Calcium-Dependent Mechanical Oscillations Occur Spontaneously in Unstimulated Mammalian Cardiac Tissues

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SUMMARY. In quiescent rat ventricular myocardium, bathed in solution of 2 mM Ca\(^{++}\) or less, it has been previously demonstrated that spontaneous microscopic oscillatory cell motion is present and interacts with an incident laser beam to produce scattered light intensity fluctuations which can be monitored to quantify the underlying motion. The present study shows that scattered light intensity fluctuations are not present under any conditions in frog atrial or ventricular preparations, but do occur in each type of mammalian cardiac tissue studied in the unstimulated state. The magnitude of scattered light intensity fluctuations in mammalian tissues varies with species and cellular Ca\(^{++}\) loading. In some tissues, e.g., rabbit or ferret ventricle, either an increase in the Ca\(^{++}\) concentration in the perfusate ([Ca\(^{++}\)],), reduction of perfusate Na\(^{+}\) concentration ([Na\(^{+}\)],), or addition of cardiac glycosides was required to elicit scattered light intensity fluctuations; in other tissues, however, e.g., the canine Purkinje fiber, atria, and ventricle, and guinea pig atria, scattered light intensity fluctuations were present at 2 mM [Ca\(^{++}\)], in the absence of experimental Ca\(^{++}\) loading. Scattered light intensity fluctuations were not affected by LaCl\(_3\), or verapamil, and were reversibly abolished by caffeine. When the pCa in the myofilament space is kept constant in detergent "skinned" fibers, scattered light intensity fluctuations are not present during contractile activation. We conclude: that scattered light intensity fluctuations are due to spontaneous intracellular Ca\(^{++}\) oscillations that require a functional sarcoplasmic reticulum; that the potential to exhibit these oscillations is a fundamental property of mammalian excitable cardiac cells; and that, in many mammalian tissues, these oscillations are present in the unstimulated state, even in the absence of experimental perturbations to enhance cell Ca\(^{++}\) loading. (Circ Res 54: 396-404, 1984)

SPONTANEOUS Ca\(^{++}\) oscillations have been demonstrated in many cell types, ranging from macrophages to slime molds, and have been implicated in cellular functions ranging from secretion to chemotaxis (Berridge and Rapp, 1979). In cardiac cell fragments stripped of sarcolemma (Fabiato, 1981a) or devoid of sarcolemmal function (Dani et al., 1979), the sarcoplasmic reticulum can generate spontaneous Ca\(^{++}\) oscillations. Evoked (e.g., after a stimulated action potential or repolarization of an externally applied voltage step) and spontaneous continuous fluctuations in force and membrane current have been observed and attributed to sarcoplasmic reticulum-generated Ca\(^{++}\) oscillations (Katzung, 1964; Glitsch and Pott, 1975; Ferrier, 1976; Lederer and Tsien, 1976; Kass et al., 1978a, 1978b; Kass and Tsien, 1982; Karagueuzian and Katzung, 1982; Matsuda et al., 1982) in cardiac cells and fibers with intact sarcolemma and which have been Ca\(^{++}\)-overloaded experimentally. More recently, studies employing the chemiluminescent protein, aequorin, have directly demonstrated both evoked and spontaneous Ca\(^{++}\) oscillations in Ca\(^{++}\)-overloaded ventricular muscle and Purkinje fiber strands (Wier, 1980; Blinks et al., 1981; Orchard et al., 1983; Wier et al., 1983). Whereas such Ca\(^{++}\) oscillations or their manifestations, i.e., mechanical or membrane current or voltage oscillations, can be relatively easily detected in the Ca\(^{++}\) overload state, at more physiological levels of Ca\(^{++}\) loading, manifestation of these oscillations may be quite subtle, particularly in the unstimulated preparation. Thus, their presence may not be detected in routine measurements of force, voltage, and current, or in measurements of cellular Ca\(^{++}\) with chemiluminescent indicators or Ca\(^{++}\) sensitive electrodes, because these techniques rely on time or space-averaged measurements.

