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Original articles

G. A. LANGER

MYOCARDIAL CALCIUM COMPARTMENTATION AND CONTRACTILE CONTROL

The Departments of Medicine and Physiology
and the Cardiovascular Research Laboratory
University of California, Los Angeles
School of Medicine, Los Angeles
California 90024-1760, USA

Under the condition of rapid perfusion, the time course of contractile response of single ventricular cells to extracellular calcium (Ca) depletion and repletion identifies „fast" and „slow" cellular Ca pools. ^{45}Ca exchange was studied in these cells under the same conditions of on-line rapid perfusion. Four kinetically-defined compartments were distinguished: (1) A „rapid" compartment containing 2.6 mmoles Ca/kg dry wt of lanthanum (La) displaceable Ca, $t^1/_2 < 1$ sec.; (2) An „intermediate" compartment(s) containing 2.1 mmoles, $t^1/_2 = 3$ and 19 sec. Caffeine displaced significant amounts of Ca from this compartment whereas La displaced none; (3) A „slow" compartment containing 1.6 mmoles, $t^1/_2 = 3.6$ min. Addition of inorganic phosphate to the perfusate adds significant amounts of Ca to this compartment; (4) An „inexchangeable" compartment, containing 1.2 mmoles. The „rapid" compartment's flux is $\geq$ 300 μmoles Ca/kg wet wt/sec. Its exchange rate indicates that it is the kinetic counterpart of the functionally-defined „fast" pool. Its subcellular locus is undefined. The „intermediate" compartment is best correlated with the „slow" pool and represents Ca in the sarcoplasmic reticulum. The „slow" compartment contains a significant fraction from the mitochondria. The results indicate that $\geq$ 40% of cellular Ca can turn over within the period of one contraction cycle. These results are consistent with the following sequence: (1) Upon sarcolemmal depolarization, Ca moves through the Ca channel to arrive at the SR and at the myofilaments. (2) Ca induced Ca release occurs via the „feet" at the SR-inner SL region. The Ca diffuses to the myofilaments or is transported across the SL via the Na-Ca exchanger. (3) Ca is pumped into the free or longitudinal SR and diffuses to the cisternae. Ca is pumped across the SL by the SL Ca pump and by the Na-Ca exchanger. (4) Mitochondrial Ca exchange via the Na-Ca exchanger and/or SL Ca pump. (Supported by NHLBI and the Laubisch and Castera Endowments.)

Key words: *heart, calcium compartmentation, contractile control*

It has been a long-term goal in the field of cardiac excitation-contraction coupling to relate subcellular calcium (Ca) compartments and Ca movements to contractile function in the intact tissue or cell. Whole cell and patch-clamping and the use of Ca-sensitive dyes in single cells (1—6) have greatly increased our knowledge of transient Ca movements during

the course of a contraction cycle. Steady-state distribution among subcellular organelles has also been evaluated by electron microprobe (7—9). The former studies provide information with respect to net Ca movements usually over short time periods; the latter indicate Ca content of cellular regions at one point in time. The evaluation of both non-steady state and steady-state Ca exchange over longer time periods is best accomplished with radioisotopic techniques. Quantitative information with respect to accurate definition of Ca compartment size and flux using these techniques has, however, been limited in the past. The limitation has centered about the problem of selection of the appropriate kinetic exchange model (series, parallel, series-parallel) for the tissue or cell under investigation. An appropriate model is required in order that realistic correction for isotopic reflux from one compartment to another be made.

The presence of reflux from the „front" compartment to the „rear" compartment in a series system will cause overestimation of the exchange rate constant and underestimation of content of the „front" compartment and vice-versa for the rear compartment (10). Correction can be made if one could be assured that a simple series arrangement of the compartments did, in fact, exist. This is impossible in whole tissue and, at best, difficult even in single cell. The best way to obviate the problem is to increase the exchange rate of the „front" compartment to the maximum level compatible with maintenance of cell integrity. This has been accomplished with rat ventricular cells, both in neonatal culture (11) and in the adult (12). Ca exchange is measured under essentially non-perfusion limited conditions. Probes for subcellular loci of Ca can be applied under these conditions and the origin of the Ca that contributes to the various exchangeable compartments can be defined more accurately than previously possible in intact cells. Contractile responses to various interventions can be measured under the non-perfusion limited conditions (13) and temporal comparisons made between function and the content of the subcellular Ca compartments.

The Compartments and Their Origin — Both neonatal cultured and adult rat myocardial cells demonstrate 4 kinetically-defined Ca compartments, 3 exchangeable and 1 inexchangeable. The exchangeable compartemnts with their exchange rates and contents are listed in Table 1. The general pattern for the two preparations is similar.

