Two Functionally Distinct Calcium Pools in the Excitation-Contraction Coupling Process

The contractile process in mammalian heart has been reported to be dependent upon the calcium contained in one kinetically discrete pool[1,2]. In this study, evidence is presented to show that this calcium pool is composed of two components each with a different functional role in the process coupling excitation to contraction in the heart.

Ten hearts obtained from kittens (0.8–1.0 kg) were perfused by the Langendorff technique with 3 m*M* Hepes buffer solution[3]. All hearts were driven electrically at 180 beats/min. After 30 min equilibration, the hearts were washed out with calcium-free Hepes solution until contractile force had decayed to 1 g or less. The hearts were then reperfused with Hepes solution containing 5 mEq/l calcium. Great care was exercised to minimize intermixing of the calcium-free and calcium containing perfusates. One group of 5 hearts was reperfused with Hepes solution containing 5 μM/l lanthanum in addition to 5 mEq/l calcium. Lanthanum in this concentration inhibits contractile activity in the heart either by displacing superficial calcium stores or by blocking calcium uptake from the extracellular space[3]. The effluent from all hearts was collected at 6 sec intervals during the reperfusion. Aliquots of the effluent samples were diluted with 1% lanthanum in 5% (v/v) HCl for the determination of calcium concentration by atomic absorption spectrophotometry.

Contractile force was completely restored by the calcium containing reperfusion medium in the 5 control hearts. In contrast, when the reperfusion fluid contained lanthanum, contractile activity was not evident during the reperfusion despite the presence of normal concentrations of calcium. The uptake of calcium in a representative experiment in which contractile force was restored by reperfusion with the solution containing 5 mEq/l calcium is shown in Figure 1 A. Subtraction of the effluent calcium concentration, $[Ca]_T$, from the calcium concentration in the perfusate yielded the curve, R. Compartmental analysis of R yielded 2 compartments, Ca_1 and Ca_2. The analysis was done as described by RIGGS[4], and the separation of the 2 compartments was based entirely on statistical criteria to minimize the bias inherent in the usual graphical analysis technique. The relationship between the accumulation of calcium by the 2 compartments and the restoration of contractile force is shown in Figure 2. It is clear that the return of contractile force was not linearly related to the accumulation of calcium by Ca_1, and moreover that this pool was filled to capacity early in the reperfusion. However, the restoration of contractile force was directly related to the quantity of calcium accumulated by Ca_2 during reperfusion (Figure 2). The mean coefficient of correlation, r, between the 2 variables for the 5 hearts was 0.93 ($P < 0.01$). This suggests that the calcium contained in Ca_2 is directly related to the maintenance of contractile force in the heart. In sharp contrast, when the reperfusion fluid contained 5 μM lanthanum in addition to the normal concentration of calcium, contractile force was not restored (Table). Furthermore, in the presence of lanthanum the calcium uptake curve, R, could be resolved into only a single compartment (Figure 1 B). The mean halftime for calcium uptake by the single compartment did not differ significantly ($P > 0.05$) from the mean halftime for the uptake of calcium by Ca_2 in the hearts not treated with lanthanum (Table). Since net calcium uptake by Ca_2 in both the control and lannthanum treated hearts ap-

1 L. E. BAILEY and P. E. DRESEL, J. gen. Physiol. *52*, 969 (1968).
2 L. E. BAILEY and H. A. SURES, J. Pharmac. exp. Ther. *178*, 259 (1971).
3 W. G. SANBORN and G. A. LANGER, J. gen. Physiol. *56*, 191 (1970).
4 D. S. RIGGS, *The Mathematical Approach to Physiological Problems* (The Williams and Wilkins Co., Baltimore 1963).

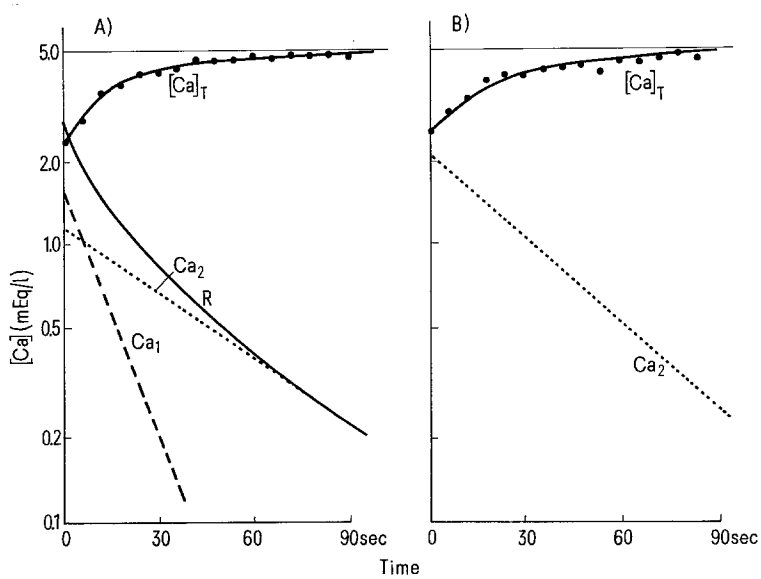

Fig. 1. The characteristics of calcium uptake in typical hearts perfused in the absence and presence of lanthanum. Panel A) Control experiment in which contractile force was restored by reperfusion with Hepes solution containing 5 mEq/l calcium. $[Ca]_T$ represents the calcium concentration measured in the effluent during the reperfusion. R is the least squares best fit line for the difference curve, 5.0 mEq/l – $[Ca]_T$. Ca_1 and Ca_2 are the 2 calcium compartments resolved by graphical analysis. Panel B) The uptake of calcium in the presence of 5 μM/l lanthanum. Contractile force was not restored and Ca_1 could not be resolved by compartmental analysis of $[Ca]_T$.