Recent studies utilizing laser spectroscopy and cinemicrography have indicated that spontaneous Ca\(^{++}\)-dependent mechanical oscillations occur in the absence of experimental Ca\(^{++}\) overload in intact rat ventricular muscle in the unstimulated state (Lappe and Lakatta, 1980; Lakatta and Lappe, 1981; Stern et al., 1983). At the microscopic level, this motion appears as myofilament activation which occurs regionally within a cell and travels in a wave-like manner for a variable distance. Because these contractile waves are asynchronous within and among cells (Stern et al., 1983), oscillations in force measured at the ends of the preparation are not readily detected. Still, the resulting microscopic mechanical...
motion is sufficient to modulate the beam of a laser passed through the tissue, which results in scattered light intensity fluctuations (SLIF). When monitored simultaneously, both SLIF and cellular microscopic motion are: (1) abruptly and reversibly abolished by caffeine, (2) not diminished under conditions that inhibit sarcolemmal Na\(^+\) and Ca\(^++\) currents or Na-Ca exchange, and (3) not present when the myoplasmic [Ca\(^++\)] concentration is kept constant by ethylene glycol bis-N,N,N',N'-tetraacetic acid (EGTA) buffering in detergent "skinned" muscles. It has therefore been inferred that the spontaneous mechanical motion that causes SLIF is a manifestation of spontaneous intracellular Ca\(^++\) oscillations of the type generated by the sarcoplasmic reticulum (Stern et al., 1983), i.e., Ca\(^++\)-induced Ca\(^++\) release (CICR), as described in cell fragments (Fabiato, 1981a).

Spontaneous Ca\(^++\) oscillations of this sort could profoundly influence both the experimental and physiological definition of myocardial automaticity (Bozler, 1943; Tsien et al., 1979), excitation-contraction coupling (Lakatta and Lappe, 1981), and metabolism. Therefore, it is important to determine whether such spontaneous Ca\(^++\) oscillations which occur in the apparent absence of experimental Ca\(^++\) overload, e.g., in rat ventricular muscle, are a property common to a variety of cardiac muscle types. The purpose of the present study was to determine whether spontaneous Ca\(^++\) oscillations, manifested as SLIF, could be demonstrated in other types of cardiac tissues in the unstimulated state.

**Methods**

Relatively thin (0.31 \(\pm\) 0.35 mm\(^2\) cross-sectional area) right ventricular papillary muscles, or atrial or ventricular trabeculae from rabbits, dogs, ferrets, guinea pigs, and rats, strips of frog atrial and ventricular muscle, canine Purkinje fibers, and rabbit sinoatrial nodal tissue \((n = 3-6\) of each type) were excised and mounted in a chamber (Stern et al., 1983). Mammalian tissues were superfused with a 100% O\(_2\) gassed solution, containing 140 mM NaCl, 2 mM CaCl\(_2\), 4.2 mM KCl, 1.2 mM MgCl\(_2\), 5 mM glucose, and 3 mM N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid (Hepes). The pH was buffered to 7.4, and temperature was maintained constant at 29°C. Frog tissues were bathed with Ringer's solution containing 117 mM Na\(^+\), 2 mM CaCl\(_2\), 2.5 mM KCl, 5 mM glucose, and 3 mM Hepes at 22°C. During the run-in period, atrial and ventricular tissues were stimulated regularly at 24 beats/min and stretched to the length at which developed force was optimal; Purkinje fibers and sinoatrial nodal tissue were not stimulated during this period and were stretched until the tissue no longer appeared slack under a dissection microscope.

