Cell Calcium in the Pathophysiology of Ventricular Fibrillation and in the Pathogenesis of Postarrhythmic Contractile Dysfunction

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The mechanism of ventricular fibrillation is poorly understood at the cellular level. We explored the role of intracellular free calcium in the pathophysiology and pathogenesis of ventricular fibrillation in perfused ferret hearts loaded with the Ca$^{2+}$ indicator 5F-BAPTA. Nuclear magnetic resonance spectroscopy was used to measure [Ca$^{2+}$]i, pH, and high-energy phosphates. During ventricular fibrillation induced by burst pacing, [Ca$^{2+}$]i rose rapidly and dramatically, exceeding by four times the control within 5 minutes. [Ca$^{2+}$]i remained markedly elevated throughout 20 minutes of fibrillation, but it returned to control values shortly after defibrillation. In a group of hearts kept isovolumic by a balloon in the left ventricle, acidosis and high-energy phosphate depletion developed despite the maintenance of normal coronary pressure. To distinguish the effects of superimposed ischemia from those of the arrhythmia itself, we lowered left ventricular volume during fibrillation in a second group of hearts. This maneuver decreased wall stress such that fibrillation had no significant adverse effect on intracellular pH, high-energy phosphates, or lactate efflux. [Ca$^{2+}$]i still increased remarkably despite the absence of ischemic changes. Developed pressure did not recover to control levels after defibrillation in either group; the hearts appeared “stunned.” We conclude that intracellular calcium increases as a direct consequence of ventricular fibrillation. The increase in [Ca$^{2+}$]i may cause the contractile dysfunction observed in postarrhythmic hearts. Its possible role in initiating or maintaining the arrhythmia is less clear. (Circulation 1989;80:369–379)
noise analysis of pressure and on electrogram recordings, not on direct measurements of [Ca\(^{2+}\)]. Another important idea that has not yet been tested adequately is the possibility that VF itself induces changes in [Ca\(^{2+}\)], rather than the converse. Does the presumed increase in [Ca\(^{2+}\)] cause the arrhythmia, or alternatively, does the arrhythmia lead to an increase in [Ca\(^{2+}\)]? In either case, calcium overload could have important metabolic consequences\textsuperscript{20} and may help explain why fibrillation can be deleterious even in the absence of superimposed ischemia.\textsuperscript{21}

The questions raised above can be most effectively addressed by directly measuring [Ca\(^{2+}\)] during VF. Such measurements present a special challenge because VF can only be sustained in a myocardial mass of several grams or more.\textsuperscript{1} Luckily, technical innovations now make it possible to measure [Ca\(^{2+}\)] in perfused hearts,\textsuperscript{14-16,22} in which VF can be induced by a variety of maneuvers.\textsuperscript{23-27} In the present study, we measured [Ca\(^{2+}\)] during VF induced by burst pacing in perfused ferret hearts. Nuclear magnetic resonance (NMR) spectroscopy enabled us not only to measure [Ca\(^{2+}\)] but also pH\textsubscript{i} and high-energy phosphates. To induce VF, we chose burst pacing over ischemia and reperfusion or digitalis toxicity: both of the latter interventions raise [Ca\(^{2+}\)], even in the absence of VF, which may bias the results toward showing a correlation even if such a conclusion were unwarranted.

Our observations with electrically induced VF do not support (but cannot entirely exclude) the notion that an increase in [Ca\(^{2+}\)] initiates or maintains the arrhythmia. Instead, we report here that [Ca\(^{2+}\)] increases to remarkably high levels (greater than those during normal systole) as a consequence of VF. The increase in [Ca\(^{2+}\)] has little deleterious effect on energy metabolism when the wall stress of the left ventricle is kept low. Even with such precautions, the recovery of contractile function is poor after VF, providing further evidence for the hypothesis\textsuperscript{28-30} that transient calcium overload leads to myocardial stunning.