The effect of 5 μM lanthanum on calcium uptake and on the restoration of contractile force in calcium depleted hearts

	Control (5)		Lanthanum (5)	
	Ca_1	Ca_2	Ca_1	Ca_2
$T_1/_2$ (sec)	5.6 ± 1.7[a]	46.2 ± 6.2	—	33.4 ± 7.9
Calcium content (mEq/kg tissue wet wt.)	0.92 ± 0.13	2.87 ± 0.33	—	3.96 ± 0.38[b]
Contractile force (g)	19.1 ± 3.1		0	

Figures in parentheses indicate the number of hearts. [a] Mean ± S.E. [b] Differs significantly from control ($P < 0.05$).

proached a steady state at approximately the same rate[2], the calcium taken up probably entered the same pool, Ca_2. Thus, on the basis of this criterion, Ca_1 was absent in the calcium uptake curve in lanthanum treated hearts. The quantity of calcium taken up by Ca_2 during reperfusion in the presence of 5 μM lanthanum was greater ($P < 0.05$) than the quntitiy of calcium taken up by Ca_2 alone, but was not significantly different ($P > 0.05$) from the total quantity of calcium (Ca_1 + Ca_2) by the control hearts.

It can be concluded from these results that 5 μM lanthanum blocked the uptake of calcium by Ca_1 but not by Ca_2, and moreover, when calcium uptake into Ca_1 was blocked, contractile force was not restored even though significantly more calcium was taken up by Ca_2. Since this concentration of lanthanum has been shown to have little or no effect on electrical activity in the myocardium[3], these results indicate that there are at least 2 pools of calcium involved in the process coupling excitation to contraction in the heart. First, a compartment which we have identified as Ca_2, and have shown to be necessary, but not sufficient by itself, to maintain contractile force.

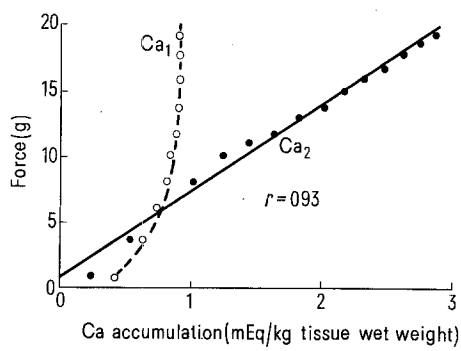

Fig. 2. The relationship between the accumulation of calcium during reperfusion by Ca_1 and Ca_2 and the restoration of contractile force. The mean coefficient of correlation, r, between the accumulation of calcium by Ca_2 and restoration of contractile force for the 5 hearts was 0.93.

Second, a smaller calcium compartment, Ca_1, whose calcium content was not related directly to the restoration of contractile force, but which was absolutely essential to the coupling process in cardiac muscle. One possible interpretation of these results is that the calcium contained in Ca_1 in some way mediates the release of calcium from Ca_2 which in turn activates the contractile mechanism. A mechanism similar to this has been proposed for skeletal muscle where it has been shown that the release of activator calcium from the sarcoplasmic reticulum of skinned skeletal muscle fibres is a regenerative process initiated by free calcium in the bathing solution[5,6]. Presumably the free ion represents calcium liberated from the sarcolemma and transverse tubular system by depolarization of the cell. According to this hypothesis, the free calcium then 'triggers' the release of reticular calcium to activate the contractile mechanism[7]. However, one can only conclude from the present results that the 2 calcium pools, Ca_1 and Ca_2, are both intimately involved in the coupling process in the heart and that their roles in this system are different.

Zusammenfassung. Es wird angenommen, dass mindestens zwei Fraktionen von intrazellulärem Kalzium am Kupplungsprozess zwischen Reizung und Kontraktion des Herzens beteiligt sind.

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[5] M. ENDO, M. TANAKA and Y. OGAWA, *Nature 228*, 34 (1970).
[6] L. E. FORD and R. J. PODOLSKY, Science *167*, 58 (1970).
[7] C. P. BIANCHI and T. C. BOLTON, J. Pharmac. exp. Ther. *157*, 388 (1967).
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Neurohumoral Regulation of Motility and Blood Flow in the Colon

The extrinsic nervous control of colonic motility and blood flow has recently been studied[1]. Electrical stimulation and reflex activation of the pelvic nerves produce an intense flushing of the mucosa in the distal two thirds of the colon. Corresponding in time with this mucosal flush there is a marked but transient increase of venous outflow and concomitantly with the vasodilatation a mucoid secretion. Intravenous administration of atropine (1 mg/kg) did not significantly interfere with the vasodilation and the motility response indicating that both might be