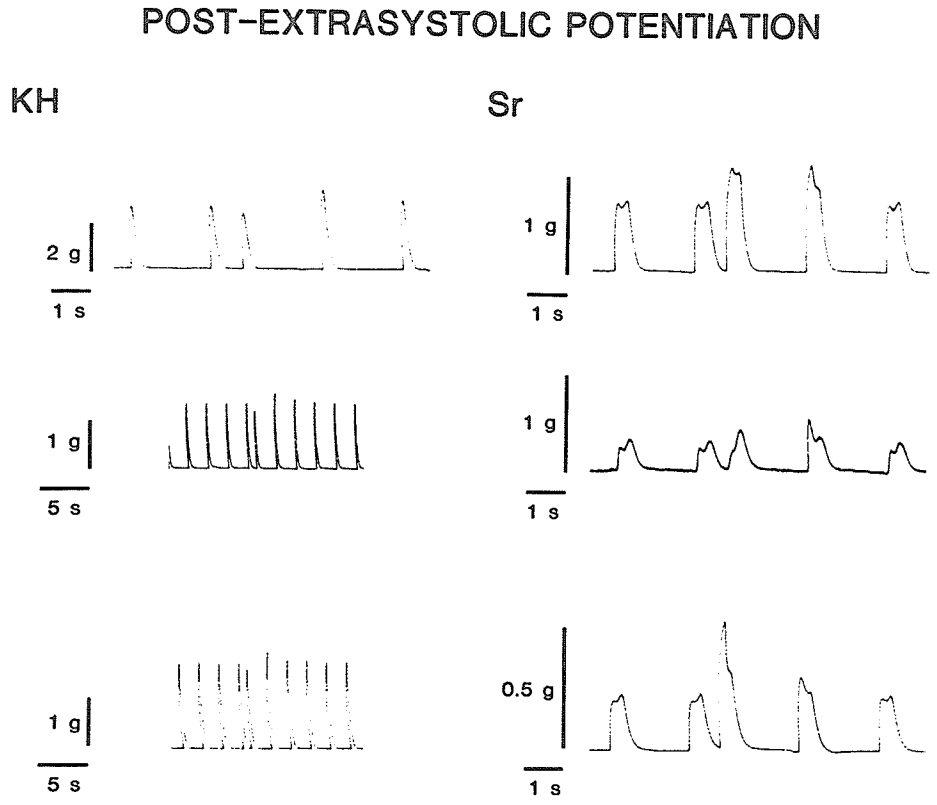


FIGURE 25

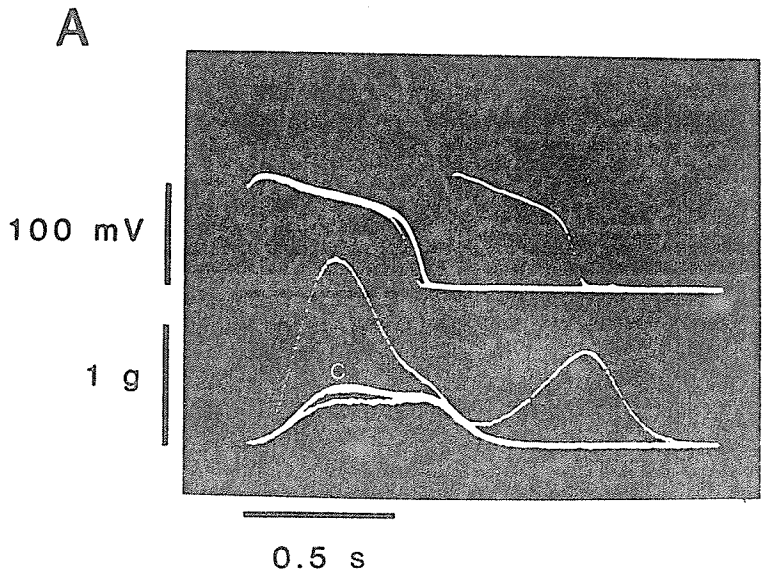
Three types of responses of biphasic contractions to a test pulse interval of 0.8 sec (an extrasystole). On the left, the extrasystolic and post-extrasystolic beats were the same in all three preparations. On the right, the same tissues under biphasic conditions produced different patterns of extrasystoles but the same pattern of post-extrasystolic beats. Note that P1 of the post-extrasystolic contraction was always potentiated, regardless of the pattern of the extrasystole.

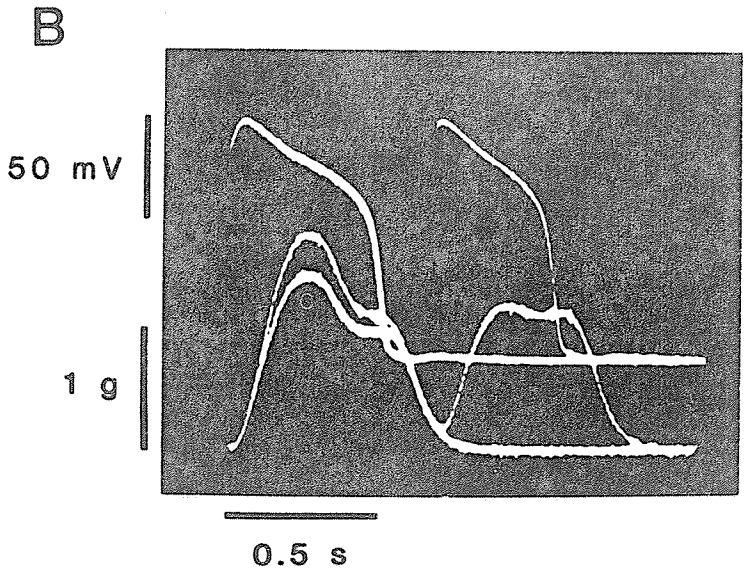
tion. No other factors, such as contraction duration, absolute tension produced, or animal weight, were found to correlate with the differences. However, since the dogs were obtained from the local dog pound, their ages were not known. As shown in fig. 26a, the action potential duration of an extrasystole triggered 0.7 sec after the preceeding steady state contraction had a shorter duration and a slightly higher plateau potential. The higher plateau is consistent with an increased slow inward current, mirrored in the contraction as an increased P2. The greater importance of slow inward current cation on extrasystolic contractions than on steady state contractions has previously been demonstrated (Hiraoka and Sano, 1978; KL Seaman and D Bose, personal communication). After the addition of 0.1 uM isoproterenol, which produces a better separation between P1 and P2 (see section I), P2 of the extrasystole was still potentiated and showed an action potential that was shortened to the same duration as that seen in the absence of the drug (fig. 26b). Thus, in this experiment, isoproterenol decreased the action potential duration during steady state by about 12% without changing that of the extrasystole. Furthermore, isoproterenol caused a much smaller increase in extrasystolic P2 tension than in steady state P2 tension.

The post-extrasystolic contraction consistently showed a large increase in P1 tension and a much less pronounced change in P2 when compared to steady state (figs. 25, 26, and 27). In addition, if the actual time between relaxation of the steady state contraction and the onset of the extrasystole is used as the test pulse interval, the curve relating P1 tension of the post-extrasystolic contraction to the

FIGURE 26

Electrophysiology of an extrasystole in a biphasic tissue. A. 0.5 Hz steady state contraction (C) followed by an extrasystole at a test pulse interval of 0.7 sec (right). The post-extrasystolic contraction had a potentiated P1 and a shorter action potential duration while the next contraction had a smaller P1 than steady state. B. The same procedure after the addition of 0.1 uM isoproterenol. There was less potentiation of P2 of the extrasystole and of P1 of the post-extrasystolic beat.


A
100 mV
1 g
0.5 s


B
50 mV
1 g
0.5 s

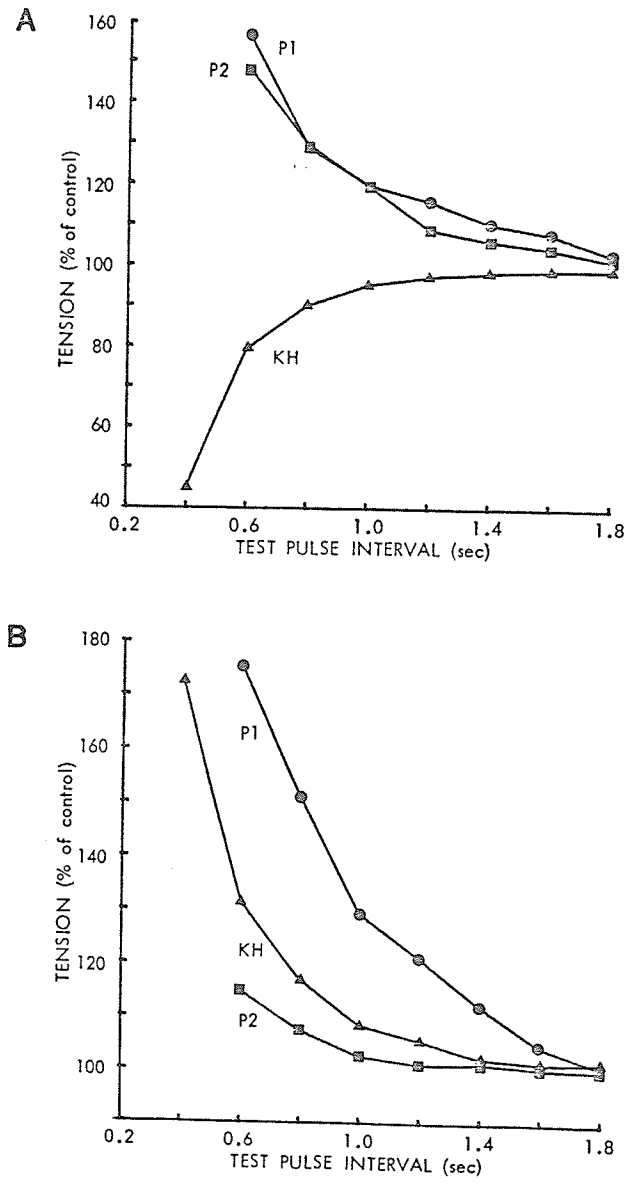


FIGURE 27

P1 and P2 of extrasystolic (A) and post-extrasystolic (B) beats in relation to the test pulse interval. P1 (circles), P2 (squares), and contractions in KH solution (triangles) are expressed as percent of the contraction immediately preceeding the extrasystole. For biphasic tissues, n = 5 and for KH tissues, n = 19.

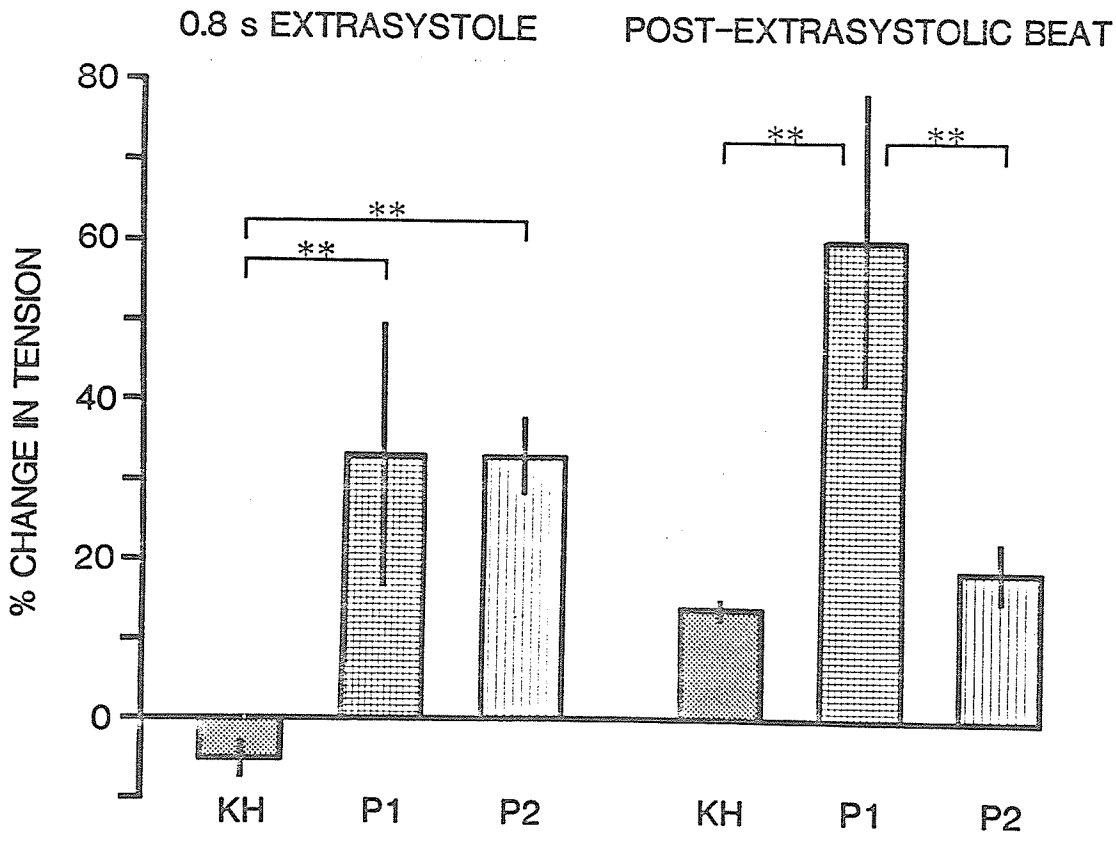


FIGURE 28

Comparison of KH, P1, and P2 contractions at a test pulse interval of 0.8 sec. Left, the extrasystolic contraction. Right, the post-extrasystolic contraction. For KH contractions, n = 14 and for biphasic contractions, n = 6. Comparisons were performed using analysis of variance and Duncan's test (** denotes $P<.01$).

test pulse interval (fig. 27b) becomes virtually superimposible on the KH curve. A number of studies have shown that contractions potentiated by an extrasystole are relatively insensitive to the entry of Ca across the sarcolemma (Bass, 1976; Seaman and Bose, 1981), as are contractions potentiated by a rest (Endoh and Iijima, 1981). Coupled with the present results, it can be seen that the tension produced by an extrasystole is more dependent on direct activation by slow inward current cation whereas the postextrasystolic contraction is potentiated primarily due to increased sarcoplasmic reticulum Ca release.