**Methods**

The method for the perfusion of isolated hearts has been described in detail elsewhere.\textsuperscript{15,21,32} Briefly, hearts were removed from 13-15-week-old ferrets that had been anesthetized with sodium pentobarbital (200 mg, i.p.) and heparinized. The aorta was cannulated rapidly, and each heart was perfused with 100% O\(_2\)-bubbled solution of the following composition (mM): 108 NaCl, 5 KCl, 1 MgCl\(_2\), 5 HEPES [N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid], 2 CaCl\(_2\), 20 Na acetate, and 10 glucose. The pH was adjusted to 7.4 by titration with NaOH at 30°C. Coronary pressure was maintained at 100 mm Hg throughout the experiments. A thin latex balloon tied to the end of a polyethylene tube was inserted into the left ventricle to measure isovolumic pressure. The balloon was filled with 1 mM 6-fluoro-tryptophan as a standard for the calcium measurements by \(^{19}\)F NMR spectroscopy. The balloon volume was set to achieve an initial end-diastolic pressure of 10-20 mm Hg. Hearts were paced by an agar wick electrode at 1.0-1.5 Hz before and after the period of VF.

**Fluorine Nuclear Magnetic Resonance Measurements of [Ca\(^{2+}\)], in Perfused Hearts**

The Ca\(^{2+}\) indicator 5F-BAPTA (the 5,5'-'difluoro derivative of 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid)\textsuperscript{33,34} was loaded into 28 hearts by perfusion with its tetra-acetoxymethyl ester derivative (5F-BAPTA-AM) as described previously.\textsuperscript{15,32} After perfusion with 5F-BAPTA-AM for 20-30 minutes, the solution was usually changed to modified Tyrode’s solution with 8 mM [Ca\(_i\)] to antagonize partially the negative inotropic effect of the calcium buffering introduced by 5F-BAPTA. In two hearts, [Ca\(_i\)] was kept at 2 mM throughout the experiment. The cytoplasmic concentration of 5F-BAPTA, estimated as described previously,\textsuperscript{15} remained quite stable from the beginning to the end of our experimental protocol (0.25±0.05 mM before VF, 0.23±0.05 mM after) so that leakage of 5F-BAPTA into the extracellular space cannot account for the observed changes in [Ca\(^{2+}\)].

The objective in 17 of the 28 hearts was to measure [Ca\(^{2+}\)]. These hearts were lowered into the bore of a superconducting magnet (8.5 Tesla) connected to a Bruker AM-360 NMR spectrometer (Billerica, Massachusetts) that was operated in the pulsed Fourier transform mode. The details of the NMR pulse protocols have been given previously.\textsuperscript{15} In the present study, spectra were averaged for periods of 1-10 minutes to achieve an acceptable signal-to-noise ratio and were acquired sequentially throughout the experiments. All chemical shifts were referenced with respect to the 6-fluoro-tryptophan signal, assigned to 0 ppm. [Ca\(^{2+}\)] can be calculated\textsuperscript{33} according to the equation $[\text{Ca}^{2+}] = K_\text{d} \cdot [B]/[F]$, where [B] and [F] represent the concentrations of the indicator bound to calcium and free in the cytoplasm, respectively. These are proportional to the areas under characteristic peaks in the \(^{19}\)F spectra (about 8 ppm for B and 2 ppm for F). We have used the $K_\text{d}$ of 285 nM previously measured at 30°C in solutions buffered with EGTA (ethyleneglycolbis-(β-aminooxyethyl ether-N,N,N',N'-tetraacetic acid)\textsuperscript{15} to calibrate our signals.

