The plants exposed to 50 mM NaCl at $CaSO_4$ concentrations below 1 mM suffered great damage (Fig. 1). At higher concentrations of $CaSO_4$, the plants grew well and, both in appearance and weight, did not differ significantly from the control plants receiving no NaCl. All control plants grew equally well, except those receiving no Ca.

The Na concentration in the roots dropped by about one-third as the $CaSO_4$ concentration in the solutions was raised from 0.1 to 10 mM. Over the same interval of $CaSO_4$ concentrations, the Na content of the stems fell by a factor of about 2.5. However, it was in the leaves that the effect of $CaSO_4$ on Na content was most dramatic. The Na concentration fell from 3.2 mg per 100 mg (dry weight) at 0.1 mM $CaSO_4$ to 0.2 mg per 100 mg at 3 mM. There was no further change at 10 mM $CaSO_4$. That is, at less than 3 mM $CaSO_4$, a massive breakthrough of Na into the leaves occurred.

The effect of $CaSO_4$ is due to the Ca ion; repetition of the experiment with $CaCl_2$ instead of $CaSO_4$ gave very similar results. Attempts to replace Ca with either Mg or K (in the presence of 0.1 mM Ca) proved futile, affording little or no protection. At the effective concentrations of Ca, Mg caused damage in addition to that attributable to NaCl.

The protective action of Ca is not related to any simple shift in internal cationic ratios without change in the total cationic content. This is evident from the fact that the changes in Na content of the tissues, induced by changes in the Ca concentration of the solutions, far exceeded any changes in the concentrations of the other cations in the tissues.

Both cellular membranes and RNA may be sites of Na-induced derangements and, specifically, displacement of Ca, as already surmised on the basis of other evidence (*4*, *8*). Since a massive intrusion of Na into the plant was the outstanding effect of salinity, both this intrusion and the decline of the plants being checked by Ca, it is likely that the site of the primary Na (and Ca) effect is the plasmalemma of the absorbing cells of the roots, for that is where entry of ions into the plant is governed (*9*).

Our experiments show the efficacy of Ca in protecting an extremely salt-sensitive species against the deleterious effects of NaCl present in the medium at about one-tenth its concentration in seawater.

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Rat Heart Papillary Muscles: Action Potentials and Mechanical Response to Paired Stimuli

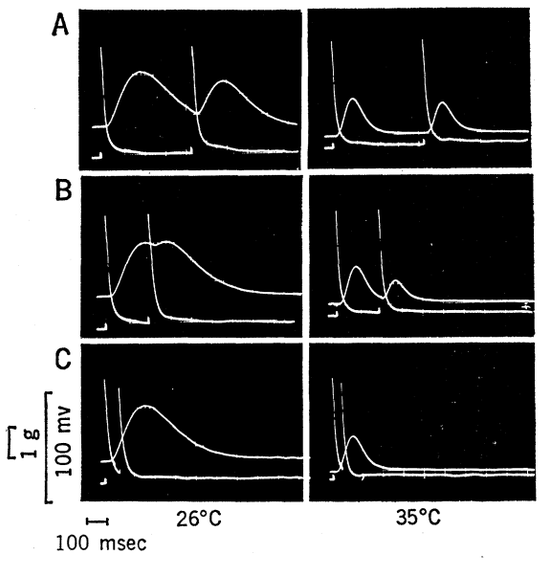
Abstract. *Electrical reexcitation of rat papillary muscle after a short interval (50 to 80 milliseconds) results in action potentials with no significant mechanical counterpart. The mechanical response recovers as the interval is increased beyond 80 milliseconds. The rate of recovery is slowed at low external calcium levels. It appears that the coupling mechanism passes through a refractory stage owing to the depletion of an intracellular "releasable calcium" fraction.*

Rat papillary muscle fibers have short action potentials compared with those found in ventricular muscle of other species (*1*). This provides an opportunity to investigate the effects of electrical reexcitation during the period of tension development, a technique that proved useful in characterizing the active state of skeletal muscle (*2*, *3*).

In order to study the effects of paired pulses, papillary muscles were isolated in a plexiglass bath and stimulated with either a pair of fine-wire platinum electrodes or platinum plate electrodes placed on either side of the muscle, 1 mm away. A modified Tyrode solution (*3*) was used to bathe the muscles. Most experiments were performed at 26°C, since this gave a better separation of electrical and mechanical activity than at higher temperatures. Intracellular action potentials were measured with glass microelectrodes, and tension was recorded on an oscilloscope by using a Statham G 1-4 strain gauge.

When paired stimulus pulses were used to excite the preparation, two action potentials could be recorded. The amplitude of the second action potential became slightly smaller than that of the first as the interval between the stimuli decreased to less than 100 msec, but the decrease was small (less than 10 percent) for most fibers until the interval was reduced to 50 msec. At shorter intervals the second action potential could not always be elicited though in some preparations double action potentials could be seen at stimulus intervals as short as 30 msec. The mechanical response on the other hand became progressively smaller as the interval decreased until at pulse intervals of 80 to 100 msec the effect of the second pulse became practically undetectable (Fig. 1). Neither the peak tension nor the duration of the concentration due to a single pulse could be increased significantly by a second pulse falling at an interval of less than 80 msec after the first. Peak tension is increased when the second pulse falls later than 80 msec but before the peak which, at this temperature, falls 200 msec after the first impulse.