4) REST POTENTIATION

Dog trabeculae show rest potentiation, where the first contraction following an interruption of stimulation produces more tension than steady state contractions, at test pulse intervals of between 3 and 17 sec when the basic driving rate is 0.5 Hz. Post-rest tension was still increasing after a 17 sec rest (fig. 21b), in contrast to results found in rabbit papillary muscle (Edman and Johannsson, 1976).

Figure 29 demonstrates some of the variability seen when a rest was imposed on a muscle contracting biphasically in SrKH at 0.5 Hz. Although the most common response to a rest of short duration (10 sec or less) was a small increase in P1 and either no change or a slight decrease in P2 (as in fig. 29a and b) a fair degree of variability was observed. With a test pulse interval of 7 sec, P1 of the test pulse (the first contraction after the rest) in 9 experiments ranged from 43% to 113% of control while P2 ranged from 74% to 127% of con-

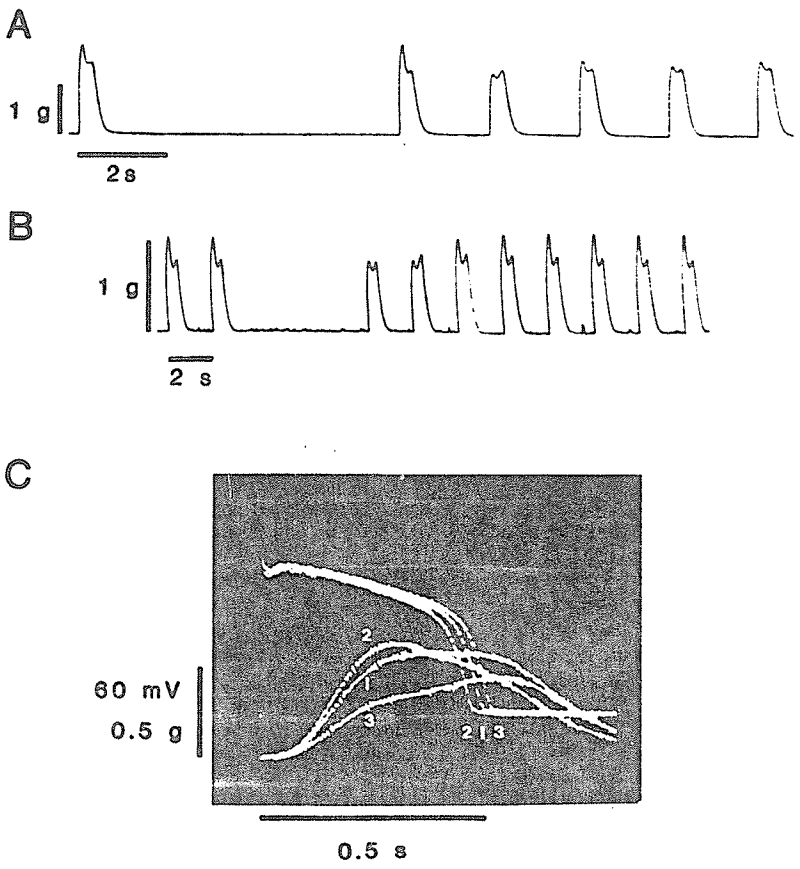


FIGURE 29

Effect of a rest on biphasic contractions. After reaching steady state at 0.5 Hz, a test pulse interval of 7 sec (corresponding to an equivalent rest of 5 sec) was introduced between two contractions. P1 usually showed a slight potentiation after the rest period (as in A) but occasionally showed a marked decline of P1 (B). In C, action potentials are superimposed on contractions. Action potential 1 and contraction 1 were at 0.5 Hz steady state, response 2 was post-rest, and 3 was due to the next normal stimulus after 2 sec.

trol (means: 91 + 8% and 96 + 5% respectively). The action potential of the first post-rest contraction was found to be slightly shorter than steady state while the second post-rest action potential was longer (fig. 29c).

D. DISCUSSION

Two component contractions provide a method of examining the non-steady state contributions of sarcoplasmic reticulum Ca release and extracellular cation on contractions. The present experiments were performed in an attempt to elucidate the mechanisms underlying the force-interval relationship.

Some work has been done on the effect of stimulation pattern on the early component, P1, and the late component, P2, of contraction. In the original abstract describing Sr-induced biphasic contractions, Braveny and Sumbera (1972) noted that shortening the interval between contractions resulted in an increased P2 in the first contraction while P1 increased on the next contraction. Similarly, a typical rested-state contraction in normal Ca-containing medium has been shown to have an increased latency of onset and a time to peak tension which is completely dependent on the action potential duration (Allen et al., 1976; Beresewicz and Reuter, 1977; Reiter et al., 1978; Seibel et al., 1978). This indicates that the contraction is similar, if not identical, to the P2 component described in this study and characterized by King and Bose (1981). Furthermore, an early component appeared in the second contraction, reaching its steady state only after

about six contractions (Seibel et al., 1978). As in the present study, then, an increment in P1 tension was preceeded by a large P2 in the previous contraction. The finding that the force staircase is accelerated (i.e. fewer contractions are required to reach maximum tension) when the bathing Ca concentration is increased (Lakatta and Spurgeon, 1980) gives further support to the importance of Ca influx on staircase phenomena.

The results presented in this section show that, prior to a potentiation of P1, the P2 component of the preceeding contraction must have been potentiated. This was demonstrated both by the beat-to-beat changes in each component during positive and negative frequency staircases and by the potentiating effect of an extrasystole. Both inotropic procedures produced greatly augmented P1 tensions one contraction after P2 was increased.

The precise origin of the P2 cation pool in contractions at the basic frequency used (0.5 Hz) is not entirely clear. As mentioned in the Results section, a "tonic" component of contraction (P3) is usually observed in tissues stimulated at 0.2 Hz or less. However, since the time course of development and frequency dependence of the magnitude of this component are not known, its contribution to the observed P1 and P2 tensions is also unknown. This may account for the unexpected increase in P1 tension seen in extrasystoles in some preparations. If the phasic P1 component were superimposed on an increased, quickly-rising P3 component, the observed contractile changes would be seen. A more likely reason is that the intracellular Sr concentration is higher than the resting level but less than threshold at the time

the extrasystole is triggered. A relatively small amount of Ca released from the sarcoplasmic reticulum would then be able to cause an inordinately large P1 tension response. The P2 staircase, on the other hand, was not entirely unexpected since a frequency-dependent staircase of slow inward current has been demonstrated in frog heart (Noble and Shimoni, 1981). In mammalian ventricle, a positive correlation between slow inward current and tension has been observed but the relationship depends on both the level of depolarization (Simurda et al., 1981) and on the voltage clamp frequency (Payet et al., 1981). This means that increasing tension is associated with decreasing current over a range of membrane potentials and frequencies. A similar negative relationship has been shown in guinea pig atrium (Schulze, 1981). This is interesting in that an increased intracellular Ca concentration (which accompanies a larger contraction), when produced by injection of Ca, results in an increased slow inward current (Isenberg, 1977). It is apparent that the relationship between frequency, slow inward current, and tension is not entirely clear.

In normal KH solution, dog trabeculae showed a considerable degree of rest potentiation (fig. 21b). It was somewhat disconcerting that a rest produced very little rest potentiation in biphasically-contracting trabeculae. One might have expected P1 to be increased due to a time-dependent over-accumulation of Ca in the sarcoplasmic reticulum release site. Instead, a number of tissues showed either no change or a decrease in P1 tension following a 5 sec rest. When it is recalled that the conditions for producing biphasic contractions include an extracellular Ca concentration of only 0.05 to

0.2 mM, the results may be explained. Allen et al. (1976), when studying rest decay curves in cat papillary muscles, found that the rate of decay of tension with increasing rest durations was inversely proportional to the bathing Ca concentration. Thus, at the low extracellular Ca concentration used in this study, the relatively rapid decay of P1 with rest is not contradictory to the basic contraction model referred to in the introduction. In any event, the important point is that P1 of the contractions following the post-rest contraction behave in the manner predicted by the model, with an initial decrease to tension levels less than steady state and then a rise to the steady state value. This is the same pattern seen in KH solution. P2 tension after a rest was reduced to a far lesser degree than was P1, indicating that the time constant of decay of P2 is less than that of P1. Therefore, in KH solution, one may visualize the decline of a P1-like component to zero while maintaining a P2-like component, as seen in rested-state contractions (Allen et al., 1976; Beresewicz and Reuter, 1977; Seibel et al., 1978).

The observation that, for a given potentiation of P2 in an extrasystole the potentiation of P1 of the post-extrasystolic contraction was 50% greater (fig. 27) implies that PESP may not be accounted for simply by an increase in the Ca content of the cell induced by the extrasystole. One possible explanation is that the rate of recycling of Ca from the uptake to the release site of the sarcoplasmic reticulum is rate-dependent or, more precisely, depends on the Ca content of the release site per unit time. Edman and Johannsson (1976) showed that, in rabbit papillary muscle, a test pulse interval of about 800 msec

produces maximum tension regardless of the "priming" frequency preceeding the test pulse. This points to a frequency-independence of the recycling mechanism. However, if it is assumed (not unreasonably) that the loss of Ca from the release site is proportional to the Ca content of the site, an increased recycling rate may be masked by an increased rate of loss induced by greater Ca content at higher frequencies.

In terms of models, we may interpret the results roughly according to the basic model mentioned in the introduction section (Wood et al., 1969; Morad and Goldman, 1973; Kaufmann et al., 1974; Allen et al., 1976; Edman and Johannsson, 1976). Sarcolemmal depolarization causes the release of Ca from an intracellular release site, initiating contraction. Simultaneously, Ca entering the cell through both the slow inward current and some unknown mechanism, possibly related to either intracellular K (Morad and Goldman, 1973) or Na (Coraboeuf, 1974; Mullins, 1981), diffuses through the sarcoplasm to the contractile apparatus. Membrane repolarization shuts off extracellular Ca entry, the "relaxing system" takes up the contractile Ca, and relaxation ensues. Ca in the sarcoplasmic reticulum uptake site is recycled to the release site at a frequency-dependent rate, Ca bound to the inside of the sarcolemma is eliminated from the cell by Na-Ca exchange (Glitsch et al., 1970), and the process is ready to be repeated.

According to this model, the positive staircase results from: 1) an increase in total cellular Ca content due to the greater fraction of time the sarcolemma is depolarized (Braveny and Sumbera, 1970), 2) an increase in the magnitude of the slow inward current (Noble and

Shimoni, 1981), and 3) an increase in the recycling rate of Ca from the uptake to the release site of the sarcoplasmic reticulum. An extrasystole would augment both cellular Ca content and the recycling rate. Finally, the degree of potentiation following a rest would depend upon the balance between three factors: 1) the extent of Ca recycling continuing beyond the previous inter-stimulus interval, 2) the rate of Ca loss from the release site to the extracellular space, and 3) the rate of decline of the late component of contraction. In this regard, it is interesting to note that Edman and Johannsson (1976) found that, with increasing test pulse intervals, the decline of twitch tension in rabbit papillary muscle followed a biexponential time course. The two phases had time constants of 2.6 ± 0.8 sec and 92 ± 13.3 sec. It is tempting to speculate that in KH solution the slow decline, which ultimately leads to a rested-state, P2-like contraction, indicates the decay of the frequency-potentiated, membrane-dependent late component of contraction while the fast decay is a measure of the loss of Ca from the sarcoplasmic reticulum release site. Testing this hypothesis will require further experimentation.

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION AND CONCLUSIONS

A. JUSTIFICATION OF THE BIPHASIC CONTRACTION METHOD

The results presented in this thesis depend primarily on the correct identification of the cation pools responsible for P1, P2, and P3. Sections I, II, and III were concerned with determining the origin of activator cation for each phase of contraction.

In section I, it was concluded that P1 is caused by Ca release from the sarcoplasmic reticulum. The Ca-dependency was a straightforward observation but localization of the pool required the use of two drugs whose specificity may be questioned. Caffeine and other methylxanthines are commonly used as inhibitors of the cardiac sarcoplasmic reticulum (e.g. Chuck and Parmley, 1980) although they also affect trans-sarcolemmal Ca fluxes (Blinks et al., 1972; Clark and Olson, 1973; Kavaler et al., 1978). It is generally thought that a caffeine-induced increase in the action potential plateau voltage is indicative of an increased slow inward current (Clark and Olson, 1973) but a number of experiments have not been supportive. Kavaler et al. (1978) found that caffeine causes a positive inotropic effect in frog ventricle when applied during tension development and that the effect was blocked instantly by extracellular Mn. This supports the role of an increased slow inward current. Eisner et al. (1979), using voltage clamped sheep Purkinje fibres, observed a complete block of slow inward current by caffeine. Finally, Ohba (1973) found no caffeine-induced change of the current in voltage clamped dog papilla-

ry muscle. Either a considerable species variation exists in the response of the current to caffeine or the differences in experimental protocol have obscured the true effect. In contrast to the ambiguous results on sarcolemmal Ca current, it is well established that caffeine causes a rapid release of Ca stored in the sarcoplasmic reticulum (Fabiato and Fabiato, 1975), decreases its rate of Ca uptake (Shine and Langer, 1971), and increases the permeability of its membrane to Ca (Blayney et al., 1978). The result is a decreased storage and release of Ca from the sarcoplasmic reticulum.