In several experiments, [Ca\(^{2+}\)], reached such high levels that the indicator was practically saturated with calcium so that the area under the free peak in the spectra was difficult to quantify reliably. Because the term [F] appears in the denominator of the equation used to calculate [Ca\(^{2+}\)], small errors in measurement of the area under the free peak can give rise to large errors in calculated [Ca\(^{2+}\)]. Thus, when the free peak was too small, we chose to set a lower limit for [Ca\(^{2+}\)], rather than to quantify it with
more precision than was warranted by the data. Except in spectra with unusually favorable signal-to-noise characteristics, we found that [B]/[F] ratios of 10 were as high as we could quantify with confidence; the values for [Ca\(^{2+}\)] that appear to equal or exceed 10 times the K\(_d\) are therefore generally reported as [Ca\(^{2+}\)] \( \geq 2.85 \mu\text{M} \). Fortunately, this value falls well within the range of [Ca\(^{2+}\)], usually achieved in physiologic excitation-contraction coupling.\(^{32}\)

Most of the spectra were time-averaged (i.e., not gated with respect to the cardiac cycle). In two experiments, we obtained time-resolved spectra that allowed us to measure the calcium transient before VF with gated \(^{31}\)P NMR spectroscopy as described previously.\(^{32}\) For these measurements, the excitatory radiofrequency pulses and subsequent NMR data acquisition were gated according to a programmable delay from the time of the pacing stimulus. One pulse was applied during each cycle, and 600 consecutive gated scans were averaged at each time in the cardiac cycle.

**Phosphorus Nuclear Magnetic Resonance Measurements**

For measurement of pH\(_i\) and high-energy phosphates in the remaining 11 hearts, we used \(^{31}\)P NMR spectroscopy and a different spectrometer. The NMR methods have been described previously.\(^{31}\) In brief, \(^{31}\)P NMR spectra were collected with 34–45° pulses delivered at 2-second intervals and averaged for 5-minute periods with a Bruker WH-180 spectrometer and a Nicolet 1280 computer (Madison, Wisconsin). The amounts of P\(_i\), phosphocreatine (PCr), and ATP in the myocardium were determined by planimetry of the areas under individual peaks, and each value was expressed as percent of control ATP. Also, pH\(_i\) was determined from the shift between the peaks for P\(_i\) and PCr.

**Lactate Efflux**

Lactate was measured in the coronary effluent of four hearts during \(^{31}\)P NMR studies with a one-filter (340 nm) end-point technique to detect NADH produced from lactic acid on a mole-for-mole basis after oxidation catalyzed by lactic dehydrogenase (Sigma, St. Louis, Missouri).\(^{35}\) From the coronary flow rate and the heart weight, effluent lactate concentrations were converted to efflux rates in units of micromoles per gram of wet weight per minute.

**Experimental Protocols**

The experimental protocols are depicted schematically in Figure 1. After 1–3 time-averaged \(^{19}\)F (or \(^{31}\)P) spectra were obtained under control conditions, ventricular fibrillation was induced by repetitive ventricular stimulation at 10–30 Hz for approximately 10 seconds. Of 28 total hearts, VF could be readily induced in 16. In seven of these hearts, VF was maintained uninterrupted for 20 minutes until defibrillation by bolus injection of lidocaine (0.2 mg). The remaining nine hearts defibrillated spontaneously at least once during 20 minutes; VF was reinduced by burst pacing within 10 seconds in each case. No hearts were included in which spontaneous defibrillation events were so frequent that more than 7% of any VF spectrum was obtained during periods when the hearts were not fibrillating. After 20 minutes, VF was terminated with lidocaine or, in one case, by spontaneous defibrillation.

As the bottom row of Figure 1 shows, left ventricular volume was either kept constant throughout the experiment (group 1, n=4) or reduced during fibrillation to keep the mean left ventricular pressure between 10 and 20 mm Hg (group 2, n=12). The hearts were paced at 1.0–1.5 Hz before and after fibrillation, then patted dry and weighed at the conclusion of each experiment.
**Statistical Analysis**

All pooled data are presented as mean±SEM. Statistical analysis was performed with paired or nonpaired $t$ tests or analysis of variance when indicated. Results were regarded as significant when $p$ was less than 0.05.