The methods for measuring SLIF have been described previously (Lakatta and Lappe, 1981; Stern et al., 1983). In brief, the light scattered at 40° from an incident He-Ne laser beam was collected on a photomultiplier tube (Malvern RR51) and then analyzed by a Malvern K7025 digital autocorrelator. The autocorrelation function obtained is related to the frequency transform of the scattered light's power spectrum, and, thus, is a method whereby the spectral components of the scattered light may be quantified. In the present study, the autocorrelation function was characterized by its half decay time, \(T_{fw}\), and expressed as \(f_w\), where \(f_w = 1/(2\pi T_{fw})\). For the case of single-order scattering, \(f_w\) is directly proportional to the root mean square velocity of the light scatterers within the illuminated tissue. Thus, \(f_w\) is not equatable with the actual frequency of the underlying mechanical oscillatory motion observed by light microscopy, but, rather, is the product of that frequency and the amplitude of its mechanical displacement in wavelengths of light (Bonner and Carlson, 1975). The scattered light in our experiments was contaminated by a very slow fluctuation in intensity observed even when an inanimate object replaced the muscle. Because these very low frequency fluctuations fail to decorrelate with the long averaging times required to measure \(f_w\) values of less than about 0.35 Hz, SLIF below this level were defined as being below the "system threshold."

Measurements of SLIF were made in the unstimulated state a minimum of 4 minutes after the last externally applied stimulus. This was sufficient time to allow the restoration of \(f_w\) to its stationary value following a period of external stimulation (Lakatta and Lappe, 1981). A tissue in the unstimulated state was defined as quiescent if it did not exhibit the spontaneous twitch-like contractions which conducting tissue, in particular, was prone to display. This type of contractile activity, even under low power microscopy, was obviously synchronous over relatively large regions of the preparation, and could easily be discriminated from the wave-like asynchronous motion that causes SLIF in the unstimulated state. SLIF were measured in tissues which were, by this definition, "quiescent" (1) at intermittent intervals during the 3-hour run in period following mounting of the tissue in [Ca\(^++\)]\(_o\) of 2 mM, and (2) in response to alterations in [Ca\(^++\)], or [Na\(^+\)], or to the addition of caffeine, strophanthidin, verapamil, LaCl\(_3\), or combinations thereof made after SLIF had reached a stationary level following the run-in period. In a few experiments, the preparation was stimulated to produce a single twitch at intervals during a perturbation to define the effect of that perturbation on twitch force. Some muscles were "chemically skinned" with a non-ionic detergent as described previously (Stern et al., 1983), and examined for the presence of SLIF both in the relaxed state and across a full range of buffered Ca\(^++\) concentrations (Fabiato and Fabiato, 1978a).

Specimens from each tissue type were mounted on a microscope stage and observed by means of phase contrast microscopy at 250X. This permitted monitoring of SLIF and direct observation of the tissue motion by changing from coherent to incoherent illumination (Stern et al., 1983). When examined under high power microscopy, unstimulated tissues exhibiting fairly rapid SLIF always exhibited a cellular motion that was distinctly wave-like in nature. In the perturbations described above, those which enhanced SLIF also enhanced the magnitude of this motion, whereas those which reduced or abolished SLIF also reduced or abolished this microscopic cellular motion. However, it is noteworthy that SLIF are responsive to displacements of less than a wavelength of light and thus are more sensitive than is light microscopy for the detection of subtle forms of this motion.

**Results**

The initial protocol was designed to detect whether SLIF were present in a preparation, and, if so, whether they varied with time subsequent to in vitro mounting. At intervals following mounting,
electrical stimulation of atrial and ventricular tissues was discontinued, and SLIF were recorded (Fig. 1). All mammalian specimens demonstrated SLIF immediately after mounting. In some tissue types, typified by the guinea pig atrium and rat ventricle (Fig. 1), $f_n$ decayed with time from its value just after mounting, eventually achieving a stationary value that was relatively stable, i.e., $<5\%$ change per hour, and which could be reliably quantified.

Among these tissues, classified as group I in Table 1, the average stationary $f_n$ was $1.4 \pm 0.17$ Hz. It is important to note that this group of tissues exhibits SLIF in the state of tissue Ca++ loading usually considered as physiological in in vitro experiments.

