BRIEF COMMUNICATIONS

The Effects of Shortening on Myoplasmic Calcium Concentration and on the Action Potential in Mammalian Ventricular Muscle

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SUMMARY. When cardiac muscle shortens during a contraction, the duration of mechanical activity is abbreviated (shortening deactivation), but the duration of the action potential is prolonged. Neither of these phenomena is fully understood, but both may be related to changes in the myoplasmic free calcium concentration. In these experiments, isolated papillary muscles from cats and ferrets were allowed to contract under various mechanical conditions while myoplasmic calcium was monitored with aequorin, or in parallel experiments the membrane potential was recorded with microelectrodes or a sucrose gap. When shortening occurred, myoplasmic calcium was increased and the membrane potential was more positive than in isometric contractions. The changes in calcium apparently precede the depolarization. We propose that muscle shortening reduces calcium binding to the contractile proteins and leads to a rise in myoplasmic calcium, and that this rise in myoplasmic calcium activates an inward current leading to the observed changes in the action potential. These processes may be important contributory factors in some arrhythmias. (Circ Res 55:825–829, 1984)

THE time course of mechanical activity during muscle contraction depends on the mechanical conditions under which the muscle contracts (Jewell and Wilkie, 1960). Such effects are particularly pronounced in cardiac muscle. For example, Brady (1963) has shown, using cat papillary muscles, that the duration of lightly loaded contraction, in which near-maximal shortening occurs, is only about 60% of an isometric contraction. This abbreviation of contraction is known as shortening deactivation. Paradoxically, the duration of the action potential in a lightly loaded contraction is prolonged when compared with an isometric contraction (Kaufmann et al., 1971). Recently, it has been demonstrated that the transient rise of intracellular calcium concentration (the calcium transient) is, like the action potential, prolonged in lightly loaded contractions (Allen et al., 1983; Housmans et al., 1983).

The experiments described in this paper were designed to investigate the causal relations between the prolongation of the calcium transient and the action potential. We have measured the free myoplasmic calcium concentration ([Ca++]j) in papillary muscles during isometric contractions, lightly-loaded contractions, and contractions in which sudden releases at various velocities were imposed on the muscle. In a parallel series of experiments, action potentials were recorded under similar mechanical conditions. Comparison of the two sets of results shows that when shortening occurs, it has similar effects on the calcium transients and the action potential. These findings support the proposal that changes in the mechanical conditions modulate calcium binding to the contractile proteins and, hence, alter the [Ca++]j (Gordon and Ridgway, 1978; Allen and Kurihara, 1982; Housmans et al., 1983). In addition, the present work provides direct evidence for the suggestion (Lab, 1982) that changes in [Ca++]j may underlie the alterations in the action potential.

Methods

Papillary muscles were dissected from the right ventricles of cats or ferrets that had been killed while anesthetized with chloroform. The muscles were placed horizontally in a bath perfused with Tyrode’s solution at 30°C and stimulated to contract at 0.2–0.33 Hz. One end of the muscle was attached to a force transducer and the other to a lever which was under feedback control. The lever could be used to produce isometric contractions, lightly loaded contractions in which shortening occurred, or contractions with rapid length changes.

[Ca++]j was measured with the calcium-sensitive aequorin (Blinks et al., 1982). Aequorin was microinjected into 50–100 cells on the surface of the preparation (Allen and Blinks, 1978; Allen and Kurihara, 1982), and the resulting light emission (a function of [Ca++]j) was monitored with a photomultiplier. The light-collecting system was designed so that movements of the muscles did not significantly affect light collection (Allen and Kurihara 1982; Cannell and Allen, 1983). Aequorin records are intrinsically noisy and the signal-to-noise ratio was improved by signal averaging.
Action potentials were obtained under comparable mechanical conditions but in different preparations. The action potentials were either recorded from individual cells, using dangling microelectrodes, or by sampling many cells with sucrose gap measurements (Kaufmann et al., 1971; Hennekes et al., 1977; Lab, 1980). The results were the same with both techniques.

The records of tension, aequorin light, and action potential were similar in cat and ferret papillary muscles, except that the time courses of all three measurements were 30–50% faster in ferret preparations than cat. The effects of length changes on mechanical responses and on aequorin transients were also similar in the two preparations.

Results

Figure 1A shows records of aequorin light, muscle tension, and muscle length during a lightly loaded contraction and during a subsequent isometric contraction. In these experiments, the muscle was stimulated to contract isometrically for a series of contractions, and then a single contraction with shortening was interposed. This protocol minimizes the changes of contractility associated with a more prolonged series of contractions with shortening (Parmely et al., 1969). In the aequorin experiments, the protocol was repeated a number of times (usually 32) to allow signal averaging. Despite the fact that the lightly loaded contraction has a shorter duration, the light response (calcium transient) associated with it is prolonged. Under similar conditions, the action potential associated with the lightly loaded contraction is longer than that in the isometric contraction (Fig. 1B). The change in the calcium signal is discernible at about the time the isometric tension has reached 50% maximum, but the action potential plateaus appear to diverge slightly later, at about the time when isometric tension has reached 75% maximum.