**Results**

**Detection of Ventricular Fibrillation**

Strong magnetic fields make it difficult to obtain high-fidelity electrical recordings during NMR experiments. We thus sought an alternative method for detecting the presence of fibrillation. Fortunately, the electrical and mechanical signals are closely correlated during VF; noise analysis has shown that both signals exhibit characteristic periodic oscillations at the same frequency. We thus inferred the presence of fibrillation from isovolumic pressure recordings rather than from extracellular electrograms. Figure 2 shows a typical record of left ventricular pressure (top panel) during VF; note the remarkable similarity between the pressure pattern and our mental image of VF from electrocardiography. The bottom panel shows a spectrum derived by fast Fourier transformation of the pressure signal during ventricular fibrillation. There is a clear-cut peak at 8–10 Hz; such spectra are quite distinctive and further support our visual identification of ventricular fibrillation as the underlying rhythm. This approach echoes the classic descriptions of fibrillation by Garrey:

*these were based not on electrograms, but rather on observations of the mechanical activity of fibrillating myocardium.*

**[$Ca^{2+}$], During Ventricular Fibrillation**

We first investigated the behavior of [$Ca^{2+}$], in hearts that were kept isovolumic throughout the entire experiment; left ventricular volume was not reduced during VF. Figure 3 shows the results from one such experiment, in which $^{19}F$ NMR spectra were collected every 10 minutes before, during, and after VF. A representative spectrum during VF (b, upper panel) reveals a striking increase in the calcium-bound peak (B) relative to the calcium-free peak (F) compared with spectra obtained during the initial (a) or post-VF (c) periods of paced rhythm. These spectra report [$Ca^{2+}$], which is plotted in the lower panel as a function of time during the experiment. The period of VF is indicated by the bar above the data and is demarcated by dashed vertical lines. [$Ca^{2+}$], increased quite remarkably during fibrillation, reaching a value three to four times control within the first 10 minutes. [$Ca^{2+}$] remained elevated throughout VF but returned quickly to control.
levels after defibrillation by a bolus injection of lidocaine (at 20 minutes). This pattern of a dramatic, reversible increase in [Ca$^{2+}$], during fibrillation was observed in a total of three hearts in which volume was kept constant.

*Is the Increase in [Ca$^{2+}$], Secondary to Ischemia?*

The increase in [Ca$^{2+}$], seen during 20 minutes of VF is faster and greater than that observed during 20 minutes of global ischemia (cf. Marban et al$^{15}$). Nevertheless, we were concerned that the increase in [Ca$^{2+}$] during VF might be at least partially attributable to ischemia despite the maintenance of a normal perfusion pressure. When hearts are kept isovolumic throughout VF, the mean wall tension is quite high (as evidenced by an average ventricular pressure of 60 mm Hg during VF in the heart that reported the data in Figure 3); there is never a period of coordinated diastole to favor coronary flow.$^{18}$ The effects of the consequent decrease in perfusion are likely to be accentuated by an increase in energy demand during VF.$^{40,41}$

To evaluate the possibility of ischemia, we measured $^{31}$P NMR spectra and lactate efflux.$^{28,29}$ Figure 4A shows the changes in [ATP], [PCr], [Pi], pH, and lactate efflux during and after VF with the heart kept isovolumic. During VF, induced at time 0, Pi clearly increases, and PCr falls concomitantly (second panel). The figure also reveals further metabolic evidence of ischemia: ATP depletion (top panel), acidosis (third panel), and increased lactate efflux (bottom panel). Given this evidence
for substantial subendocardial ischemia, we were not entirely comfortable with attributing the observed increase in $[\text{Ca}^{2+}]_i$, during VF to the rhythm itself. Therefore, in a second group of hearts, we reduced the left ventricular volume during fibrillation such that the mean left ventricular pressure was $10-20$ mm Hg; we hoped to remove the confounding effect of ischemia and thus to determine the primary consequences of the arrhythmia itself.