Other mammalian tissues, typified by the rabbit atrium in Figure 1, exhibited a similar equilibration decay in $f_n$ that continued below our system threshold (dotted line in Fig. 1). In these tissues (group II in Table 1) [Ca++]$_t$ was increased from its initial 2 mM until SLIF could be accurately detected. The average [Ca++]$_t$ required for this in each tissue is given in brackets.

A third type of behavior characterized the only non-mammalian specimens studied, frog atrium and ventricle (group III in Table 1) in which SLIF were not detectable either upon mounting or at any time during this protocol, which included the titration of [Ca++]$_t$ up to 40 mM.

All sinoatrial nodal preparations exhibited spontaneous synchronous twitches, and to measure SLIF in the quiescent state, we employed verapamil, a Ca++ channel blocker, to terminate this spontaneous rhythmic twitch-like activity. In sinoatrial nodal preparations the value for [Ca++]$_t$ in Table 1 was obtained in the presence of verapamil (10$^{-6}$M). In Purkinje fibers, similar spontaneous synchronous contractile activity in parts of specimens was occasionally observed following a train of externally applied electrical stimulation. The addition of verapamil, in this case, as in sinoatrial nodal tissue, terminated the spontaneous organized contractile activity. This reduced both the level and the variation about the mean in consecutive $f_n$ measurements to the values observed prior to the period of external stimulation. However, verapamil was without effect when added to quiescent fibers (Fig. 2) (also see Stern et al., 1983). Note also, that verapamil failed to abolish the characteristic increase in $f_n$ in response to an increase in [Ca++]$_t$.

### Table 1

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<tr>
<th>Group I. Tissues that exhibit a stable stationary value for SLIF in the quiescent state in [Ca++]$_t$ = 2 mM</th>
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<tr>
<td>Rat ventricle</td>
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<td>Canine Purkinje fiber</td>
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<td>Canine atrium</td>
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<td>Canine ventricle</td>
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<td>Guinea pig atrium</td>
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<th>Group II. Tissues that exhibit SLIF transiently after mounting but in which a stable stationary value is not reliably detectable in the quiescent state in [Ca++]$_t$ = 2 mM</th>
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<tr>
<td>Average [Ca++]$_t$ in mM required to generate a stationary stable SLIF</td>
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<tr>
<th>Rabbit atrium</th>
<th>3.8 ± 1.1</th>
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<tr>
<td>Ferret ventricle</td>
<td>8.0 ± 0.57</td>
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<tr>
<td>Rabbit sinoatrial nodal tissue</td>
<td>9.0 ± 1.0</td>
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<tr>
<td>Rabbit ventricle</td>
<td>16 ± 1.5</td>
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<th>Group III. SLIF never observed in quiescent state at any level of [Ca++]$_t$</th>
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<tr>
<td>Frog ventricle</td>
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<td>Frog atrium</td>
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It is reasonable to suspect that a passive movement of Ca++ along its electrochemical gradient may have a role in the [Ca++]_e dependence of SLIF in the unstimulated state. If this were the case, LaCl₃, which displaces Ca++ from the cell surface and prevents passive influx and cellular Ca++ efflux as well (Langer et al., 1979), might be expected to abolish SLIF. The effect of incremental concentrations of LaCl₃ on f_y, in four preparations is illustrated in Figure 3. Note that the average f_y was not substantially altered. At each concentration of LaCl₃, the preparation was stimulated to contract, and tension developed in the twitch was recorded. Note that LaCl₃, although not affecting f_y, markedly reduced twitch tension in a concentration-dependent manner.