We have suggested (Allen et al., 1983) that changes in actin and myosin interaction during shortening could lead to the rise in [Ca++]_i, and that this rise might then cause the observed changes in the action potential (Lab, 1982). Three consequences of this hypothesis are that (1) the rate of shortening should affect the magnitude of changes in [Ca++]_i, (2) there should be some correlation between the magnitude of the changes in [Ca++]_i and the action potential, and (3) the changes in [Ca++]_i should precede the changes in the action potential. To test these predictions, we have released the muscle at a constant time during the rising phase of the contraction, but at different rates, so that the rate of muscle shortening varied over a wide range (Fig. 2). In these experiments, the preparations were allowed to reach a steady state under each of the different mechanical conditions. Figure 2A shows the aequorin light of isometric contractions and for releases at two different rates. The faster shortening produces a sudden rise in the calcium signal. The slower shortening produces a slower and smaller increase in the calcium signal. Thereafter, both calcium signals decline with a similar time course, but both are greater (or more prolonged) than that in isometric contraction. During the prolonged fall of calcium, both contractions are at the same muscle length, show similar degrees of redevelopment of tension, and both demonstrate shortening deactivation (Brady, 1965). Figure 2B displays action potentials recorded under similar mechanical conditions. In both cases, the release caused a small depolarization of the membrane and a subsequent prolongation of the action potential. The faster release caused the associated action potential to deviate rapidly from that in the isometric contraction, whereas the slower release had a longer lag and deviated more slowly from that in the isometric contraction. Both action potentials then showed repolarization phases with similar time

**Figure 1.** Comparison of the effects of an isometric contraction (isom) and a lightly loaded contraction (II) on the calcium transients (panel A) and the action potential (panel B) on a ferret papillary muscle. Traces show from above: aequorin light (a function of [Ca++]_i), tension, and muscle length. Panel B shows action potential measurements from a cat papillary muscle made with a “floating” microelectrode. Traces show from above: membrane potential, tension, and muscle length.
Figure 2. Comparison of the effects of an isometric contraction (isom) and quick releases (1 and 2) at two velocities on calcium transients (panel A), and action potentials (panel B). The results from the three different mechanical conditions are superimposed in each panel. Panel A shows results from an aequorin-injected ferret papillary muscle. Traces show from above: aequorin light, tension, and muscle length. Panel B shows action potential measurements (sucrose gap) from a cat papillary muscle. Traces show from above: membrane potential, tension, and muscle length.

Courses, with the action potentials following the release delayed with respect to that in the isometric control. During the period of repolarization, the mechanical conditions are similar to those described for Figure 2A.

Figure 3 shows records obtained when the muscle is released progressively later during an isometric contraction. As seen in the previous figures, muscle shortening leads to both a rise in $[Ca^{++}]_i$ and slower subsequent fall, and to a depolarization and subsequent prolongation in the action potential. Measurements in which both the calcium transients and the action potentials are from cat papillary muscles show that the delay between the release and the change in the calcium signal is $10.5 \pm 1.0$ (mean $\pm$ se) msec, whereas the delay between the release and the change in the membrane potential is $27 \pm 2.5$ msec ($n = 20$). When the release occurs early in the contraction, the effect on the action potential is initially very small and appears to have a long delay [35 $\pm$ 5 msec, $n = 19$; e.g., Fig. 3B (1)], whereas, when the release is near the peak of contraction, the delay is shorter (21.5 $\pm$ 2.0 msec, $n = 24$), and the effect on the membrane potential is larger [Fig. 3B (2)]. Nonetheless, this small delay is still significantly longer than the delay between the release and the calcium change ($P < 0.0005$, unpaired $t$-test). To compare the magnitude of the calcium changes associated with releases, it is necessary to correct for the nonlinear nature of the relation between ae-
would lead to both prolongation of the calcium bound to contractile proteins is released into the myoplasm by the process of shortening, this should lead to a prolongation of mechanical activity. (3) Calcium release from contractile proteins. If calcium uptake by intracellular stores. These mechanisms are unlikely, because, as discussed above, the absolute change in [Ca++] is bigger in the middle panel. Thus, the changes in both membrane potential and [Ca++] are greater when a release is imposed at 200 msec as opposed to 100 msec. When the release occurs late in the contraction, the aequorin response is very small, but, because of the aequorin nonlinearity, this does not mean that the [Ca++] response is proportionately small. The membrane potential response can be substantial, and as it occurs during the relative refractory period, it can trigger a new action potential (Kaufmann et al., 1971).

Discussion

Our results confirm earlier findings that in contractions in which shortening occurs, both the calcium transient (Allen et al., 1983; Housmans et al., 1983) and the action potential (Kaufman et al., 1971; Lab, 1980) are prolonged. Further comparison of the changes in the calcium transient and the changes in the action potential under various mechanical conditions show that they have similar characteristics.