The observed depression of P1 by caffeine was concluded to be due to an effect on the sarcoplasmic reticulum after similar results were obtained with ryanodine. The latter alkaloid has no effect on amphibian ventricle but profoundly depresses contractility in mammalian heart containing a well-defined sarcoplasmic reticulum (Ciofalo, 1973; Penefsky, 1974a and b; Sutko et al., 1979). At low concentrations, ryanodine selectively eliminates rest potentiation without affecting steady state contractions by a sarcolemmal action (Frank and Sleater, 1975). The drug does not appreciably affect the slow inward current (c.f. Sutko et al., 1979) but dramatically increases the Ca uptake velocity and storage capacity of cardiac sarcoplasmic reticulum vesicles while inhibiting Ca efflux (Jones et al., 1979). Ryanodine has no effect on Ca transport in sarcolemmal vesicles (Besch et al., 1981). Furthermore, electron micrographs have shown a disrupted connection between the T tubules and the sarcoplasmic reticulum (Penefsky, 1974b). Taken together, the published data show that ryanodine eliminates the sarcoplasmic reticulum as a major release site of acti-

vator Ca. The consistent attenuation of P1 by two drugs which block sarcoplasmic reticulum Ca release by different mechanisms proves that P1 is a consequence of activator cation release from that pool. However, a possibility that cannot be excluded with the present results is that the sarcoplasmic reticulum releases Sr to yield P1 but that Sr is unable to replace Ca as the "trigger" (i.e. Ca-induced Sr release).

If the late component of contraction (P2) were the result of sarcoplasmic reticulum release of Ca, as suggested by Seibel et al. (1978) and Reiter et al. (1978), caffeine and ryanodine should have had a negative inotropic effect on P2. This was never seen. Instead, the results with Mn and Ni ions (sections I and II) have shown that P2 is the result of contractile activation by Sr which crosses the sarcolemma during the action potential. The precise origin of P2 cation has not been resolved as clearly as that of P1 cation but the results suggest that Sr derived from the enhanced slow inward current is superimposed on an increasing P3 component, probably due to Na-Sr exchange (Horackova and Vassort, 1976a). This accounts for the depression of P2 seen with Mn, Ni, and TTX and the increase of P2 seen with ouabain and isoproterenol. It is not surprising that such a "blending" of slow inward current and Na-Sr exchange activator Sr should occur because both sources are presumably mobilized at about the same time (at the onset of the action potential plateau) and both must diffuse from the sarcolemma to the contractile proteins.

In the General Introduction, a study by Wier (1980) on aequorin transients in dog Purkinje fibres was alluded to. The author found

two components of intracellular Ca increase during contraction with the late component being more frequency-dependent than the early component. The results in section V of this thesis show that, in SrKH, P1 (the early component of contraction) was much more frequency-dependent than P2. The conclusion by Wier (1980) that the early Ca transient originates from the slow inward current is not inconsistent with the present results for two reasons: 1) it is very possible that Sr originating from the sarcolemma enters the sarcoplasm prior to Ca release from the sarcoplasmic reticulum but, due to a greater diffusional distance, activates contraction later, and 2) the intracellular distribution of aequorin may favour earlier detection of slow inward current Ca. Furthermore, the results presented by Wier (1980) and the results shown in section V on the force-interval relationship are mutually supportive since in both cases the slow inward current is seen as the primary source of activator cation for an extrasystole.

Beeler and Reuter (1970c) and others have noted that, in some tissues during prolonged voltage clamps, tension development follows its normal time course, relaxing well before the clamp is withdrawn. When the clamp is extended for more than one sec in dog trabeculae, a tonic tension is seen, corresponding to the P3 tension described in this thesis. The lack of complete relaxation between P1 and P2 thus deserves comment. It may be safely assumed that relaxation of P1 is due to reuptake of Ca by the sarcoplasmic reticulum. Binding and extrusion of Ca by the sarcolemma may be discounted in this regard since the membrane is still depolarized at this time. Under voltage clamp

conditions in normal, Ca-containing solution, the trans-sarcolemmal Ca flux analogous to P2 and P3 cation continues for the duration of the clamp (with slow inward current declining with time) but the Ca is largely taken up by the sarcoplasmic reticulum. In this case, tonic tension would only develop when the uptake capacity of the sarcoplasmic reticulum is exhausted. When Sr is the cation in this situation, secondary tension development could occur earlier since: 1) the slow inward current carried by Sr has a larger magnitude and longer duration than that carried by Ca (Kohlhardt et al., 1973a and b; Reuter and Scholz, 1977a; Noble and Shimoni, 1981a), 2) the sarcoplasmic reticulum needs a much higher Sr concentration for half maximal uptake (Edwards et al., 1966; Moisescu and Thieleczek, 1978), in spite of a greater maximal uptake velocity of Sr (Mermier and Hasselbach, 1976), and 3) the contractile proteins of heart muscle are equally sensitive to Ca or Sr (Donaldson et al., 1978; Kerrick et al., 1979 and 1980). Therefore, a larger amount of activator cation, less damped by uptake from the sarcoplasmic reticulum, would reach the contractile proteins in a sufficient concentration to initiate contraction in a shorter time.

The precise origin of P3 cation was not extensively studied but the results with ouabain and TTX (section III) are more consistent with a Na-Sr exchange model rather than the K-divalent cation exchange model proposed by Morad and Goldman (1973). In any case, P3 is considered to be identical to the "tonic" tension described by a number of researchers using voltage clamp techniques (see General Introduction part A3).

The pronounced prolongation of the action potential in Sr-containing solution is probably related, in part, to a much greater inactivation time constant of the slow inward current (Kohlhardt et al., 1973a and b; Noble and Shimoni, 1981). Although the slow inward current is thought to be the primary determinant of the action potential duration in ventricular muscle (but not in Purkinje fibres; New and Trautwein, 1972b; Trautwein, 1973; Vassalle, 1979), the finding that TEA shortens the duration in SrKH solution indicates that blockade of K conductance by Sr is also important under these conditions. TEA has not been shown to alter the inactivation kinetics of the slow inward current. It is interesting that, despite inhibiting K conductance, Sr has been shown to dramatically increase cellular K loss and Na gain when no Ca is present (Thomas, 1957; Weyne, 1966b) but not when 0.2 mM Ca is added (Thomas, 1957). An Sr-induced inhibition of Na-K ATPase must be considered. With this in mind, it should be noted that digitalis glycosides increase the rate of development of the Sr-induced positive inotropic effect (Thomas, 1957) and that Ca replacement by Sr potentiates the inotropic effect of K-free solutions (Eisner and Lederer, 1979). Both digitalis and K-free perfusion inhibit Na-K ATPase.

B. THE MECHANISM OF Ca RELEASE FROM THE SARCOPLASMIC RETICULUM

The results have shown that Sr replacement of Ca produces prolonged contractions with a delayed onset in canine ventricular muscle, as has previously been shown in a number of other mammalian ventricular pre-

parations (see the General Introduction part B1). Significantly, Sr does not delay the onset of contraction in amphibian ventricle, where the sarcoplasmic reticulum is sparse (Bass et al., 1975; Henderson and Cattell, 1976). Brutsaert and Claes (1974) noted the similar effects of caffeine and Sr on myocardial contractions but, as shown in section I, the time courses of the effects are different. Caffeine in KH solution caused an initial transient stimulation, then depression, and finally the slowly-developing positive inotropic effect characteristic of the drug (Blinks et al., 1972). Replacing Ca with Sr, on the other hand, produced only the depression and subsequent positive inotropic effect. It seems likely that the first effect of Sr is to reduce sarcoplasmic reticular Ca release during the action potential whereas caffeine transiently potentiates the release (Fabiato and Fabiato, 1975; Endo, 1977). The similarity of the steady state contractions in Ca-free SrKH solution to those in caffeine-containing KH solution indicates that Sr is unable to be released from the sarcoplasmic reticulum. As mentioned in the General Introduction part B1, Sr is accumulated by the sarcoplasmic reticulum (Edwards et al., 1966; Winegrad, 1973; Mermier and Hasselbach, 1976; Kawata and Hatae, 1977) and can be released by caffeine or extrareticular Sr (skeletal muscle; Moisescu and Thieleczek, 1978). If it is accepted that Sr can be taken up and released by the sarcoplasmic reticulum, then it follows that the coupling between membrane depolarization and release of Sr from the organelle is blocked. The coupling cannot be merely current flow through the slow channel since this is actually increased by Sr (Kohlhardt et al., 1973a and b; Noble and Shimoni, 1981a).

Furthermore, a loss of sarcoplasmic reticular membrane polarization in SrKH solution appears unlikely since the concentration of unbound Sr in the organelle may actually be greater than that attained by Ca (Mermier and Hasselbach, 1976), adding to the intrareticular free cation concentration and possibly supporting polarization. It is possible, however, that the greater free Sr concentration in the lumen leads to a hyperpolarization of the membrane, inhibiting release or that the increased intracellular Na concentration induced by Sr (Thomas, 1957) depolarizes the sarcoplasmic reticulum. These possibilities await further experimentation.

The easiest way to explain the loss of sarcoplasmic reticulum release of divalent cation in SrKH solution is to assume that Sr cannot replace Ca as the "trigger" in a divalent cation-induced release mechanism. The Ca-induced Ca release mechanism championed by Fabiato and Fabiato (1972; 1975; 1979) is based on the observation that application of a sub-contraction threshold concentration of Ca to skinned muscle fibres is able to trigger the release of Ca from the sarcoplasmic reticulum. Endo et al. (1970) found that reticulum isolated from frog skeletal muscle can release Ca in response to a raised concentration of either Ca or Sr but that 50 times more Sr was needed for an equivalent release. In the study by Moisescu and Thieleczek (1978) alluded to above, Sr-induced Sr release in skinned frog skeletal muscle fibres was seen with a Sr concentration of about 30 uM, a level greater than that necessary for maximal contractile activation in cardiac muscle (Donaldson et al., 1978; Kerrick et al., 1980). When the well-described competition between Ca and Sr at the sarcolem-

ma is considered (see the General Introduction part B2), it is reasonable to propose that the transient contractile depression caused by Sr is the result of decreased Ca-triggered release of Ca from the sarcoplasmic reticulum in response to the action potential. The eventual loss of P1 in SrKH solution could then be ascribed to complete suppression of Sr release from that site. The reappearance of P1 when low concentrations of Ca were reintroduced into the solution further supports this mechanism. Finally, it was noted that the loss of P1 and the increase of P2 in SrKH solution was more time-dependent than rate-dependent, supporting a role of some sarcolemmal divalent cation binding site in the production of P1 where Sr cannot replace Ca.

In conclusion, the results tend to support the Ca-induced Ca release hypothesis of E-C coupling rather than a depolarization-induced mechanism (Endo, 1977).

C. A MODEL OF E-C COUPLING

The results presented in this thesis point to the model of mammalian ventricular E-C coupling illustrated schematically in fig. 30. In the model, the sarcolemma depolarizes when conductance channels for Na and Ca are opened. The increased Na at the inside of the sarcolemma should be almost immediately available for reverse electrogenic Na-Ca exchange, where 3 or more Na ions are transported out of the cell for each Ca entering. This Ca, plus the Ca entering the cell as the slow inward current, would diffuse throughout the cell water. Meanwhile, as the action potential prodeeds down the T-tubules, Ca is released

from the sarcoplasmic reticulum by, probably, Ca-induced Ca release (see above). The longitudinal sarcoplasmic reticulum continually takes up some fraction of the activator Ca of all origins before it can activate the contractile proteins. At the risk of placing too much faith in the specificity of ryanodine (section I), perhaps 30% of contractile activation is caused by sarcolemmal cation while the remaining 70% of activator Ca is from the sarcoplasmic reticulum at a stimulating frequency of 0.5 Hz. Since the longitudinal sarcoplasmic reticulum continues to lower the sarcoplasmic Ca concentration concurrently with the inactivation of the slow inward current, the tissue can relax even if the membrane voltage is clamped. Since there is certainly a limit to the amount of Ca the sarcoplasmic reticulum can take up, prolonged depolarization would cause a tonic tension due to the continued reverse Na-Ca exchange mechanism. The electrogenic Na-Ca exchange would change direction when the membrane repolarizes, thus contributing to relaxation. The Ca at the sarcoplasmic reticular uptake site is recycled to the release site, possibly (but not necessarily) at a stimulation frequency-dependent rate. The cycle is then ready to be repeated.