Reduction of the left ventricular volume during ventricular fibrillation (see Figure 1, group 2) is expected to improve substantially the myocardial metabolic supply-demand ratio.\textsuperscript{19,20} In fact, such is the case. Pooled data from three hearts in group 2, shown in Figure 4B, reveal the absence of any significant changes in [ATP] (top panel), [P] and [PCr] (second panel), or pH\textsubscript{H} (third panel) during fibrillation. The bottom panel of Figure 4B further shows that there is no increase in lactate production during ventricular fibrillation in group 2. These findings reassure us that myocardial ischemia does not occur during VF when ventricular volume is reduced. The small, statistically insignificant changes in the metabolites hint that there may still be an increase in $[\text{Ca}^{2+}]_i$, during VF in unloaded hearts because calcium overload, even in the absence of ischemia, taxes cellular energy metabolism.\textsuperscript{20,42}

**Figure 5.** Changes in $[\text{Ca}^{2+}]_i$ during and after ventricular fibrillation (VF) in group 2. Panel A: Representative \textsuperscript{19}F nuclear magnetic resonance spectra in control, during the early phase of ventricular fibrillation (0–5 minutes) and in the postventricular fibrillation period (20–25 minutes). Panel B: Plot of pooled data from six hearts (except the data during $-15$ to $-10$ minutes [n=2] and $-10$ to $-5$ minutes [n=3]).

**Figure 6.** Top panel: 1-minute \textsuperscript{19}F nuclear magnetic resonance spectra during control (a) and during the early phase of ventricular fibrillation (VF) (b, 0–1 minute; c, 6–7 minutes) from an experiment with an unusually favorable signal-to-noise ratio. Bottom panel: Plot of time course of the changes in $[\text{Ca}^{2+}]_i$, has been fit by eye to two exponential curves. Values that equal or exceed 2.85 $\mu$M are plotted as $\geq$2.85 $\mu$M.

**Does $[\text{Ca}^{2+}]_i$, Increase in Hearts With a Reduced Left Ventricular Volume During Ventricular Fibrillation?**

Figure 5A shows representative \textsuperscript{19}F NMR spectra from a heart in group 2. During fibrillation, the area under the bound peak is greatly increased at the expense of the area under the free peak; as seen in the right-hand spectrum of Panel A, the changes were fully reversible upon defibrillation. Figure 5B shows pooled data from six hearts at 5-minute resolution, depicting the time course of $[\text{Ca}^{2+}]_i$, before, during, and after 20 minutes of VF. $[\text{Ca}^{2+}]_i$ is already significantly elevated in the first 5 minutes and remains high throughout the period of VF ($p<0.01$ vs. control). Upon defibrillation, the recovery of $[\text{Ca}^{2+}]_i$ is very rapid (NS vs. control within the first 5 minutes).

To define more precisely the time course of the increase in $[\text{Ca}^{2+}]_i$ during fibrillation, we obtained spectra at 1-minute resolution in the experiment shown in Figure 6, from a heart that exhibited a particularly favorable signal-to-noise ratio. In the control period, $[\text{Ca}^{2+}]_i$ was quite stable. Fibrillation was induced at time 0. Close inspection of the time course reveals a trend for $[\text{Ca}^{2+}]_i$, to increase incrementally during the first 5 minutes of fibrillation: the full increase in $[\text{Ca}^{2+}]_i$ is clearly not immediate, and $[\text{Ca}^{2+}]_i$ only reaches steady state after 5–10 minutes.

**Effect of Perfsusate Calcium Concentration**

As the $[\text{Ca}]_o$ is routinely elevated to 8 mM after loading to antagonize the negative inotropic effect of calcium buffering introduced by 5F-BAPTA, the
increase in \([Ca^{2+}]\) during VF shown in Figures 5 and 6 may be an artifact of working in high \([Ca]\). To exclude this possibility, we performed the same protocol but with perfusate containing only 2 mM \([Ca]\). Figure 7 shows the results of such an experiment. The \(^{19}\text{F}\) NMR spectra still show a sizable increase in the area of the bound peak, and a decrease in the free peak, during VF. Although the absolute values of \([Ca^{2+}]\) are lower during fibrillation than those generally observed with 8 mM \([Ca]\), in the perfusate, this is also true during the control period as expected from previous results.\(^{15}\) A second experiment with 2 mM \([Ca]\) exhibited an even greater increase in \([Ca^{2+}]\), during VF than that shown in Figure 7. Thus, the changes in \([Ca^{2+}]\) are qualitatively similar with either 2 or 8 mM \([Ca]\).