We consider, first, possible mechanisms by which muscle shortening leads to an increase (or a prolongation) of the calcium transient. (1) An increase in sarcolemmal calcium influx. This proposal is initially attractive because it can explain both the increase in the calcium transient and the increase is the action potential duration. The main argument against this proposal is that an increase in [Ca++]; would be expected to increase mechanical activity, whereas in fact mechanical activity is reduced after shortening. In addition, if sarcolemmal calcium influx were increased, one would expect the effect on the action potential to precede the effect on measured [Ca++]; since it will take some time for calcium to diffuse to the aequorin molecules which are thought to be distributed throughout the myoplasm. In fact, the changes in [Ca++]; appear to precede the change in membrane potential. (2) An increase in calcium release from intracellular stores or a decrease in calcium uptake by intracellular stores. These mechanisms are unlikely, because, as discussed above, they should lead to a prolongation of mechanical activity. (3) Calcium release from contractile proteins. If calcium bound to contractile proteins is released into the myoplasm by the process of shortening, this would lead to both prolongation of the calcium transient and a reduced duration of mechanical activity (Allen and Kurihara, 1982; Allen et al., 1983; Housmans et al., 1983; Ridgway and Gordon, 1984). One argument against this hypothesis is that a stretch during contraction has either no effect on the calcium transient (Gordon and Ridgway, 1978; Allen and Kurihara, 1982) or a much smaller effect than the corresponding release (Ridgway and Gordon, 1984). This may be because the change in tension as a fraction of the isometric control is much smaller for stretches than it is for releases. Alternatively, the differences may be related to the nature of the crossbridge attachment and detachment cycle under the different mechanical conditions.

A biochemical basis for changes in calcium binding with mechanical conditions is suggested by the work of Bremel and Weber (1972) who showed that attachment of rigor crossbridges to thin filaments leads to an increase in the calcium affinity of troponin. It is not yet clear from biochemical studies whether the attachment of crossbridges in a normal contraction has the same effect or how rapid cycling of crossbridges, such as occurs in shortening, would affect the troponin affinity for calcium. Alternatively, it is possible that changes in muscle length and myofilament overlap lead directly to changes in affinity of troponin for calcium. It is unlikely that this is the major mechanism in the present situation, as Allen and Kurihara (1982) and Housmans et al. (1983) have shown that active shortening from a long to a short length leads to a larger change in [Ca++]; than is observed when calcium transients at the long and the short length are compared.

Our results also demonstrate that muscle shortening produces changes in the action potential and in the calcium transient which are strikingly similar. A further similarity is that stretches, in contrast to releases, have little or no effect on the action potential (Kaufmann et al. 1971; Hennekes et al., 1977), and, as noted above, stretches have much less effect than the release on the calcium transients. However, correlation does not imply causality, and we need to see if other interventions influence free myoplasmic calcium and action potential in parallel. Nonetheless, the findings so far, together with the observation that changes in [Ca++]; probably precede the changes in membrane potential, suggest that the mechanically induced changes in [Ca++]; may have a direct effect on the membrane properties. There are a number of calcium-activated currents that might be involved (for discussion, see Eisner and Vaughan-Jones, 1983). (1) Calcium-activated K+ conductance. This conductance has been established in other tissues (Meech, 1974) and possibly exists in ventricular muscle (Bassingthwaighte et al., 1976). However, increases in [Ca++]; should always lead the membrane potential toward the potassium equilibrium potential (~80 mV) which is in the opposite direction to the observed changes in membrane potential. Thus, our results cannot be explained in this way. (2) Electrogenic Na-Ca exchange. Although the presence of Na-Ca exchange is established in heart...
muscle (Reuter and Seitz, 1968), its stoichiometry is unknown. If more than two Na\(^+\) exchange for a single Ca\(^{++}\), then the exchange will be electrogenic, and this has important implications for calcium movements (Mullins, 1981). On such an electrogenic model, irrespective of the initial direction of the exchange or the membrane potential, an increase in [Ca\(^{++}\)], will lead to increased Ca\(^{++}\) efflux/Na\(^+\) influx, and will tend to make the membrane potential more positive. Thus, this mechanism is compatible with our findings. (3) Calcium-activated nonspecific cation conductance (Kass et al., 1978; Colquhoun et al., 1981). This [Ca\(^{++}\)]-activated conductance carries both Na\(^+\) and K\(^+\), and it has an effective reversal potential of about −5 mV. Thus, an increase in [Ca\(^{++}\)] should always lead the membrane potential to change in the direction of this potential. This is certainly true of our results when shortening occurs during the declining phase of the action potential. When shortening occurs early in the contraction (i.e., when the membrane potential is more positive than −5 mV), the effect on [Ca\(^{++}\)] is smaller, and the effect on the membrane potential is also small, but always seems to be in a positive direction. Thus, this mechanism alone cannot explain our results.

In summary, present evidence supports the hypothesis that shortening reduces the binding of calcium to contractile proteins, and this leads to the observed rise in [Ca\(^{++}\)]. The increased [Ca\(^{++}\)], leads to a positive change in the membrane potential, possibly by activation of an electrogenic Na–Ca exchange, perhaps combined with opening of nonspecific channels which carry Na\(^+\) and K\(^+\). The importance of the resulting depolarization is that it can, in some circumstances, trigger new action potentials, and may be involved in the production of arrhythmias (Lab, 1982).

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