When a sufficient concentration of extracellular Sr is present to overcome the competition with Ca, some fraction of both the slow inward current and the Na-divalent cation exchange mechanism would begin to carry Sr. The sarcoplasmic reticulum is a fairly poor sink for this Sr and as a result contractile activation due to the extracellular pool begins relatively early. With sarcolemmal repolarization, Na-Sr exchange would begin removing Sr from the cell, contributing to

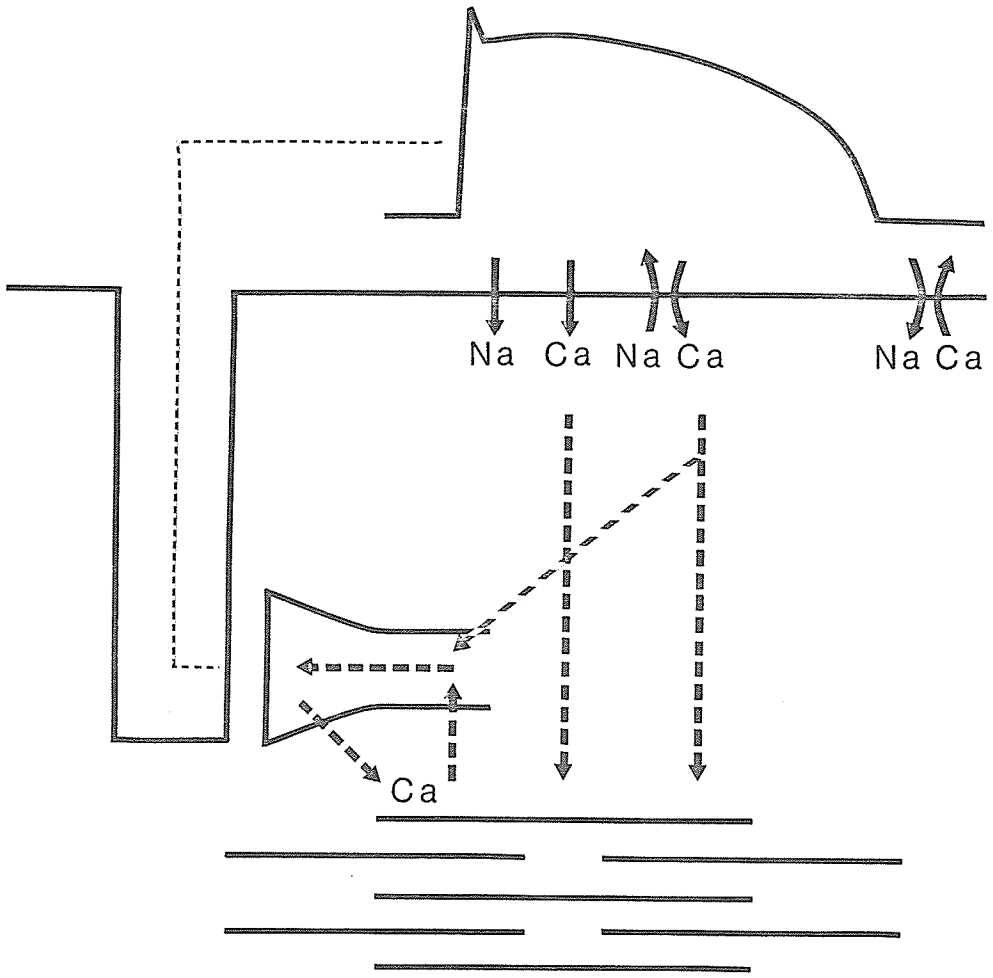


FIGURE 30

Model of excitation-contraction coupling in mammalian ventricle. The dashed lines show the intracellular movements of activator Ca, the straight, solid arrows are transmembrane ion fluxes, and the curved, solid arrows indicate a carrier-mediated sarcolemmal exchange mechanism. See text for further discussion.

the relaxation process.

D. USES AND LIMITATIONS OF BIPHASIC CONTRACTIONS

The Sr-induced biphasic contraction model should provide an easy method for preliminary determinations of the locus of action of cardioactive drugs and toxins. With the agents used in sections I to IV, some expected effects on contraction were seen (Mn, caffeine, ryanodine, isoproterenol, TTX, and ouabain) in addition to the unexpected effects of TEA and Ni. It is probably not coincidental that the last two agents are the least studied.

Secondly, biphasic contractions should be useful in probing the mechanisms underlying basic physiological phenomena such as the force-interval relationship. (The use of the term "biphasic" may actually be a misnomer since P3 is also present but, at the basic stimulating frequency used in these studies (0.5 Hz), only two peaks of contraction were routinely seen.) In addition to the force-interval relationship studies of section V, preliminary results (not shown) indicate that stretching a trabecula between contractions increases sarcoplasmic reticulum cation release (P1), as has been shown by Chuck and Parmley (1980). It is obvious that the model has wide applicability to both pharmacological and physiological studies.

The major limitations of the technique include: 1) experiments with any agent which chelates Ca must be done with low drug concentrations since P1 is maintained by only 0.1 to 0.2 mM Ca, 2) drugs which affect K conductance may not show the expected effects in SrKH since

Sr also blocks K conductance, and 3) the prolonged action potentials and contractions preclude the maintained separation of P1 and P2 at high stimulation frequencies. It must be concluded, however, that these limitations are quite minor when compared to the number of situations where biphasic contractions would be useful.

BIBLIOGRAPHY

BIBLIOGRAPHY

Allen DG (1977). On the relationship between action potential duration and tension in cat papillary muscle. Cardiovasc. Res. 11: 210-218.

Allen DG and Blinks JR (1978). Calcium transients in aequorin-injected frog cardiac muscle. Nature 273: 509-513.

Allen DG, Jewell BR, and Wood EH (1976). Studies of the contractility of mammalian myocardium at low rates of stimulation. J. Physiol. 254: 1-17.

Anderson PAW, Manring A, Sommer JR, and Johnson EA (1976). Cardiac muscle: an attempt to relate structure to function. J. Mol. Cell. Cardiol. 8: 123-143.

Anderson TW, Hirsch C, and Kavaler F (1977). Mechanism of activation of contraction in frog ventricular muscle. Circ. Res. 41: 472-480.

Armstrong CM (1975). Ionic pores, gates, and gating currents. Q. Rev. Biophys. 7: 179-210.

Attwell D, Cohen I, Ohba M, and Ojeda C (1979). The steady state TTX-sensitive ("window") sodium current in cardiac Purkinje fibres. Pflugers Arch. 379: 137-142.

Attwell D, Cohen I, and Eisner DA (1981). The effects of heart rate on the action potential of guinea-pig and human ventricular muscle. J. Physiol. 313: 439-461.

Bailey LE and Dresel PE (1968). Correlation of contractile force with a calcium pool in the isolated cat heart. J. Gen. Physiol. 52: 969-982.

Baker PF, Blaustein MP, Hodgkin AL, and Steinhardt RA (1969). The influence of calcium on sodium efflux in squid axons. J. Physiol. 200: 431-458.

Bass BG, Ciulla EM, Klop P, and van Baal S (1975). Some electrical and mechanical effects of strontium on toad ventricular muscle: comparison to calcium. J. Physiol. 252: 547-564.

Bass O (1976). The decay of the potentiated state in sheep and calf ventricular myocardial fibers. Influence of agents acting on transmembrane Ca^{2+} flux. Circ. Res. 39: 396-399.

Bassingthwaighte JB, Fry CH, and McGuigan JAS (1976). Relationship between internal calcium and outward current in mammalian ventricular muscle; a mechanism for the control of the action potential duration? J. Physiol. 262: 15-37.

Bayer R, Kalusche D, Kaufmann R, and Mannhold R (1975). Inotropic and

electrophysiological actions of verapamil and D600 in mammalian myocardium III. Effects of the optical isomers on transmembrane action potentials. Naunyn-Schmied. Arch. Pharmacol. 290: 81-97.

Beeler GW and McGuigan JAS (1978). Voltage clamping of multicellular myocardial preparations: capabilities and limitations of existing methods. Prog. Biophys. Mol. Biol. 34: 219-254.

Beeler GW and Reuter H (1970a). Voltage clamp experiments on ventricular myocardial fibres. J. Physiol. 207: 165-190.

Beeler GW and Reuter H (1970b). Membrane calcium current in ventricular myocardial fibres. J. Physiol. 207: 191-209.

Beeler GW and Reuter H (1970c). The relation between membrane potential, membrane currents and activation of contraction in ventricular myocardial fibres. J. Physiol. 207: 211-229.

Beresewicz A and Reuter H (1977). The effects of adrenaline and theophylline on action potential and contraction of mammalian ventricular muscle under "rested-state" and "steady-state" stimulation. Naunyn-Schmied. Arch. Pharmacol. 301: 99-107.

Besch HR, Jones LR, and Maddock KA. "Effects of calcium antagonists on sarcoplasmic reticular and sarcolemmal vesicles from canine heart" in New Perspectives on Calcium Antagonists, GB Weiss, ed. Baltimore: Waverly Press Inc., 1981, pp. 47-57.

Blayney L, Thomas H, Muir J, and Henderson A (1978). Action of caffeine on calcium transport by isolated fractions of myofibrils, mitochondria and sarcoplasmic reticulum from rabbit heart. Circ. Res. 43: 520-526.

Blinks JR, Olson CB, Jewell BR, and Braveny P (1972). Influence of caffeine and other methylxanthines on mechanical properties of isolated mammalian heart muscle. Evidence for a dual mechanism of action. Circ. Res. 30: 367-392.

Bogdanov KY, Zakharov SI, and Rosenshtraukh LV (1979). The origin of two components in contraction of guinea pig papillary muscle in the presence of noradrenaline. Can. J. Physiol. Pharmacol. 57: 866-872.

Bozler E (1952). Evidence for an ATP-actomyosin complex in relaxed muscle and its response to calcium ions. Am. J. Physiol. 168: 760-765.

Braveny P and Sumbera J (1970). Electromechanical correlations in the mammalian heart muscle. Pflugers Arch. 319: 36-48.

Braveny P and Sumbera J (1971). Interrelations between action potential and contractile response of heart muscle. Cardiovasc. Res. suppl. 1: 109-111.

Bravený P and Šumbera J (1972). Biphasic activation of the myocardial contraction. Physiol. Bohemoslov. 21: 73-74.

Bremel RD and Weber A (1972). Cooperation within actin filament in vertebrate skeletal muscle. Nature (New Biol.) 238: 97-101.

Brutsaert DL (1967). Frequency potentiation and paired stimulation potentiation of cat papillary muscles in calcium and in strontium containing media. Arch. Int. Physiol. Biochim. 75: 229-244.

Brutsaert DL and Claes VA (1974). Onset of mechanical activation of mammalian heart muscle in calcium- and strontium-containing solutions. Circ. Res. 35: 345-357.

Carmeliet E and Willems J (1971). The frequency dependent character of the membrane capacity in cardiac Purkyně fibres. J. Physiol. 213: 85-93.

Chapman RA (1979). Excitation-contraction coupling in cardiac muscle. Prog. Biophys. Molec. Biol. 35: 1-52.

Chapman RA and Ellis D (1977). The effects of manganese ions on the contraction of the frog's heart. J. Physiol. 272: 331-354.

Chiesi M and Inesi G (1981). Mg^{2+} and Mn^{2+} modulation of Ca^{2+} transport and ATPase activity in sarcoplasmic reticulum vesicles. Arch. Biochem. Biophys. 208: 586-592.

Chuck LHS and Parmley WW (1980). Caffeine removal of length-dependent changes in myocardial contractile state in the cat. Circ. Res. 47: 592-598.

Ciofalo FR (1973). Relationship between ^{3}H-ryanodine uptake and myocardial contractility. Am. J. Physiol. 225: 324-327.

Clark A and Olson CB (1973). Effects of caffeine and isoprenaline on mammalian ventricular muscle. Br. J. Pharmacol. 47: 1-11.

Coraboeuf E (1974). Membrane electrical activity and double component contraction in cardiac tissue. J. Mol. Cell. Cardiol. 6: 215-225.

Coraboeuf E, Deroubaix E, and Coulombe A (1979). Effect of tetrodotoxin on action potentials of the conducting system in the dog heart. Am. J. Physiol. 236: H561-H567.

Coraboeuf E, Deroubaix E, and Tazieff-Depierre F (1975). Effect of toxin II isolated from scorpion venom on action potential and contraction of mammalian heart. J. Mol. Cell. Cardiol. 7: 643-653.

Coraboeuf E, Gautier P, and Guiradou P (1981). Potential and tension changes induced by sodium in dog Purkinje fibres: role of an electrogenic sodium-calcium exchange. J. Physiol. 311: 605-622.

Deck KA and Trautwein W (1964). Ionic currents in cardiac excitation. Pflugers Arch. 280: 63-80.

Donaldson SKB, Best PM, and Kerrick WGL (1978). Characterization of the effects of Mg^{2+} on Ca^{2+}- and Sr^{2+}-activated tension generation of skinned rat cardiac fibers. J. Gen. Physiol. 71: 645-655.

Donaldson SKB and Kerrick WGL (1975). Characterization of the effects of Mg^{2+} on Ca^{2+}- and Sr^{2+}-activated tension generation of skinned skeletal muscle fibers. J. Gen. Physiol. 66: 427-444.

Dudel J, Peper K, Rudel R, and Trautwein W (1967). The potassium component of membrane current in Purkinje fibres. Pflugers Arch. 296: 308-327.

Ebashi S (1976). Excitation-contraction coupling. Ann. Rev. Physiol. 38: 293-313.

Ebashi S and Endo M (1968). Ca ion and muscle contraction. Prog. Biophys. Mol. Biol. 18: 123-183.