Rapid Compartment — Both cultured and adult cells demonstrate a large (67% and 41% of exchangeable cellular Ca in cultured and adult, respectively) very rapidly exchangeable compartment. Non-perfusion limited flux from this compartment is greater than 1000 μmoles/kg dry cell wt/sec (200—300 μmoles/kg wet cell wt/sec), This flux is 3—5 times greater than the amount of Ca calculated to be required by the cell for greater than 50% maximum force development (14, 15). The Ca in this compartment is displaced by appli-

cation of extracellular La with a $t_{\frac{1}{2}} < 1.5$ sec (11). Since there is no intracellular penetration of La in this short time (16) the Ca in the rapid compartment is in the sarcolemma and/or in an intracellular location in virtual instantaneous equilibrium with sarcolemmal sites.

The region of the cell which seems appropriately structured to deal with the large Ca fluxes measured from the rapid compartment is the subsarcolemmal-sarcotubular lateral cisternal junction. This is the space which is spanned by the so-called „feet" which extend from the SR cisternal membrane to the inner leaflet of the T tubule sarcolemma. It is from these „feet" that Ca is released from the SR to diffuse to the myofilaments and/or

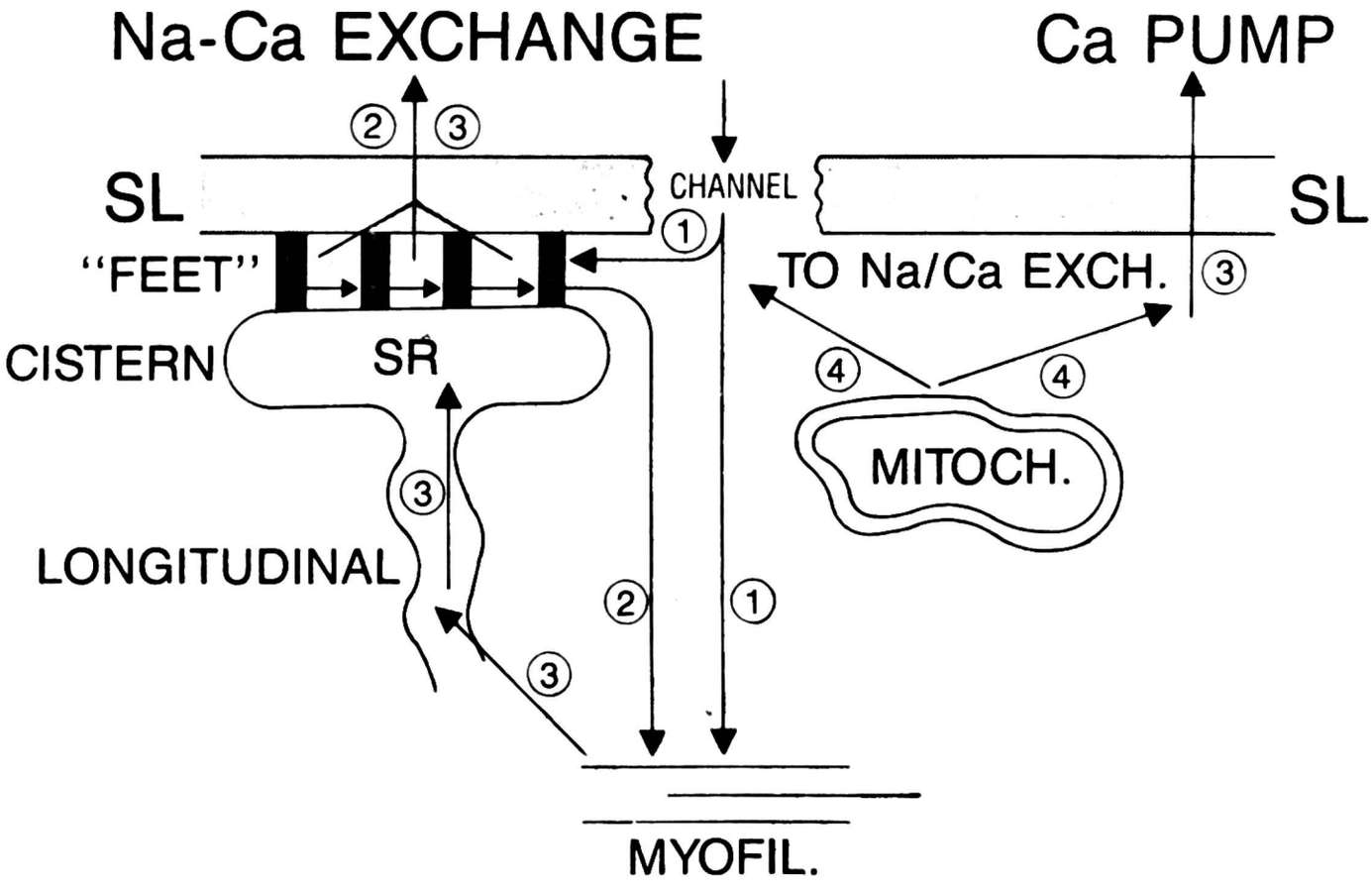

Fig. 1. Compartmentation and movement of Ca in the cardiac cell. See text for discussion.

exit from the cell (17—18). The release of Ca into this restricted space (approx. 25 nm between membranes) could result in a transiently high Ca concentration at the inner sarcolemmal surface.