Is the Rapid Decrease in \([Ca^{2+}]\) Upon Defibrillation Induced by Lidocaine?

Lidocaine can modify \([Ca^{2+}]\), secondarily by blocking voltage-dependent sodium channels and decreasing \([Na]\).\(^{43}\) Therefore, the rapid decrease in \([Ca^{2+}]\), upon defibrillation might be attributed to this agent even though it was present only transiently in the perfusate. This seems unlikely given the result in Figure 8, for a rapid decrease in \([Ca^{2+}]\), was also observed in this heart in which VF terminated spontaneously without lidocaine. We conclude that the fall in \([Ca^{2+}]\) is due not to the drug but rather to cessation of the arrhythmia.

Postarrhythmic Contractile Dysfunction

We have previously found that transient cellular calcium overload leaves behind long-lasting contractile dysfunction practically indistinguishable from that in postischemic "stunned" myocardium.\(^{29,44}\) If, as concluded before, a history of calcium overload suffices to stun the heart, we might expect postfibrillatory contractility to be low as a direct after-effect of the substantial rise in \([Ca^{2+}]\), during fibrillation. Figure 9 shows that this is indeed the case, even in group 2 hearts. Panel A shows records of left ventricular pressure in one experiment before and 20 minutes after fibrillation. Developed pressure in the post-VF period falls by about 50% compared with the response before fibrillation. The pooled data in Panel B show that this finding was consistent. The postfibrillatory dysfunction is not due to the transient exposure to lidocaine because a similar reduction in developed pressure was seen in the heart that recovered spontaneously from ventricular fibrillation (\(\square\) symbols). The observed postarrhythmic contractile dysfunction does not occur only in hearts loaded with the \([Ca^{2+}]\) indicator; in a heart containing no cytoplasmic calcium buffer and perfused with 2 mM \([Ca]\), (data not shown), developed pressure fell from 112 mm Hg before VF to 46 mm Hg afterward (group 2 protocol).

Discussion

We have found that \([Ca^{2+}]\), increases rapidly during VF, even in the absence of superimposed ischemia. Left ventricular developed pressure is markedly decreased as an after-effect of fibrillation. We will now consider the implications of our observations for the cellular mechanisms of fibrillation and postarrhythmic contractile dysfunction.

What Factors Initiate or Maintain Ventricular Fibrillation?

In these experiments, we excluded 12 hearts (46%) because VF was not readily induced or
sustained. We checked a number of variables that might reveal a clue to the origin of the differences in inducibility. Myocardial mass is an important variable to consider because fibrillation is favored by a large substrate.\(^1\) However, the mean wet weights of the inducible and noninducible hearts were fortuitously identical (5.26±0.28 vs. 5.26±0.31 g). As a second potential contributor, we compared \([\text{Ca}^{2+}]\), in the two groups in the control period before burst pacing. Again, there was no significant difference between the two groups (0.29±0.03 vs. 0.33±0.03 \(\mu\)M). We suspect that the differences in inducibility arise largely from nonbiologic factors such as the high impedance of our pacing system, which was adapted specifically for NMR experiments; if so, inducibility should be more uniform with conventional pacing electrodes, but we have not yet tested this idea.