Ebashi S, Kodama A, and Ebashi F (1968). Troponin I. Preparation and physiological function. J. Biochem. 64: 465-477.

Ebashi S, Ohnishi S, Abes S, and Maruyana K (1975). A spin-label study on calcium-induced conformational changes of troponin components. J. Biochem. 75: 211-213.

Edman KAP and Johannsson M (1976). The contractile state of rabbit papillary muscle in relation to stimulation frequency. J. Physiol. 254: 565-581.

Edwards C, Lorkovic H, and Weber A (1966). The effect of the replacement of calcium by strontium on excitation-contraction coupling in frog skeletal muscle. J. Physiol. 186: 295-306.

Eisner DA and Lederer WJ (1979). Inotropic and arrhythmogenic effects of potassium-depleted solutions on mammalian cardiac muscle. J. Physiol. 294: 255-277.

Eisner DA, Lederer WJ, and Noble D (1979). Caffeine and tetracaine abolish the slow inward current in sheep cardiac Purkinje fibres. J. Physiol. 293: 76P-77P.

Endo M (1977). Calcium release from the sarcoplasmic reticulum. Physiol. Rev. 57: 71-108.

Endo M and Nakajima Y (1973). Release of calcium induced by "depolarisation" of the sarcoplasmic reticulum membrane. Nature (New Biol.) 246: 216-218.

Endo M, Tanaka M, and Ogawa Y (1970). Calcium induced release of calcium from the sarcoplasmic reticulum of skinned skeletal fibres. Nature

228: 34-36.

Endoh M and Iijima T (1981). Twitch potentiation by rest in canine ventricular muscle: effects of theophylline. Am. J. Physiol. 241: H583-H590.

Fabiato A and Fabiato F (1972). Excitation-contraction coupling of isolated cardiac fibers with disrupted or closed sarcolemmas. Calcium-dependent cyclic and tonic contractions. Circ. Res. 31: 293-307.

Fabiato A and Fabiato F (1975). Contractions induced by a calcium-triggered release of calcium from the sarcoplasmic reticulum of single skinned cardiac cells. J. Physiol 249: 469-495.

Fabiato A and Fabiato F (1979). Calcium and cardiac excitation-contraction coupling. Ann. Rev. Physiol. 41: 473-484.

Fawcett DW and McNutt NS (1969). The ultrastructure of the cat myocardium. I. Ventricular papillary muscle. J. Cell. Biol. 42: 1-45.

Fleckenstein A (1977). Specific pharmacology of calcium in myocardium, cardiac pacemaker, and vascular smooth muscle. Ann. Rev. Pharmacol. Toxicol. 17: 149-166.

Ford LE and Podolsky RJ (1970). Regenerative calcium release within muscle cells. Science 167: 58-59.

Fozzard HA (1977). Heart: excitation contraction coupling. Ann. Rev. Physiol. 39: 201-220.

Fozzard HA and Gibbons WR (1978). Electrical aspects of excitation-contraction coupling. Rec. Adv. Stud. Card. Struct. Metab. 11: 3-10.

Frank GB (1962). Utilization of bound calcium in the action of caffeine and certain multivalent cations on skeletal muscle. J. Physiol. 163: 254-268.

Frank M and Sleator WW (1975). Effect of ryanodine on myocardial calcium. Naunyn-Schmied. Arch. Pharmacol. 290: 35-47.

Garb S (1951). The effects of potassium, ammonium, calcium, strontium, and magnesium on the electrogram and myogram of mammalian heart muscle. J. Pharmacol. Exp. Ther. 101: 317-326.

Gergely J (1976). Excitation-contraction coupling - cardiac muscle events in the myofilament. Fed. Proc. 35: 1283-1287.

Gibbons WR and Fozzard HA (1971a). High potassium and low sodium contractures in sheep cardiac muscle. J. Gen. Physiol. 58: 483-510.

Gibbons WR and Fozzard HA (1971b). Voltage dependence and time depen-

dence of contraction in sheep cardiac Purkinje fibers. Circ. Res. 28: 446-460.

Glitsch HG, Reuter H, and Scholz H (1970). The effect of the internal sodium concentration on calcium fluxes in isolated guinea-pig auricles. J. Physiol. 209: 25-43.

Greenspan AM and Morad M (1975). Electromechanical studies on the inotropic effects of acetylstrophanthidin in ventricular muscle. J. Physiol. 253: 357-384.

Hagiwara S and Byerly L (1981). Membrane biophysics of calcium currents. Fed. Proc. 40: 2220-2225.

Hagiwara S and Naka K (1964). The initiation of spike potential in barnacle muscle fibers under low intracellular Ca++. J. Gen. Physiol. 48: 141-162.

Hagiwara S and Nakajima S (1965). Tetrodotoxin and manganese ion: effects on action potential of the frog heart. Science 149: 1254-1255.

Hagiwara S and Takahashi K (1967). Surface density of calcium ions and calcium spikes in the barnacle muscle fiber membrane. J. Gen. Physiol. 50: 583-601.

Haldimann C (1963). Effet de tétraéthylammonium sur les potentiels de repos et d'action du coeur de mouton. Arch. Int. Pharmacodyn. Ther. 146: 1-9.

Heilbrunn LV and Wiercinski FJ (1947). The actions of various cations on muscle protoplasm. J. Cell. Comp. Physiol. 29: 15-32.

deHemptinne A, Weyne J, and Leusen I (1967). Dynamic parameters of myocardial contractility under influence of calcium and strontium. Arch. Int. Physiol. Biochim. 75: 96-108.

Henderson AH and Cattell MR (1976). Prolonged biphasic strontium-mediated contractions of cat and frog heart muscle and their response to inotropic influences. J. Mol. Cell. Cardiol. 8: 299-319.

Hermsmeyer K, Rulon R, and Sperelakis N (1972). Loss of the plateau of the cardiac action potential in hypertonic solutions. J. Gen. Physiol. 59: 779-793.

Hiraoka M and Sano T (1978). Role of slow inward current on premature excitation in ventricular muscle. Rec. Adv. Stud. Card. Struct. Metab. 11: 31-36.

Hoar PE and Kerrick WGL (1979). Rabbit diaphragm: two types of fibres determined by calcium strontium activation and protein content. J. Physiol. 295: 345-362.

Holloway JH and Reilly CN (1960). Metal chelate stability constants of amino polycarboxylate ligands. Analyt. Chem. 32: 249-256.

Honerjäger P and Reiter M (1975). The relation between the effects of veratridine on action potential and contraction in mammalian ventricular myocardium. Naunyn-Schmied. Arch. Pharmacol. 289: 1-28.

Horackova M and Vassort G (1976a). Regulation of tonic tension in frog atrial muscle by voltage dependent Na-Ca exchange. J. Physiol. 258: 77P-78P.

Horackova M and Vassort G (1976b). Calcium conductance in relation to contractility in frog myocardium. J. Physiol. 259: 597-616.

Huxley AF and Taylor RE (1958). Local activation of striated muscle fibres. J. Physiol. 144: 426-441.

Inomata H and Kao CY (1979). Ionic mechanisms of repolarization in the guinea-pig taenia coli as revealed by the actions of strontium. J. Physiol. 297: 443-462.

Isenberg G (1977a). Cardiac Purkinje fibres. Resting, action, and pacemaker potential under the influence of $[Ca^{2+}]i$ as modified by intracellular injection techniques. Pflugers Arch. 371: 51-59.

Isenberg G (1977b). Cardiac Purkinje fibres. The slow inward current component under the influence of modified $[Ca^{2+}]i$. Pflugers Arch. 371: 61-69.

Isenberg G (1977c). Cardiac Purkinje fibres. $[Ca^{2+}]i$ controls steady state potassium conductance. Pflugers Arch. 371: 71-76.

Isenberg G (1977d). Cardiac Purkinje fibres. $[Ca^{2+}]i$ controls the potassium permeability via the conductance components gk1 and gk2. Pflugers Arch. 371: 77-85.

Johnson EA and Lieberman M (1971). Heart: excitation and contraction. Ann. Rev. Physiol. 33: 479-532.

Jones LR, Besch Jr HR, Sutko JL, and Willerson JT (1979). Ryanodine-induced stimulation of net Ca++ uptake by cardiac sarcoplasmic reticulum vesicles. J. Pharmacol. Exp. Ther. 209: 48-55.

Kass RS and Tsien RW (1975). Multiple effects of calcium antagonists on plateau currents in cardiac Purkinje fibers. J. Gen. Physiol. 66: 169-192.

Kass RS and Tsien RW (1976). Control of action potential duration by calcium ions in cardiac Purkinje fibers. J. Gen. Physiol. 67: 599-617.

Katz B and Miledi R (1969). Tetrodotoxin-resistant electric activity in presynaptic terminals. J. Physiol. 203: 459-487.

Kaufmann R, Bayer R, Furniss T, Krause H, and Tritthart H (1974). Calcium-movement controlling cardiac contractility II. Analog computation of cardiac excitation-contraction coupling on the basis of calcium kinetics in a multi-compartment model. J. Mol. Cell. Cardiol. 6: 543-559.

Kavaler F (1959). Membrane depolarization as a cause of tension development in mammalian ventricular muscle. Am. J. Physiol. 197: 968-970.

Kavaler F, Anderson TW, and Fisher VJ (1978). Sarcolemmal site of caffeine's inotropic action on ventricular muscle of the frog. Circ. Res. 42: 285-290.

Kawata H and Hatae J (1977). Contractile behavior of cardiac ventricular muscle in strontium solution. Jap. J. Physiol. 27: 167-184.

Kenyon JL and Gibbons WR (1979). Influence of chloride, potassium, and tetraethylammonium on the early outward current of sheep cardiac Purkinje fibers. J. Gen. Physiol. 73: 117-138.

Kerrick WGL, Hoar PE, Malencik DA, Stamps L, and Fischer EH (1979). Characterization of Ca^{2+}- and Sr^{2+}-activated tension in functionally skinned chicken fibers of normal and dystrophic skeletal and normal cardiac muscle. Pflugers Arch. 381: 53-62.

Kerrick WGL, Malencik DA, Hoar PE, Potter JD, Coby RL, Pocinwong S, and Fischer EH (1980). Ca^{2+} and Sr^{2+} activation: comparison of cardiac and skeletal muscle contraction models. Pflugers Arch. 386: 207-213.

King BW and Bose D (1981). Strontium-induced biphasic contractions of canine ventricle. Pharmacologist 23: 185.

Koch-Weser J and Blinks JR (1963). The influence of the interval between beats on myocardial contractility. Pharmacol. Rev. 15: 601-652.

Kohlhardt M, Bauer B, Krause H, and Fleckenstein A (1973a). Selective inhibition of the transmembrane Ca conductivity of mammalian myocardial fibres by Ni, Co and Mn ions. Pflugers Arch. 338: 115-123.

Kohlhardt M, Haastert HP, and Krause H (1973b). Evidence of non-specificity of the Ca channel in mammalian myocarcial fibre membranes. Substitution of Ca by Sr, Ba or Mg as charge carriers. Pflugers Arch. 342: 125-136.

Kohlhardt M, Herdey A, and Kubler M (1973c). Interchaneability of Ca ions and Sr ions as charge carriers of the slow inward current in mammalian myocardial fibres. Pflugers Arch. 344: 149-158.

Kohlhardt M, Mnich Z, and Haap K (1979). Analysis of the inhibitory effect of Ni ions on slow inward current in mammalian ventricular myocardium. J. Mol. Cell. Cardiol. 11: 1227-1243.

Lakatta EG and Spurgeon HA (1980). Force staircase kinetics in mammalian cardiac muscle: modulation by muscle length. J. Physiol. 299: 337-352.

Langer GA (1973). Heart: excitation-contraction coupling. Ann. Rev. Physiol. 35: 55-86.

Langer GA (1977). Ionic basis of myocardial activity. Ann. Rev. Med. 28: 13-20.

Lee KS and Klaus W (1971). The subcellular basis for the mechanism of inotropic action of cardiac glycosides. Pharmacol. Rev. 23: 193-261.

Levitsky DO, Benevolensky DS, Levchenko TS, Smirnov VN, and Chazov EI (1981). Calcium-binding rate and capacity of cardiac sarcolasmic reticulum. J. Mol. Cell. Cardiol. 13:785-796.

Linden J and Brooker G (1980). Properties of cardiac contractions in zero sodium solutions: intracellular free calcium controls slow channel conductance. J. Mol. Cell. Cardiol. 12: 457-478.

Locke FS and Rosenheim O (1907). Contributions to the physiology of the isolated heart. The consumption of dextrose by mammalian cardiac muscle. J. Physiol. 36: 205-220.

MacLennan DH and Wong PTS (1971). Isolation of a calcium-sequestering protein from sarcoplasmic reticulum. Proc. Natl. Acad. Sci. USA 68: 1231-1235.

Marban E (1981). Inhibition of transient outward current by intracellular ion substitution unmasks slow inward calcium current in cardiac Purkinje fibers. Pflugers Arch. 390: 102-106.

McAllister RE and Noble D (1966). The time and voltage dependence of the slow outward current in cardiac Purkinje fibres. J. Physiol. 186: 632-662.

McAllister RE, Noble D, and Tsien RW (1975). Reconstruction of the electrical activity of cardiac Purkinje fibres. J. Physiol. 251: 1-59.

McDonald TF, Nawrath H, and Trautwein W (1975). Membrane currents and tension in cat ventricular muscle treated with cardiac glycosides. Circ. Res. 37: 674-682.