The sarcolemmal system primarily responsible for Ca efflux from the cell is the Na-Ca exchanger (19). If exchanger sites were located at the SR-sarcolemmal junction (there is no evidence for or against this) the proposed transiently high Ca concentration would maximally stimulate the efflux of Ca via the exchanger. It has recently been shown (4) in cardiac single cells that Ca release from the SR fully activates, within 200 msec, a transient inward current. This reflects the electrogenic efflux of Ca across the sarcolemma by

the Na-Ca exchanger. The capacity of the exchanger has recently been defined in canine sarcolemmal vesicles (20). The V_{max} for the fast phase of Na-Ca exchange was 37 nmol Ca/mg SL protein/sec. Based on the value of 21 mg SL protein/g dry cell wt., (as calculated from Tibbits (21)) this translates to a maximum Ca turnover of > 700 μmoles Ca/sec/kg dry wt cell via the Na-Ca exchanger which is in the range for rapid compartment exchange in the non-perfusion limited intact cell. It, therefore, seems a real possibility that the sarcolemmal Na-Ca exchanger mediates the Ca exchange represented by the rapid compartment. The specific origin(s) of the compartment are not, as yet, defined. However, it could be speculated that Ca released from the sub-sarcolemmal lateral cisternae at the inner leaflet of the sarcolemma is that which contributes to the rapid compartment. In this context, it should be noted that the exchanger's activity is greatly augmented by the addition of anionic phospholipid to sarcolemmal vesicles (22) where it acts to increase the Ca affinity of the exchanger. Moreover, all anionic phospholipid in the in vivo sarcolemma is located in the inner leaflet (23) where it could act to facilitate interaction of Ca with the exchanger and facilitate its transsarcolemmal movement.

Intermediate Compartment(s) — This is a biexponential compartment which is present in both cultured and adult rat cells (*Table 1*). At this point

Table 1. Exchangeable Calcium Compartments

Compartment	Rapid			Intermediate			Slow		
	$t^1/_2$(sec)	λ(min^{-1})	cont(mmol)	$t^1/_2$(sec)	λ(min^{-1})	cont(mmol)	$t^1/_2$(min)	λ(min^{-1})	cont(mmol)
Neonatal	<1.5	>28	2.6	19 103	2.2 0.4	0.4 0.2	19	0.04	0.7
Adult	<1.0	>42	2.6	3.5 19	12 2.2	1.3 0.8	3.6	0.2	1.6

it has been most extensively studied in the adult cell preparation in which it is found that the compartment(s) contains more Ca and exchanges at a greater rate than in the cultured preparation. In the adult, the compartment contains 33% of the cell's exchangeable Ca and contributes > 250 μmoles/kg dry wt/sec (50—60 μmoles/kg wet cell wt/sec) to the total Ca flux or approximately 20% of total steady-state flux under non-perfusion limited conditions.

Ca in this compartment is released specifically and rapidly by application of caffeine and the amount of this release is greatly dimished by pre-treatment of the cells with 10^{-6} M ryanodine (12). This places the

origin of this compartment, at least in part, in the SR. Caffeine, at maximum, released only 21% of the intermediate compartment(s) Ca from the cell (440 μmoles/kg wet wt). This indicates that either caffeine does not release all of SR Ca or that other cellular loci exchange across the sarcalemma with similar kinetic characteristics. Since mitochondrial exchange has been kinetically localized (see below) it is difficult to assign such a large component of exchangeable Ca to some other organelle. It is possible that intracellular cytoplasmic binding sites (e.g. protein) might contribute but it seems more likely that Ca from non-caffeine releasable SR sites contributes.

The biexponential nature of the intermediate compartment ($t_{\frac{1}{2}}$ 3.5 and 19 sec) is of interest. The more rapid component accounts for 60% of the compartment. It has been recognized that the SR has a diverse structure with junctional, corbular and longitudinal components. Jorgensen et al. (9), using electron microprobe technique, describe two structurally distinct SR Ca storage sites: a) junctional and corbular with higher Ca content which contain calsequestrin. 2) Anastomosing or longitudinal with lower Ca content lacking calsequestrin. It might be speculated that these anatomically distinct SR components account for the biexponential character of the intermediate compartment. It is not, at present, possible to examine this possibility since specific probes for these components are not available.