Replacement of [Na⁺]_o with lithium not only inactivates the fast Na⁺ channel but also transiently loads the myoplasm with Ca++ via Na-Ca exchange, producing a substantial transient increase in resting force (Mullins, 1979). In Figure 4, notice that in both canine (panel A) and rabbit ventricles (panel B), in which SLIF were present and absent, respectively, prior to Na⁺ removal, transients in f_y paralleled those in force upon removal of [Na⁺]_o. However, in the frog ventricle (panel C), although the force transient was comparable to that in the mammalian tissues, a corresponding transient in f_y was virtually nonexistent. Microscopical examination also failed to show the characteristic wave-like motion.

In Purkinje tissues that have been Na⁺ and Ca++-loaded with cardiac glycosides, evidence for cellular Ca++ oscillations, either evoked by the repolarization of an action potential or voltage clamp, or occurring spontaneously, has been presented (Katzung, 1964; Glitsch and Pott, 1975; Ferrier, 1976; Lederer and Tsien, 1976; Kass et al., 1978a, 1978b; Kass and Tsien, 1982). Figure 5 demonstrates the effect of strophanthidin (2.5 μM) on f_y and resting force in a quiescent canine Purkinje fiber. Note that SLIF are present at [Ca++]_e of 2 mM, even before the addition of the glycoside. Progressive increases in f_y, and a small but definite increase in resting force, occurred with time after the addition of strophanthidin. In other experiments in unstimulated Purkinje fibers, similar increases in f_y occurred even when this

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**FIGURE 2.** The effect of dl-verapamil, 10^(-4)M, in a typical quiescent Purkinje fiber strand. Note that verapamil is without effect on the stable level of SLIF and does not prevent the increase in SLIF when [Ca++]_e is increased.

**FIGURE 3.** The effect of LaCl₃ on f_y and twitch force in four rat papillary muscles. The data have been normalized to the pre-LaCl₃ level of f_y, which was 3.7 ± 1.2 Hz. In each [LaCl₃], the muscle was excited to produce a twitch, and the developed tension, normalized to the pre-LaCl₃ control, is also plotted in the figure. Control twitch force = 8.3 ± 1.4 g/mm². [Ca++]_e was 2.5 mM and muscle cross-sectional area was 0.22 ± 0.05 mm².

**FIGURE 4.** Typical examples of the effect of replacing [Na⁺]_o with lithium on f_y and force (redrawn from original record) in unstimulated ventricular muscles from three species. [Ca++]_e = 2 mM in each panel.

**FIGURE 5.** The effects of the cardiac glycoside, strophanthidin, removal of [Na⁺]_o and caffeine, on f_y and force in a typical Purkinje fiber strand (redrawn from the original record). [Ca++]_e = 2 mM.
experiment was performed in the presence of verapamil. This progressive increase in \( f_a \) with time in the presence of glycosides occurred in all tissues studied except those from the frog. Note (in Fig. 5) that, when \([Na^+]_c\) was removed, \( f_a \) and resting force, as would be expected from the operation of Na-Ca exchange (Fig. 4), exhibited transient increases followed by gradual declines. Interestingly, this decline continued to levels below those preceding the removal of Na\(^+\), approaching the pre-strophanthidin control levels. Reintroduction of \([Na^+]_c\) produced a rapid transient decrease (undershoot), followed by an increase in \( f_a \) and resting tension (see Mullins, 1979).

Whereas SLIF are not present in quiescent rabbit ventricular muscle in \([Ca^{++}]_c\) of 2 mM or less (Table 1), cardiac glycosides resulted in first the appearance of, and subsequently in progressive increases in, \( f_a \) (Fig. 6). In this experiment, a single twitch was produced each minute subsequent to the measurement of \( f_a \). Progressive increases in twitch force were accompanied by increases in \( f_a \). Removal of \([Ca^{++}]_c\) in the presence of EGTA abolished both the twitch and \( f_a \).