Mermier P and Hasselbach W (1976). Comparison between strontium and calcium uptake by the fragmented sarcoplasmic reticulum. Eur. J. Biochem. 69: 79-86.

Mines GR (1913). On functional analysis by the action of electrolytes. J. Physiol. 46: 189-235.

Moisescu DG and Thieleczek R (1978). Calcium and strontium concentration changes within skinned muscle preparations following a change in the external bathing solution. J. Physiol. 275: 241-262.

Moisescu DG and Thieleczek R (1979). Sarcomere length effects on the Sr^{2+}- and Ca^{2+}- activation curves in skinned frog muscle fibres. Biochim. Biophys. Acta 546: 64-76.

Morad M and Goldman Y (1973). Excitation-contraction coupling in heart muscle: membrane control of development of tension. Prog. Biophys. Molec. Biol. 27: 257-313.

Morad M and Orkand RK (1971). Excitation-contraction coupling in frog ventricle: evidence from voltage clamp studies. J. Physiol. 219: 167-189.

Morad M and Trautwein W (1968). The effect of the duration of the action potential on contraction in the mammalian heart muscle. Pflugers Arch. 299: 66-92.

Mullins LJ. Ion transport in the heart. New York: Raven Press, 1981.

Nayler WG and Emery PF (1962). Effect of strontium on cardiac contractility and membrane resting potential. Am. J. Physiol. 203: 844-848.

New W and Trautwein W (1972a). Inward membrane currents in mammalian myocardium. Pflugers Arch. 334: 1-23.

New W and Trautwein W (1972b). The ionic nature of slow inward current and its relation to contraction. Pflugers Arch. 334: 24-38.

Niedergerke R (1957). The rate of action of calcium ions on the contraction of the heart. J. Physiol. 138: 506-515.

Niedergerke R and Harris EJ (1957). Accumulation of calcium (or strontium) under conditions of increasing contractility. Nature 179: 1068-1069.

Niedergerke R and Orkand RK (1966). The dual effect of calcium on the action potential of the frog's heart. J. Physiol. 184: 291-311.

Noble D and Tsien RW (1968). The kinetic and rectifier properties of the slow potassium current in cardiac Purkinje fibres. J. Physiol. 195: 185-214.

Noble D and Tsien RW (1969). Outward membrane currents activated in the plateau range of potentials in cardiac Purkinje fibres. J. Physiol. 200: 205-231.

Noble S and Shimoni Y (1981a). The calcium and frequency dependence of the slow inward current "staircase" in frog atrium. J. Physiol. 310: 57-75.

Noble S and Shimoni Y (1981b). Voltage-dependent potentiation of the slow inward current in frog atrium. J. Physiol. 310: 77-95.

Ochi R (1970). The slow inward current and the action of manganese ions in guinea-pig's myocardium. Pflugers Arch. 316: 81-94.

Ochi R (1977). Electrical uncoupling by Sr action potentials in cardiac muscle. Experientia 33: 1187-1189.

Ochi R and Trautwein W (1971). The dependence of cardiac contraction on depolarization and slow inward current. Pflugers Arch. 323: 187-203.

Ohba M (1973). Effects of caffeine on tension development in dog papillary muscle under voltage clamp. Jap. J. Physiol. 23: 47-58.

Ong SD (1972). Studies on excitation-contraction coupling in cardiac muscle. Ph.D. thesis. University of Manitoba.

Ong SD and Bailey LE (1973). Uncoupling of excitation by Ni in cardiac muscle. Am. J. Physiol. 224: 1092-1098.

Pappano AJ (1970). Calcium-dependent action potentials produced by catecholamines in guinea pig atrial muscle fibers depolarized by potassium. Circ. Res. 27: 379-390.

Payet MD, Schanne OF, and Ruiz-Ceretti E (1981). Frequency dependence of the ionic currents determining the action potential repolarization in rat ventricular muscle. J. Mol. Cell. Cardiol. 13: 207-215.

Penefsky ZJ (1974a). Studies on mechanism of inhibition of cardiac muscle contractile tension by ryanodine. Mechanical response. Pflugers Arch. 347: 173-184.

Penefsky ZJ (1974b). Ultrastructural studies of the site of action of ryanodine on heart muscle. Pflugers Arch. 347: 185-198.

Philipson KD and Langer GA (1979). Sarcolemmal-bound calcium and contractility in the mammalian myocardium. J. Mol. Cell. Cardiol. 11: 857-875.

Pidgeon J, Lab M, Seed A, Elzinga G, Papadoyannis D, and Noble MIM (1980). The contractile state of cat and dog heart in relation to the interval between beats. Circ. Res. 47: 559-567.

Porter KR and Palade GE (1957). Studies on the endoplasmic reticulum: III its form and distribution in striated muscle cells. J. Biophys. Biochem. Cytol. 3: 269-300.

Reder RF, Miura DS, Danilo Jr P, and Rosen MR (1981). The electrophysiological properties of normal neonatal and adult canine cardiac Purkinje fibers. Circ. Res. 48: 658-668.

Reeves JP and Sutko JL (1980). Sodium-calcium exchange activity generates a current in cardiac membrane vesicles. Science 208: 1461-1464.

Reiter M, Seibel K, and Karema E (1978). The inotropic action of noradrenaline on rested-state contractions of guinea-pig cardiac ventricular muscle. Life Sci. 22: 1149-1158.

Reuter H (1967). The dependence of slow inward current in Purkinje fibres on the extracellular calcium-concentration. J. Physiol. 192: 479-492.

Reuter H (1973). Divalent cations as charge carriers in excitable membranes. Prog. Biophys. Mol. Biol. 26. 3-43.

Reuter H (1974). Exchange of calcium ions in the mammalian myocardium. Mechanisms and physiological significance. Circ. Res. 34: 599-605.

Reuter H (1979). Properties of two inward membrane currents in the heart. Ann. Rev. Physiol. 41: 413-424.

Reuter H and Scholz H (1977a). A study of the ion selectivity and the kinetic properties of the calcium dependent slow inward current in mammalian cardiac muscle. J. Physiol. 264: 17-47.

Reuter H and Scholz H (1977b). The regulation of the calcium conductance of cardiac muscle by adrenaline. J. Physiol. 264: 49-62.

Reuter H and Seitz N (1968). The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. J. Physiol. 195: 451-470.

Ringer S (1883a). A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. J. Physiol. 4: 29-42.

Ringer S (1883b). An investigation regarding the action of rubidium and caesium salts compared with the action of potassium salts on the ventricle of the frog's heart. J. Physiol. 4: 370-

Ringer S and Sainsbury H (1883). An investigation regarding the action of strontium and barium salts compared with the action of lime on the ventricle of the frog's heart. Practitioner 31: 81-93.

Rosenshtraukh LV, Bogdanov KY, and Zakharov SI (1980). The complex structure of mammalian heart contraction. J. Mol. Cell. Cardiol. 12 suppl. 1: 138.

Roulet M, Mongo KG, Vassort G, and Ventura-Clapier R (1979). The dependence of twitch relaxation on sodium ions and on internal Ca^{2+} stores in voltage clamped frog atrial fibres. Pflugers Arch. 379: 259-268.

Sanborn WG and Langer GA (1970). Specific uncoupling of excitation and contraction in mammalian cardiac tissue by lanthanum. J. Gen.

Physiol. 56: 191-217.

Sandow A (1952). Excitation-contraction coupling in muscular response. Yale J. Biol. Med. 25: 176-201.

Sassine A and Bernard C (1972). Étude comparée des effets des ions calcium et strontium sur les permeabilités de la membrane myocardique. J. Physiol. (Paris) 65 suppl. 3: 497A.

Schulze JJ (1981). Observations on the staircase phenomenon in guinea pig atrium. Pflugers Arch. 391: 9-16.

Seaman KL and Bose D (1981). The effects of caffeine and calcium antagonists on the force-frequency relationship in dog papillary muscle. Pharmacologist 23(3): 198.

Seibel K, Karema E, Takeya K, and Reiter M (1978). Effect of noradrenaline on an early and a late component of the myocardial contraction. Naunyn-Schmied. Arch. Pharmacol. 305: 65-74.

Sheu S (1981). Effect of ouabain on intracellular Na and Ca activities in sheep cardiac Purkinje fibers. Fed. Proc. 40: 562.

Shine KI and Langer GA (1971). Caffeine effects upon contractions and calcium exchange in rabbit myocardium. J. Mol. Cell. Cardiol. 3: 255-270.

Siegelbaum SA, Tsien RW, and Kass RS (1977). Role of intracellular calcium in the transient outward current of calf Purkinje fibres. Nature 269: 611-613.

Šimurda J, Šimurdova M, Braveny P, and Šumbera J (1981). Activity-dependent changes of slow inward current in ventricular heart muscle. Pflugers Arch. 391: 277-283.

Solaro RJ and Briggs FN (1974). Estimating the functional capabilities of sarcoplasmic reticulum in cardiac muscle. Circ. Res. 34: 531-540.

Solaro RJ, Wise RM, Shiner JS, and Briggs FN (1974). Calcium requirements for cardiac myofibrilar activation. Circ. Res. 34: 525-530.

Somlyo AV and Somlyo AP (1971). Strontium accumulation by sarcoplasmic reticulum and mitochondria in vascular smooth muscle. Science 174: 955-958.

Sonnenblick EH (1967). Active state in heart muscle. Its delayed onset and modification by inotropic agents. J. Gen. Physiol. 50: 661-676.

Standen NB and Stanfield PR (1978). A potential- and time-dependent blockade of inward rectification in frog skeletal muscle fibres by barium and strontium ions. J. Physiol. 280: 169-191.

Steel RGD and Torrie JH. Principles and Procedures of Statistics. New York: McGraw-Hill, 1960.

Sutko JL, Willerson JT, Templeton GH, Jones LR, and Besch Jr HR (1979). Ryanodine: its alterations of cat papillary muscle contractile state and responsiveness to inotropic interventions and a suggested mechanism of action. J. Pharmacol. Exp. Ther. 209: 37-47.

Thomas LJ (1957). An antagonism in the action of calcium and strontium ions on the frog's heart. J. Cell. Comp. Physiol. 50: 249-264.

Trautwein W (1973). Membrane currents in cardiac muscle fibers. Physiol. Rev. 53: 793-835.

Trautwein W, McDonald TF, and Tripathi O (1975). Calcium conductance and tension in mammalian ventricular muscle. Pflugers Arch. 354: 55-74.

Tritthart H, Kaufmann R, Volkmer HP, Bayer R, and Krause H (1973a). Ca-movement controlling myocardial contractility I. Voltage-, current- and time-dependence of mechanical activity under voltage clamp conditions (cat papillary muscles and trabeculae). Pflugers Arch. 338: 207-231.

Tritthart H, MacLeod DP, Stierle HE, and Krause H (1973b). Effects of Ca-free and EGTA-containing Tyrode solution on transmembrane electrical activity and contraction in guinea pig papillary muscle. Pflugers Arch. 338: 361-376.

Van Kerkhove E and Carmeliet E (1971). ^{85}Sr movements in cardiac Purkyne fibres. J. Physiol. (Paris) 63: 147A.

Van Winkle WB and Entman ML (1979). Comparative aspects of cardiac and skeletal sarcoplasmic reticulum. Life Sci. 25: 1189-1200.

Vassalle M (1979). Electrogenesis of the plateau and pacemaker potential. Ann. Rev. Physiol. 41: 425-440.

Vassort G (1973). Influence of sodium on the regulation of frog myocardial contractility. Pflugers Arch. 339: 225-240.

Vassort G and Rougier O (1972). Membrane potential and slow inward current dependence of frog cardiac mechanical activity. Pflugers Arch. 331: 191-203.

Verdonck F and Carmeliet E (1971). Isometric contractions in cardiac Purkyne fibres: characteristics in Na free Sr Tyrode. Cardiovasc. Res. suppl. 1: 76-83.

Vereecke J and Carmeliet E (1971a). Sr action potentials in cardiac Purkyne fibres I. Evidence for a regenerative increase in Sr conductance. Pflugers Arch. 322: 60-72.

Vereecke J and Carmeliet E (1971b). Sr action potentials in cardiac

Purkyne fibres II. Dependence of the Sr conductance on the external Sr concentration and Sr-Ca antagonism. Pflugers Arch. 322: 73-82.

Vierling W and Reiter M (1979). An intracellularly induced positive inotropic effect of manganese in guinea-pig ventricular myocardium. Naunyn-Schmied. Arch. Pharmacol. 306: 249-253.

Vitek M and Trautwein W (1971). Slow inward current and action potential in cardiac Purkinje fibres. The effect of Mn++-ions. Pflugers Arch. 323: 204-218.

Wakabayashi S and Goshima K (1981). Kinetic studies on sodium-dependent calcium uptake by myocardial cells and neuroblastoma cells in culture. Biochem. Biophys. Acta 642: 158-172.

Weidmann S (1974). Heart: electrophysiology. Ann. Rev. Physiol. 36: 155-169.

Weyne J (1966a). Effects of strontium ions on heart muscle I. Influences on contractility. Arch. Int. Physiol. Biochim. 74: 449-460.

Weyne J (1966b). Effects of strontium ions on heart muscle II. Influence on excitability, conduction and potassium efflux. Arch. Int. Physiol. Biochim. 74: 461-475.

Wiemer G, Kaiser G, and Palm D (1978). Effects of Mg^{2+}, Mn^{2+} and Ca^{2+} on adenylcyclase activity. Evidence for a metallic site. Naunyn-Schmied. Arch. Pharmacol. 303: 145-152.