The value Tr, which is the relative increase in area under the tension time curve due to the second pulse, expressed as a fraction of the area resulting from a single pulse, was calculated from planimeter measurements. We found Tr to be indistinguishable from zero ($< .02$) at pulse intervals of 80 msec or less. At longer pulse intervals Tr increases steadily to a value of 0.3 at 200 msec. Recovery is more rapid at higher temperatures, and at 35°C Tr becomes measurable ($> .02$) at 50 msec and increases to 0.5 at 200 msec; but the response to the second pulse is much less than the first, even when the second pulse falls after the mechanical



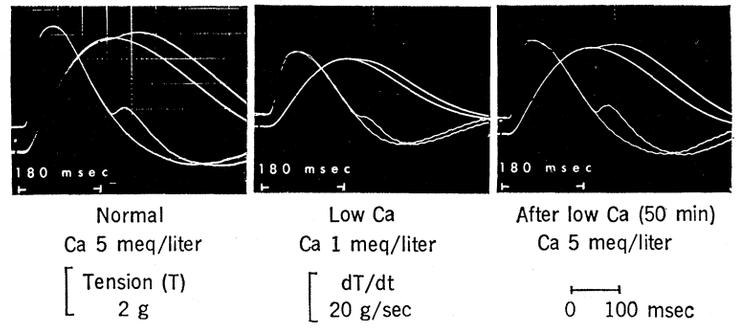


Fig. 1 (left). Effect of paired electrical stimuli on the electrical and mechanical response of isolated rat papillary muscle. Pulse interval: (A) 400, (B) 200, (C) 50 msec. Upper tracing, muscle tension; lower tracing, intracellular potential. Fig. 2 (above). Effect of reducing the calcium concentration on tension development and its derivative when paired stimulus pulses are placed 180 msec apart. Superimposed tracings of tension development (lower trace) and its derivative (upper trace) when single and double shocks are used before, during, and after exposure to low calcium (1 meq/liter). Pulses are signaled by a break in the tension curve and indicated by the vertical lines below on either side of "180 msec."

response to the first is over (Fig. 1B, right side).

The apparent uncoupling of electrical and mechanical activity at pulse intervals of 50 to 80 msec was investigated by superimposing tracings of tension development and its derivative in response to single and paired stimuli. Some small effect on the shape of the derivative curve could be detected in some preparations even at pulse intervals of 50 msec, but the maximum rate of rise was never increased and peak tension could not be significantly increased at these short intervals. Electrical and mechanical activity are thus virtually uncoupled, since the action potential of the second stimulus has practically no mechanical counterpart. Two possible explanations of this uncoupling phenomenon have been considered:

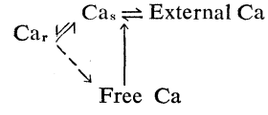
1) that the contractile system is fully saturated by the coupling "activator" released by the first action potential and is unable to respond further to the second pulse;

2) that the coupling mechanism itself goes through a refractory phase, followed by a gradual recovery.

Since there is good evidence that the intracellular release of calcium activates the contractile system during a normal contraction (*4*), it should be possible to test the first hypothesis by reducing the calcium available. Lowering the external calcium level results in a decrease in both the rate of rise of tension and the peak developed tension in response to a single stimulus. It is assumed that this is because less calcium is liberated intracellularly in response to a single action potential, and so the contractile mechanism is not fully activated. Clearly, if the first of the above explanations is true, then a second impulse during the "uncoupling period" should lead to a relatively greater increase in tension development at low external calcium levels. Experiments on a series of ten papillary muscles failed to confirm this, and in every case the response to a second pulse falling 80 to 200 msec after the first gave a smaller response in low calcium (1 meq/liter) solutions than the control (5 meq/liter), both on an absolute and a relative scale (as a fraction of the response due to a single pulse) (Fig. 2). This suggests that the coupling mechanism itself or the availability of calcium is the limiting factor and that recovery of the coupling following the refractory phase is dependent upon the external calcium concentration.

Since action potentials are recorded from single cells, while tension development is a property of the whole syncytium, it was necessary to see if the action potential was representative of electrical activity throughout the preparation. This was done by recording external action currents with a pair of platinum electrodes. An "all or none" type of response was found. The amplitude of the response to the second pulse was close to that of the first and was not significantly affected by the pulse interval as long as this exceeded the refractory period.

The simplest explanation of the uncoupling followed by slow recovery of the mechanical response appears to be as follows: each action potential liberates completely a small discrete fraction of intracellular-bound calcium (Ca_r), which results in the activation of the contractile mechanism. During recovery the calcium is taken up into a second fraction, Ca_s. Calcium recycles to Ca_r at a slower rate, limited by the amount of calcium in Ca_s.



Thus the mechanical response of the papillary muscle to the second of two pulses appears to be limited, not by the electrical refractory period or the contraction mechanism but rather by the coupling mechanism, which has its own refractory period and recovery cycle determined by the time required to replenish Ca_r.

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