In subcellular fragments and disaggregated cardiac cells that exhibit electrochemical shunting, spontaneous \( Ca^{++} \) oscillations are blocked by moderate concentrations of caffeine (Bloom et al., 1974; Dani et al., 1979; Fabiato, 1981a). Addition of caffeine (10 mM) resulted in rapid abolition of \( f_a \) in all tissues, regardless of the level of \( Ca^{++} \) loading. This effect of caffeine was reversible in all tissues, and, generally, upon removal of caffeine, the \( f_a \) returned to control levels somewhat more gradually than it fell after the introduction of caffeine. An example of the caffeine effect is illustrated in Figure 5. In other recent experiments not reported here, ryanodine, also known to inhibit sarcoplasmic reticulum \( Ca^{++} \) transport (Sutko et al., 1979), abolished SLIF, but, unlike caffeine, this effect was irreversible (Sutko and Willerson, 1980; Sutko et al., 1983; Wier et al., 1983).

To attribute the microscopic tissue motion that generates SLIF to oscillations in cellular \([Ca^{++}]_c\), myofilament-generated oscillations which are \( Ca^{++}\)-dependent, but which can occur when the \([Ca^{++}]_c\) surrounding the myofibrils is constant must be excluded (Fabiato and Fabiato, 1978a). This requires measurements of SLIF when the myofilaments are \( Ca^{++}\)-activated in the absence of \( Ca^{++} \) oscillations. Treatment of muscles with the non-ionic detergent Triton destroys membranes and allows buffering of \([Ca^{++}]_c\) in the myofilament space at a steady level. As shown in Figure 7B, when \( Ca^{++} \) oscillations are prevented by EGTA, \( Ca^{++} \) activation, from threshold to maximum, does not generate SLIF. Note that, before detergent treatment, SLIF were present in the same rabbit ventricular preparation when cellular \( Ca^{++} \) was increased with the glycoside (panel A). Sinoatrial nodal and Purkinje tissues were not studied in this protocol, but, in all atrial and ventricular preparations, direct \( Ca^{++} \) activation of the myofilaments (as in Fig. 7B) was not accompanied by SLIF.

**Discussion**

The present results demonstrate that all intact mammalian cardiac tissues initially exhibit SLIF in vitro. The presence of SLIF in the unstimulated state in \([Ca^{++}]_c\) of 2 mM is not, therefore, unique to rat myocardium. Whether the relatively high levels of SLIF observed shortly after mounting mammalian tissues indicates that these levels of SLIF were present prior to removal of the specimen from the heart or whether they resulted from \( Ca^{++} \) or Na\(^+\) overload during the period between excision and mounting, cannot be determined from the present results. With time after mounting, the level of SLIF fell in all tissues (Fig. 1). It is noteworthy that resting heat decays with time after mounting in some mammalian preparations (Loiselle and Gibbs, 1979), and this decay might be related to the decay of SLIF noted during this time. In some tissues, the level of SLIF decayed to a stable detectable level in a \([Ca^{++}]_c\) of 2 mM (Table 1, group I), whereas, in the others, SLIF decayed to levels that could not be reliably detected by the present technique. However, in these tissues in the quiescent unstimulated state, SLIF could be induced by enhancing cell \( Ca^{++} \) loading, either by an increase in \([Na^+]_c\), reduction of \([Na^+]_c\), or addition of glycosides.