Wier WG (1980). Calcium transients during excitation-contraction coupling in mammalian heart: aequorin signals of canine Purkinje fibers. Science 207: 1085-1087.

Winegrad S (1968). Intracellular calcium movements of frog skeletal muscle during recovery from tetanus. J. Gen. Physiol. 51: 65-83.

Winegrad S (1973). Intracellular calcium binding and release in frog heart. J. Gen. Physiol. 62: 693-706.

Winegrad S. "Electromechanical coupling in heart muscle" in Handbook of Physiology section II: the circulation, RM Berne, ed. Baltimore: Waverly Press, 1979, pages 393-428.

Wood EH, Heppner RL, and Weidmann S (1969). Inotropic effects of electric currents. I. Positive and negative effects of constant electric currents or current pulses applied during cardiac action potential. II. Hypothesis: calcium movements, excitation-contraction coupling and inotropic effects. Circ. Res. 24: 409-445.

APPENDIX

```
REM *************** SAMANA.BAS *************** BRIAN KING
20 REM
30 REM  PROGRAM TO ACCEPT VALUES FROM DIGITIZER AND
40 REM    STORE THEM IN SEQUENTIAL FILES
50 REM  CONTAINS CALCULATIONS FOR AREA UNDER A CURVE AND
60 REM    FORCE-INTERVAL RELATIONSHIP DATA (PAUSE, PESP,
70 REM    AND STEADY STATE TENSION)
80 REM
90 DIM C5(50)
100 PRINT "INPUT, ANALYZE (A), OR PRINTOUT (P)--"; \ INPUT B$
110 IF B$="A" GO TO 670
120 IF B$="P" GO TO 1080
130 PRINT "FILENAME"; \ INPUT F$
140 IF F$="" THEN F$="D" \ REM  DEFAULT FILENAME IS "D"
150 OPEN F$ FOR OUTPUT AS FILE #1
160 PRINT "AREA CALCULATION (Y)--"; \ INPUT A$
170 IF A$="Y" GO TO 1640
180 PRINT "CALCULATE BASELINE (Y)--"; \ INPUT B$
190 IF B$="Y" GO TO 390
200 REM ********** DIGITIZE TOP AND BOTTOM OF PEAK **********
210 PRINT "WHEN FINISHED ENTERING DATA, TYPE 'END'"
220 PRINT "RESET CURSOR AT BOTTOM LEFT AND START"
230 PRINT "TERMINAL BEEPS WHEN A POINT HAS BEEN ENTERED"
240 IF B$="Y" GO TO 390
250 INPUT V$
260 FOR I=1 TO 1.00000E+06
270 FOR J=1 TO 2
280 INPUT V$
290 IF V$="END" GO TO 370
300 IF LEN(V$)<>13 GO TO 280
310 Y$=SEG$(V$,9,13) \ Y(J)=VAL(Y$)
320 PRINT CHR$(7)
330 NEXT J
340 Y=Y(2)-Y(1)
350 PRINT #1,Y \ PRINT Y
360 NEXT I
370 PRINT  \ GO TO 620
380 REM ********** CALCULATE BASELINE POINTS OF PEAKS **********
390 PRINT "ENTER BASELINE POINT OF FIRST CONTROL PEAK AND LAST PEAK"
400 PRINT "THEN ENTER PEAK POINTS" \ PRINT
410 INPUT V$ \ REM-- RESET CURSOR AT BOTTOM LEFT
420 FOR I=1 TO 2
430 INPUT V$
440 IF V$="END" GO TO 620
450 IF LEN(V$)<>13 GO TO 430
460 X$=SEG$(V$,3,7) \ X(I)=VAL(X$)
470 Y$=SEG$(V$,9,13) \ Y(I)=VAL(Y$)
480 PRINT CHR$(7)
490 NEXT I
500 S=(Y(2)-Y(1))/(X(2)-X(1))
510 B=(Y(1)+Y(2)-(S*(X(1)+X(2))))/2 \ REM-- B= Y INTERCEPT
520 FOR I=1 TO 100000
530 INPUT V$
540 IF V$="END" GO TO 620
```

```
550 IF LEN(V$)<>13 GO TO 530
560 X$=SEG$(V$,3,7) \ X=VAL(X$)
570 Y$=SEG$(V$,9,13) \ Y=VAL(Y$)
580 Y1=S*X+B \ Y=Y-Y1
590 PRINT #1,Y \ PRINT Y
600 PRINT CHR$(7)
610 NEXT I
620 CLOSE #1 \ PRINT  \ PRINT "ANALYZE (Y)--"; \ INPUT B$
630 IF B$="Y" GO TO 680
640 GO TO 1040
650 REM ********** CALCULATION OF TENSION AND % OF CONTROL **********
660 REM   CAPABLE OF STORING RESULTS ON FILE
670 PRINT "FILENAME--"; \ INPUT F$
680 OPEN F$ FOR INPUT AS FILE #1
690 PRINT "STORE RESULTS ON FILE (Y)"; \ INPUT R$
700 PRINT "DO YOU WANT A PRINTOUT (Y)--"; \ INPUT P9$
710 PRINT "TITLE--"; \ INPUT T$
720 IF R$<>"Y" GO TO 760
730 PRINT "RESULT FILENAME"; \ INPUT F1$
740 OPEN F1$ FOR OUTPUT AS FILE #2
750 PRINT #2,F1$+".DAT" \ PRINT #2 \ PRINT #2,"********** ";T$;" **********"
760 PRINT "PAUSE, PESP, OR S.S. DATA (PA, P, OR S)--"; \ INPUT R1$
770 IF R1$="P" GO TO 1340
780 IF R1$="S" GO TO 820
790 REM ********** PAUSE DATA CALCULATION **********
800 PRINT "HOW MANY CONTROL PEAKS"; \ INPUT N
810 PRINT "HOW MANY PEAKS AFTER"; \ INPUT P
820 PRINT "CALIBRATION (mm/gm)"; \ INPUT C1
830 IF R1$="S" GO TO 1120
840 FOR I=1 TO 1.00000E+06 \ C=0
850 IF END #1 GO TO 1020
860 PRINT  \ PRINT "PAUSE #";I \ PRINT
870 IF R$="Y" THEN PRINT #2,"PAUSE #";I
880 FOR J=1 TO N
890 IF END #1 GO TO 1020
900 INPUT #1,Y
910 C=C+Y
920 NEXT J
930 C=C/N
940 FOR K=1 TO P
950 IF END #1 GO TO 1020
960 INPUT #1,Y
970 Q%=INT(Y/C*100) \ REM-- Q%=% OF CONTROL
980 G=Y/10/C1 \ REM-- G=GRAMS OF TENSION
990 IF R$="Y" THEN PRINT #2,G;" g",Q%;" %"
1000 PRINT G;" g",Q%;" %"
1010 NEXT K \ NEXT I
1020 PRINT  \ CLOSE
1030 IF P9$="Y" THEN  GOSUB 1940
1040 PRINT "INPUT (I), ANALYZE (A), PRINTOUT (P), OR STOP--"; \ INPUT B$
1050 IF B$="A" GO TO 670
1060 IF B$="I" GO TO 130
1070 IF B$<>"P" GO TO 1100
1080 PRINT "FILENAME--"; \ INPUT F1$
```

```
1090 GOSUB 1940 \ CLOSE  \ GO TO 1040
1100 STOP
1110 REM ********** STEADY STATE DATA CALCULATIONS **********
1120 PRINT "HOW MANY STEADY STATES--"; \ INPUT S1
1130 PRINT "HOW MANY CONTRACTIONS AT EACH S.S.--"; \ INPUT C3
1140 PRINT "WHAT ARE THE CYCLE LENGTHS (sec)--"
1150 FOR I=1 TO S1 \ INPUT C5(I) \ NEXT I
1160 FOR J=1 TO S1
1170 PRINT "CYCLE LENGTH";C5(J);"SECONDS"
1180 PRINT "GRAMS" \ G9=0
1190 IF R$="Y" THEN PRINT #2,"CYCLE LENGTH";C5(J);"SECONDS" \ PRINT #2
1200 IF R$="Y" THEN PRINT #2,"STEADY STATE GRAMS TENSION"
1210 FOR J1=1 TO C3
1220 IF END #1 THEN PRINT  \ GO TO 1020
1230 INPUT #1,Y \ G=Y/10/C1 \ REM-- G=GRAMS TENSION OR mmHg
1240 G9=G9+G \ PRINT G;
1250 IF R$="Y" THEN PRINT #2,G;
1260 NEXT J1
1270 PRINT
1280 G9=INT(1000*G9/C3+.5)/1000 \ PRINT "AVERAGE--";G9
1290 IF R$="Y" THEN PRINT #2 \ PRINT #2,"AVERAGE--";G9 \ PRINT #2
1300 PRINT  \ NEXT J
1310 PRINT  \ CLOSE
1320 IF P9$="Y" THEN  GOSUB 1940
1330 GO TO 1040
1340 REM ********** PESP DATA CALCULATIONS **********
1350 PRINT "HOW MANY DIFFERENT INTERVALS--"; \ INPUT S1
1360 PRINT "HOW MANY CONTROL CONTRACTIONS--"; \ INPUT N
1370 PRINT "HOW MANY POST-CONTROL CONTRACTIONS--"; \ INPUT C3
1380 PRINT "BIPHASIC CONTRACTIONS (Y)--"; \ INPUT B$
1390 PRINT "STANDARD INTERVALS (N)--"; \ INPUT A$
1400 IF A$="N" GO TO 1450
1410 PRINT "FREQUENCY (in Hz)--"; \ INPUT F \ IF F=1 THEN J=.1
1420 IF F=.5 THEN J=.2
1430 IF F=.2 THEN J=.5
1440 K=1 \ FOR I=J TO 10*J STEP J \ C5(K)=I \ K=K+1 \ NEXT I \ GO TO 1470
1450 PRINT "INPUT CYCLE DELAYS--"
1460 FOR I=1 TO S1 \ INPUT C5(I) \ NEXT I
1470 PRINT "CALIBRATION (mm/gm or mm/10 mm Hg)--"; \ INPUT C1
1480 FOR I=1 TO S1
1490 PRINT "PESP INTERVAL";C5(I) \ A=0
1500 IF R$="Y" THEN PRINT #2,"PESP INTERVAL";C5(I)
1510 IF B$="Y" THEN  GOSUB 2010 \ GO TO 1580
1520 FOR J=1 TO N \ IF END #1 THEN 1020 \ INPUT #1,Y \ A=A+Y \ NEXT J
1530 A=A/N \ REM-- A=CONTROL AVERAGE
1540 FOR J=1 TO C3 \ IF END #1 THEN 1020 \ INPUT #1,Y
1550 Y1=INT(Y/C1*100+.5)/1000 \ Y%=INT(Y/A*100+.5) \ PRINT Y1;"g";TAB(15);Y%;"%"
1560 IF R$="Y" THEN PRINT #2,Y1;"g";TAB(15);Y%;"%"
1570 NEXT J
1580 PRINT  \ IF R$="Y" THEN PRINT #2
1590 NEXT I
1600 PRINT  \ CLOSE
1610 IF P9$="Y" THEN  GOSUB 1940
1620 GO TO 1040
```

```
1630 REM ********** AREA CALCULATION **********
1640 PRINT "TURN SWITCH ON WALL FROM PDP-11 TO DIGITIZER"
1650 PRINT "USING POINT MODE, RESET CURSOR AT BOTTOM LEFT"
1660 PRINT "PRESS STREAM OR SWITCH-STREAM MODE BUTTON"
1670 PRINT "DIGITIZE BASELINE FIRST, THEN ENTER A POINT AT BOTTOM RIGHT"
1680 PRINT "DIGITIZE CURVE NEXT, THEN ENTER A POINT AT BOTTOM RIGHT"
1690 PRINT "BE CAREFUL NOT TO MOVE THE TRACING AFTER DOING THE BASELINE"
1700 INPUT V$
1710 FOR I=1 TO 2
1720 FOR J=1 TO 100000
1730 INPUT V$
1740 IF LEN(V$)<>13 GO TO 1730
1750 X=VAL(SEG$(V$,3,7)) \ Y=VAL(SEG$(V$,9,13))
1760 IF X<2600 GO TO 1770 \ IF Y<200 GO TO 1790
1770 PRINT #1,Y \ PRINT #1,X
1780 NEXT J
1790 PRINT CHR$(7) \ PRINT "OKAY" \ PRINT  \ N(I)=J-1
1800 NEXT I
1810 CLOSE #1 \ OPEN F$ FOR INPUT AS FILE #1
1820 FOR I=1 TO 2
1830 INPUT #1,Y1 \ INPUT #1,X1 \ A=0
1840 FOR J=1 TO N(I)-1
1850 INPUT #1,Y2 \ INPUT #1,X2
1860 A=A+((Y1+Y2)/2*(X2-X1))
1870 Y1=Y2 \ X1=X2 \ NEXT J
1880 A(I)=A
1890 NEXT I \ A=A(2)-A(1) \ REM-- AREA IN SQUARE mm/100
1900 PRINT "CALIBRATION OF Y AXIS (mm/g)--"; \ INPUT Y1