Slow Compartment — This compartment is also represented in both cultured and adult cells (Table 1) but is larger and more rapid in the adult cells. The compartment is defined by the fact that addition of NaH_2PO_4 to the perfusate spectifically adds Ca to the compartment in both cultured and adult cells (12,24). Phosphate acts as a proton donor (25) and exchanges for mitochondrial matrix hydroxide ions on the phosphate hydroxide anit-antiporter. The resulting excess anion provides the milieu for accumulation of Ca as the phosphate salt, specifically within the mitochondria. The accumulated Ca exchanges with the characteristics of the slow compartment.

The slow compartment, without phosphate supplementation, contained 18% of exchangeable cellular Ca in the cultured cells and 25% in the adult cells. A recent electron microprobe study of rabbit ventricular muscle indicated that approximately 20% of total cell Ca was localized to the mitochondria (7) in good agreement with the kinetically-defined content. The slow mitochondrial flux contributes considerably less than 1% to the total steady-state Ca flux from the cell.

Compartment-Contraction Relations — With the definition of the compartmental exchange characteristics it becomes possible to compare these with cellular contractile responses under non-perfusion limited conditions (13). Adult cells show a biphasic contractile response to rapid removal (within 100 msec) and return of Ca to the perfusate. Upon Ca removal, the next stimulation, as little as 500 msec later, evokes no contraction. Immediate

repletion of Ca, within 5 seconds, evokes a contraction nearly 100% that of control. These responses indicate that an extracellular source of Ca is mandatory for contraction. The simplest explanation is that Ca removal abolishes the slow inward current and Ca-induced Ca release and, therefore, completely abolishes contraction. This is, indeed, likely with the Ca involved being *extracellular* in origin. However, the contractile response to rapid Na removal and replacement indicates that there is a rapidly exchangeble *cellular* Ca pool which is also important. Reduction of $[Na]_o$ will not change extracellular Ca but will only produce changes in cellular Ca. In the rabbit cell reduction of $[Na]_o$ produces a positive inotropic response that is fully developed within two beats. Upon return to control $[Na]_o$ within a single diastolic interval, the control contraction level is completely achieved at the next contraction. This is clear evidence of the presence of a cellular Ca pool which exchanges within a few seconds. This functionally-defined „fast" pool would be expected to involve Ca located within the kinetically-defined rapid compartment. Its possible origin has been previously discussed.

As the period of Ca depletion is extended over tens of seconds or minutes, repletion of Ca does not produce control level force within one or two beats. Though the „fast" pool is fully repleted within few seconds, return to control level force requires 60 seconds or so (at 12 beats/min). This functionally-defined „slow" pool contributes relatively more, as compared to the „fast" pool, in the rat than in the rabbit cell (13). The temporal characteristics of the functionally defined „slow" pool match most closely with the kinetically-defined intermediate compartment and, therefore, it is likely that the Ca represented is localized to the SR.

The slow compartment's rate of exchange is too slow for its Ca to participate in beat-to-beat contractile control. Its mitochondrial origin indicates that it is the compartment which, under conditions of high cellular Ca-loading, could accommodate large amounts of Ca.

Overview — In the context of the data reviewed above, it is possible to suggest a correlation among function, Ca movement and subcellular compartments. Referring to Figure 1:

1) Upon sarcolemmal depolarization, Ca moves through the Ca channel to arrive at the SR and at the myofilaments.
2) Ca induced Ca release occurs via the „feet" at the SR-inner SL region. The Ca diffuses to the myofilaments or is transported across the SL via the Na-Ca exchanger.
3) Ca is pumped into the free or longitudinal SR and diffuses to the cisternae. Ca is pumped across the SL by the SL Ca pump and by the Na-Ca exchanger.
4) Mitochondrial Ca exchange via the Na-Ca exchanger and/or SL Ca pump.

The movements depicted by 1) occur between excitation and onset of contraction; through 2) and the direct activation arrow of 1) contraction proceeds and Ca begins to efflux via the Na-Ca exchanger; during 3) relaxation is occurring as Ca is pumped into the longitudinal SR and acrossthe SL via the SL Ca pump as well as via the continued operation of Na-Ca exchange.

The movemenets during 1) through 3) occur within the bounds of a single contraction cycle. The *rapid compartment*, under this scheme, is represented by the component of SR cisternal Ca transported across the SL via the Na-Ca exchanger. The *intermediate compartment* involves more slowly exchangeable Ca from the SR which may also be transported by the Na-Ca exchanger. The mitochondria are represented by the slow compartment ($t\frac{1}{2}$ of minutes). The rate is probably limited at the mitochondrial membrane with subsequent exchange across the SL through either the Na-Ca exchanger or the SL Ca pump.

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Author's address: Prof. Dr. G. A. Langer, The Departments of Medicine and Physiology and the Cardiovascular Research Laboratory University of California, Los Angeles School of Medicine, Los Angeles California 90024-1760, USA