The uniformity of response among mammalian tissues upon exposure to an increased cellular \( Ca^{++}\)
load (Table 1) clearly demonstrates the dependence of SLIF on intracellular Ca++. Since SLIF are not seen when the muscle is activated at a constant, buffered, level of Ca++ (Fig. 7B) it follows that SLIF are probably caused by fluctuations in the level of activating Ca++. Caffeine (Fig. 5) rapidly and reversibly abolished SLIF in all tissues, under all conditions studied. Since conditions which increase cAMP, i.e., the administration of sufficient isoproterenol to produce a maximum effect on contractility, does not cause a decrease in fA in unstimulated muscle (Lakatta and Lappe, 1981), the phosphodiesterase activity of caffeine cannot explain this effect. Furthermore, caffeine does not reduce the myofilament affinity for Ca++ (Fabiato and Fabiato, 1975a), nor does it, in this concentration, appear to have a substantial effect on mitochondrial Ca++ fluxes (Blayney et al., 1978), nor has there been an effect demonstrated on transmembrane potential in non-stimulated fibers (Kimoto et al., 1974). We therefore interpret the effect of caffeine in the present study to result from its well-documented effect on sarcoplasmic reticulum Ca++ loading (Weber, 1968; Blayney et al., 1978) and inhibition of Ca++-induced Ca++ release (Fabiato, 1981a). In addition, the fact that Na+-free superfusion does not abolish SLIF and that verapamil is without effect in the unstimulated quiescent preparation suggests that oscillations of ions through the known selective sarcolemmal channels do not have a direct role in the generation of cellular Ca++ oscillations. In addition, the failure of LaCl3 to abolish SLIF suggests that transsarcolemmal flux of Ca++ is not a causal factor in initiating the contractile wave. Taken together, these data strongly suggest that the asynchronous, wave-like, cellular motion which originates regionally within a cell and propagates through the cell and which causes SLIF (Stern et al., 1983), results from sarcoplasmic reticulum-generated CICR, as described in cardiac cells and cell fragments (Bloom et al., 1974; Dani et al., 1979; Fabiato and Fabiato, 1975a; Fabiato, 1981a). It is noteworthy that stretch itself appears to enhance sarcoplasmic reticulum Ca++ release (Fabiato and Fabiato, 1975b), and, therefore, that an approaching wavefront, by stretching the preceding inactive region, may serve to enhance the propagation of that wave.

The species and tissue variations in SLIF shown in Table 1 bear a striking similarity to the pattern observed by Fabiato and Fabiato (1978b) for CICR in mechanically skinned fibers. The rat showed the lowest Ca threshold for oscillations. Atrial tissues exhibited a lower threshold than ventricular tissues from the same species. The rabbit ventricle exhibited the highest threshold. This further supports the hypothesis that SLIF are a manifestation of a spontaneously occurring CICR. Note, that although this tissue-species variation appears not to be intrinsic to the myofilaments (Fabiato and Fabiato, 1978b), it cannot be determined whether the pattern observed
in Table 1 results from tissue-species differences intrinsic to the sarcoplasmic reticulum (Nayler et al., 1975; Fabiato, 1982), from differences in diffusion distances between regions of the sarcoplasmic reticulum (i.e., intracellular geometry of sarcoplasmic reticulum), from differences in the ability of other Ca++ sinks to buffer Ca++ released by the sarcoplasmic reticulum, or from differences in cellular Ca++ loading in a given experimental milieu.

That frog myocardium does not exhibit microscopic mechanical oscillations or "detectable" SLIF, even when [Na⁺], is removed, is in agreement with previous observations in which frog myocardium (1) has a paucity of sarcoplasmic reticulum relative to various mammalian species (Staley and Benson, 1968), (2) failed to exhibit cellular "squirming motion" during Ca++ loading with Na⁺-free perfusion (Anderson et al., 1976), (3) failed to demonstrate CICR in skinned cell fragments (Fabiato and Fabiato, 1978b), and (4) failed to exhibit a sarcoplasmic reticulum generated optical signal subsequent to depolarization (Weiss and Morad, 1981). This evidence suggests that the absence of detectable Ca++ oscillations in frog myocardium is causally related to the failure of its sarcoplasmic reticulum to exhibit significant CICR.