1910 PRINT "CALIBRATION OF X AXIS (mm/s)--"; \ INPUT X1
1920 PRINT  \ PRINT "AREA IS";A/X1/Y1/100;"gram-seconds"
1930 GO TO 1020
1940 REM ********** PRINTOUT ROUTINE **********
1950 OPEN F1$ FOR INPUT AS FILE #1
1960 OPEN "LP:" FOR OUTPUT AS FILE #2
1970 IF END #1 THEN CLOSE  \ RETURN
1980 INPUT #1,F9$ \ PRINT #2,F9$
1990 GO TO 1970
2000 STOP
2010 REM ********** BIPHASIC PESP SUBROUTINE **********
2020 IF END #1 THEN RETURN
2030 INPUT #1,P1 \ INPUT #1,P2 \ REM CONTROL VALUES
2040 FOR J=1 TO C3 \ IF END #1 THEN RETURN
2050 INPUT #1,Y \ Y1=INT(Y/C1*100+.5)/1000 \ Y%=INT(Y/P1*100+.5)
2060 PRINT "P1";Y1;"g";Y%;"%";TAB(35);
2070 IF R$="Y" THEN PRINT #2,"P1";Y1;"g";Y%;"%";TAB(35);
2080 INPUT #1,Y \ Y1=INT(Y/C1*100+.5)/1000 \ Y%=INT(Y/P2*100+.5)
2090 PRINT "P2";Y1;"g";Y%;"%"
2100 IF R$="Y" THEN PRINT #2,"P2";Y1;"g";Y%;"%"
2105 NEXT J
2110 RETURN
```

```
10 REM *************** SAMAN2.BAS *************** BRIAN KING
20 REM
30 REM   PROGRAM TO FIND PEAK HEIGHT AND % OF CONTROL OF
40 REM   NK AND BIPHASIC CONTRACTIONS
50 REM
60 DIM Y(200)
70 PRINT
80 PRINT "RESET CURSOR AT BOTTOM LEFT" \ PRINT
90 INPUT V$ \ REM-- RESET CURSOR AT BOTTOM LEFT
100 PRINT "FOR NK EXPT, PUT POINT AT TOP RIGHT"
110 PRINT "FOR BIPHASIC EXPT, PUT POINT AT BOTTOM RIGHT"
120 INPUT V$ \ P=VAL(SEG$(V$,9,13))
130 IF P>2600 THEN N1=1 \ GO TO 160
140 IF P<200 THEN N1=2 \ GO TO 160
150 GO TO 100
160 FOR I=1 TO 100000
170 FOR J=1 TO 2
180 INPUT V$
190 PRINT CHR$(7)
200 IF V$="E" GO TO 260
210 IF LEN(V$)<>13 GO TO 180
220 Z(J)=VAL(SEG$(V$,9,13))
230 NEXT J
240 Y(I)=Z(2)-Z(1)
250 NEXT I
260 PRINT "CALIBRATION (mm/gm)"; \ INPUT C1
270 PRINT "TITLE--" \ INPUT T$
280 PRINT "SEND RESULTS TO LP (Y)--"; \ INPUT V$
290 IF V$="Y" THEN OPEN "LP:" FOR OUTPUT AS FILE #1 \ PRINT #1,T$
300 N9=I
310 FOR I=1 TO N9-1 STEP N1
320 PRINT
330 PRINT I/N1-.5 \ PRINT "P1";Y(I)/10/C1;"g";INT(Y(I)/Y(1)*100);"%",
340 IF V$<>"Y" GO TO 360
350 PRINT #1,I/N1-.5 \ PRINT #1,"P1";Y(I)/10/C1;"g";INT(Y(I)/Y(1)*100);"%",
360 IF P>2600 GO TO 400
370 PRINT TAB(35);"P2";Y(I+1)/10/C1;"g";INT(Y(I+1)/Y(2)*100);"%"
380 IF V$<>"Y" GO TO 400
390 PRINT #1,TAB(35);"P2";Y(I+1)/10/C1;"g";INT(Y(I+1)/Y(2)*100);"%"
400 NEXT I
410 IF V$="Y" THEN CLOSE #1
420 PRINT "PRESS RETURN TO RUN AGAIN, ANY OTHER KEY TO STOP"; \ INPUT V$
430 IF V$="" GO TO 70
440 END
```

```
10 REM *************** EPHYSIOL.BAS *************** BRIAN KING
20 REM
30 REM  PROGRAM TO ANALYZE ELECTROPHYSIOLOGY DATA FROM A PAPER CHART
40 REM  WITH THE HIPAD DIGITIZER.
50 REM
60 REM  MEASURES:  (1) ACTION POTENTIAL DURATION, AMPLITUDE, AREA,
70 REM                 AND TIMES TO 50% AND 90% REPOLARIZATION
80 REM             (2) dF/dt MAX AND MIN AND TIME TO BOTH
90 REM             (3) TENSION PEAK, LATENCY, DURATION, AND AREA
100 REM
110 DIM X(500),Y(500)
120 PRINT  \ PRINT "STORE PERMANENTLY ON FILE (Y)"; \ INPUT S$
130 IF S$<>"Y" GO TO 160
140 PRINT "FILENAME--------------"; \ INPUT F$
150 OPEN F$ FOR OUTPUT AS FILE #1 \ GO TO 170
160 OPEN "EPHYS.DAT;1" FOR OUTPUT AS FILE #1
165 PRINT #1,F$
170 PRINT "DATE AND TITLE--------"; \ INPUT T$ \ PRINT #1,T$
180 PRINT "CHART SPEED (mm/sec)--"; \ INPUT C0
190 PRINT "VOLTAGE (mm/50 mV)----"; \ INPUT C1
200 PRINT "RATE (mm/(10 g/s))----"; \ INPUT C2
210 PRINT "TENSION (mm/g)--------"; \ INPUT C3
220 PRINT  \ PRINT "EVENT TITLE-----------"; \ INPUT T$
230 PRINT "DIGITIZE IN THE ORDER:  (1) ACTION POTENTIAL"
240 PRINT "                        (2) RATE"
250 PRINT "                        (3) TENSION"
260 PRINT  \ PRINT "WHEN FINISHED ANY OF (1), (2), OR (3) JUST DIGITIZE ONE"
270 PRINT "POINT AT THE BOTTOM RIGHT CORNER OF THE DIGITIZING TABLET"
280 REM
290 REM ***** ACTION POTENTIAL *****
300 PRINT CHR$(7) \ PRINT "     ACTION POTENTIAL" \ PRINT
310 GOSUB 780 \ C=C1/50 \ GOSUB 1060
320 GOSUB 1120 \ GOSUB 1240 \ PRINT  \ PRINT
325 M=INT(M*10+.5)/10 \ A=INT(A+.5)
330 PRINT "MAXIMUM VOLTAGE DEFLECTION = ";M;"mV"
340 PRINT "A.P. DURATION              = ";D;"sec"
350 PRINT "TIME TO 50% REPOL.         = ";T5;"sec"
360 PRINT "TIME TO 90% REPOL.         = ";T9;"sec"
370 PRINT "AREA UNDER A.P.            = ";A;"V-sec"
380 PRINT #1 \ PRINT #1,T$
390 PRINT #1,"AP";TAB(10);M;"mV";TAB(23);D;"sec";TAB(36);T5;"sec";
400 PRINT #1,TAB(49);T9;"sec";TAB(62);A;"V-sec"
410 REM
420 REM ***** RATE *****
430 PRINT CHR$(7) \ PRINT "     RATE" \ PRINT
440 GOSUB 780 \ C=C2/10 \ GOSUB 1120 \ PRINT  \ PRINT
445 M=INT(M*100+.5)/100
450 PRINT "MAXIMUM RATE = ";M;"g/s  AT";M1;"sec"
460 PRINT #1,"dF/dt";TAB(10);M;"g/s AT";M1;"sec";
470 GOSUB 1180
475 M=INT(M*100+.5)/100
480 PRINT "MINIMUM RATE = ";M;"g/s  AT";M1;"sec"
490 PRINT #1,TAB(40);M;"g/s AT";M1;"sec"
500 REM
```

```
510 REM ***** TENSION *****
520 PRINT CHR$(7) \ PRINT "     TENSION" \ PRINT
530 GOSUB 780 \ C=C3 \ GOSUB 1060 \ GOSUB 1120 \ GOSUB 1240
540 PRINT  \ PRINT
545 M=INT(M*100+.5)/100 \ A=INT(A*100+.5)/100
550 PRINT "MAX TENSION = ";M;"grams"
560 PRINT "t TO PEAK   = ";M1;"sec"
570 PRINT "LATENCY     = ";L;"sec"
580 PRINT "DURATION    = ";D;"sec"
590 PRINT "AREA        = ";A;"gram-sec"
600 PRINT #1,"CONTR'N";TAB(10);M;"grams";TAB(23);M1;"sec";TAB(36);L;"sec";
610 PRINT #1,TAB(49);D;"sec";TAB(62);A;"g-sec" \ PRINT #1
620 REM
630 REM ***** PROGRAM CONTROL *****
640 PRINT  \ PRINT
650 PRINT "TO STOP PROGRAM, ENTER A POINT IN THE LOWER RIGHT CORNER."
660 PRINT "TO CONTINUE, ENTER A POINT ANYWHERE ELSE."
670 GOSUB 740 \ IF X<2600 GO TO 690
680 IF Y<200 THEN CLOSE  \ STOP
690 PRINT "TO CHANGE ANY CALIBRATIONS, ENTER A POINT IN THE LOWER R. CORNER"
700 PRINT "TO CONTINUE, ENTER A POINT ANYWHERE ELSE."
710 GOSUB 740 \ IF X<2600 GO TO 220
720 GO TO 180
730 REM ***** CALCULATION *****
740 INPUT V$ \ IF LEN(V$)<>13 GO TO 740
750 X=VAL(SEG$(V$,3,7)) \ Y=VAL(SEG$(V$,9,13)) \ PRINT CHR$(7)
760 RETURN
770 REM
780 REM ***** INPUT SUBROUTINE *****
790 PRINT "PROCEED AS FOLLOWS:  (1) TURN SWITCH ON WALL TO DIGITIZER"
800 PRINT "                     (2) RESET AT BOTTOM LEFT"
810 PRINT "                     (3) ENTER BASELINE ENDPOINTS"
820 PRINT "                     (4) PRESS SWITCH-STREAM BUTTON"
830 PRINT "                     (5) DIGITIZE CURVE"
840 INPUT V$ \ REM-- RESET CURSOR AT BOTTOM LEFT
850 FOR I=1 TO 2
860 INPUT V$
870 IF LEN(V$)<>13 GO TO 860
880 X(I)=VAL(SEG$(V$,3,7)) \ Y(I)=VAL(SEG$(V$,9,13))
890 IF X(I)<2600 GO TO 910
900 IF Y(I)<200 GO TO 1040
910 PRINT CHR$(7)
920 X1=X(1) \ NEXT I
930 S=(Y(2)-Y(1))/(X(2)-X(1)) \ REM-- S= SLOPE
940 B=(Y(1)+Y(2)-(S*(X(1)+X(2))))/2 \ REM-- B= Y INTERCEPT
950 FOR I=1 TO 100000
960 INPUT V$
970 IF LEN(V$)<>13 GO TO 960
980 X(I)=VAL(SEG$(V$,3,7)) \ Y(I)=VAL(SEG$(V$,9,13))
990 IF X(I)<2600 GO TO 1010
1000 IF Y(I)<200 GO TO 1020
1010 NEXT I
1020 N=I-1 \ FOR I=1 TO N
1030 Y=S*X(I)+B \ Y(I)=Y(I)-Y \ X(I)=X(I)-X1
```

```
1040 NEXT I \ RETURN
1050 REM
1060 REM ***** AREA SUBROUTINE *****
1070 A=0 \ FOR I=2 TO N
1080 A=A+(Y(I)+Y(I-1))/2*(X(I)-X(I-1))
1090 NEXT I
1100 A=A/2/C0/C \ RETURN
1110 REM
1120 REM ***** MAXIMUM SUBROUTINE *****
1130 M=Y(1) \ FOR I=2 TO N
1140 IF Y(I)>M THEN M=Y(I) \ M1=X(I) \ M2=I \ M3=M
1150 NEXT I
1160 M=M/10/C \ M1=INT(M1/C0*100+.5)/1000 \ RETURN
1170 REM
1180 REM ***** MINIMUM SUBROUTINE *****
1190 M=Y(1) \ FOR I=2 TO N
1200 IF Y(I)<M THEN M=Y(I) \ M1=X(I)
1210 NEXT I
1220 M=M/10/C \ M1=INT(M1/C0*100+.5)/1000 \ RETURN
1230 REM
1240 REM ***** DURATION & LATENCY SUBROUTINE *****
1250 F=0 \ FOR I=1 TO N
1260 IF F=1 GO TO 1280
1270 IF Y(I)>.05*M3 THEN L=X(I) \ F=1
1280 IF I<M2 GO TO 1300
1290 IF Y(I)<.05*M3 THEN D=(X(I)-L) \ GO TO 1310
1300 NEXT I
1310 F1=0 \ FOR I=N TO M2 STEP -1
1320 IF F1=1 GO TO 1340
1330 IF Y(I)>.1*M3 THEN F1=1 \ T9=(X(I)+X(I+1))/2
1340 IF Y(I)>.5*M3 THEN T5=(X(I)+X(I+1))/2 \ GO TO 1360
1350 NEXT I
1360 T5=INT(T5/C0*100+.5)/1000 \ T9=INT(T9/C0*100+.5)/1000
1370 L=INT(L/C0*100+.5)/1000 \ D=INT(D/C0*100+.5)/1000
1380 RETURN
```