The presence of spontaneous CICR has a profound significance for cardiac function. Depending on cell Ca++ loading, CICR-generated Ca++ oscillations vary in frequency from less than 0.1 to 3-4 Hz in mammalian tissues (Wier et al., 1983; Kort and Lakatta, 1984), and can produce transient localized increases in myoplasmic [Ca++] as high as those seen following excitation of the cell by sarcolemmal depolarization (Fabiato, 1981b; Goshima and Wakahayashi, 1981). The local level of myoplasmic free [Ca++] modulates the transsarcolemmal fluxes of Ca++ as well as those of other cell ions, e.g., H⁺, K⁺, and Na⁺ via channels (Colquhoun et al., 1981; Tsien, 1983), or carrier mechanisms, e.g., Na-Ca exchange (Mullins, 1979). It has already been suggested that cell Ca++ oscillations caused by CICR may be implicated in the initiation of arrhythmias due to glycosides, i.e., that described as "triggered" automaticity during cell Ca++ overload (Ferrier, 1976; Kass et al., 1978a; Kass and Tsien, 1982). Recent studies suggest that some arrhythmias induced by ischemia may have a similar basis (Gough et al., 1983). Even in the absence of abnormal Ca++ homeostasis, CICR could have a role in sinoatrial pacemaker function (Noma et al., 1979). That CICR occurs in intact mammalian myocardium even in the absence of Ca++ overload renders it a potential mechanism for the modulation of excitation-contraction coupling (Fabiato and Fabiato, 1975a). In this regard, it is noteworthy that over an optimal range of cell Ca++ loading, progressive increases in inotropic state are accompanied by progressive increases in SLIF frequency (see Fig. 6, and Lakatta and Lappe, 1981).

The spatiotemporal inhomogeneity in myoplasmic free [Ca++] can affect both diastolic and systolic force production within the myocardium. In the absence of stimulation, local variations in free [Ca++] result in asynchronous myofilament activation, with progressive inhomogeneity being manifest as progressive broadening of the peaks of the diastolic sarcomere deflection pattern (Lakatta and Lappe, 1981). This asynchronous myofilament displacement summates to produce a resting "tone" (Stern et al., 1983) which varies with the inotropic state (Lakatta and Lappe, 1981). Thus, CICR likely accounts for an "active component" of myocardial diastolic compliance observed in earlier studies in isolated cardiac muscle (Meek, 1927; Scherlag et al., 1966; Feigl, 1967; Hoffman et al., 1968), and in the intact heart could also cause changes in the diastolic pressure-volume relationship (Grossman and Barry, 1980).

The presence of spontaneous CICR also implies inhomogeneity of diastolic sarcoplasmic reticulum Ca++ loading within and among cells. Systolic force production, i.e., twitch force, will be affected not only by the inhomogeneity in diastolic sarcomere length, as noted above, but also the spatiotemporal inhomogeneity of sarcoplasmic reticulum Ca++ loading which may result in inhomogeneous Ca++ release at the time of cell depolarization. Thus, twitch force will be compromised when excessive spontaneous CICR occurs prior to an excitation, e.g., in states of high Ca++ loading, and this may account at least in part for the "myocardial failure" associated with cell Ca++ overload.

In addition to having functional implications, the spatial inhomogeneity in free [Ca++], caused by CICR can interfere with the experimental measurement of average free [Ca++], and with the estimation of transsarcolemmal Ca++ ionic gradients. Nonlinear Ca++ indicators, e.g., Ca++ ion-selective electrodes or the chemiluminescent protein, aequorin, provide erroneous estimates of the average free [Ca++], in the presence of gross Ca++ oscillations (Orchard et al., 1983; Wier et al., 1983). Also, because of the highly nonlinear nature of the force-pCa relation, changes in the average free [Ca++], cannot be accurately quantified from changes in "tonic" force when spontaneous CICR are present (Lappe and Lakatta, 1980; Eisner et al., 1983; Chapman et al., 1983). Thus the true force-pCa relationship in the intact myocardium cannot be determined from measurements of "average" [Ca++], with nonlinear probes and tonic force when spontaneous Ca++ oscillations occur (Lakatta et al., 1983). Finally, the presence of Ca++ oscillations implies that neither the myoplasm nor sarcoplasmic reticulum [Ca++] is in a steady state, and this interferes with the interpretation of Ca++ flux data in the context of the steady state concept of "Ca++ pools" within the myocardium (Langer, 1974).
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