With regard to the maintenance of fibrillation, we considered the possibility that \([\text{Ca}^{2+}]\) might have been higher in the hearts that did not defibrillate spontaneously. The results in Figure 10 argue strongly against this idea. Each point represents the average \([\text{Ca}^{2+}]\) in a given spectrum. The points have been sorted into two columns that show whether or not the heart defibrillated spontaneously at least once during the accumulation of the spectrum. There is clearly no predictive value of mean \([\text{Ca}^{2+}]\) with regard to the likelihood of spontaneous defibrillation. This lack of correlation is even more striking when one considers that the values of \([\text{Ca}^{2+}]\) in the spontaneous defibrillation column are underestimated by up to 7% (the greatest fraction of time not in VF in any given spectrum). Therefore, our findings do not support the hypothesis that the increase in \([\text{Ca}^{2+}]\) during VF is sufficient to sustain the arrhythmia.

**Role of \([\text{Ca}^{2+}]\), Considered in the Context of Earlier Work**

Several reports have implicated an increase in \([\text{Ca}^{2+}]\) in the initiation of VF, but the evidence for this hypothesis remains circumstantial as reviewed briefly here. Catecholamines raise intracellular calcium and promote VF\(^4,8\); the associated fall in the VF threshold can be prevented by calcium influx blocking agents.\(^27\) Similarly, pretreatment with calcium blocking agents blunts the decrease in the threshold of VF during coronary occlusion.\(^7,45\) These observations are consistent with a direct role of calcium ions in the initiation of VF during ischemia or catecholamine infusion,\(^8\) but other factors (such as improved collateral perfusion or a decrease in myocardial oxygen consumption) were not excluded. Not all the evidence favors an etiologic role for \([\text{Ca}^{2+}]\). Lynch et al\(^46\) found that diltiazem and KB-944 (another calcium antagonist) had no effect on the electrical current threshold required to induce ventricular fibrillation after a recent myocardial infarction. Experiments in nonischemic myocardium, which are the most comparable to our conditions, have shown that verapamil does not significantly affect the strength of electrical stimuli required to elicit VF.\(^47\) Thus, the evidence in the literature does not uniformly support the idea that an increase in \([\text{Ca}^{2+}]\) initiates VF.

Our technique for measuring \([\text{Ca}^{2+}]\) does not have the temporal or spatial resolution that would be required to assess this idea critically. Ideally, \([\text{Ca}^{2+}]\) should be measured in real time at or near the pacing site during initiation of the arrhythmia.
What we can definitely conclude is that \([\text{Ca}^{2+}]_i\) increases as a consequence of VF induced by burst pacing. After burst pacing, several minutes are required for \([\text{Ca}^{2+}]_i\) to reach a new, elevated steady state. Upon defibrillation, \([\text{Ca}^{2+}]_i\) returns to control levels quickly, indicating the dependence of the increase in \([\text{Ca}^{2+}]_i\) on the presence of the arrhythmia. The calcium buffering effect of 5F-BAPTA may well attenuate the amplitude of the increase in \([\text{Ca}^{2+}]_i\), while affecting its time course less profoundly (as determined by the effects of calcium buffering on cytoplasmic \([\text{Ca}^{2+}]_i\) transients\(^{12}\)). The greatest slowing would be expected in the rate of fall of \([\text{Ca}^{2+}]_i\) after VF because of competition between calcium extrusion or sequestration and the unbinding of calcium from buffer molecules; this would not affect our basic conclusions because the observed fall in \([\text{Ca}^{2+}]_i\) upon defibrillation is already near the limits of temporal resolution with this technique. In summary, our findings clearly support the view that the increase in \([\text{Ca}^{2+}]_i\) is secondary to the arrhythmia, but we cannot exclude an additional, primary role under some conditions (particularly during ischemia or reperfusion).

Mechanisms of the Increase in \([\text{Ca}^{2+}]_i\), During Ventricular Fibrillation

Figure 11 shows an experiment designed to compare time-averaged \([\text{Ca}^{2+}]_i\) during fibrillation to the time-resolved values of \([\text{Ca}^{2+}]_i\) during physiologic excitation-contraction coupling. We first obtained gated \(^{19}\text{F}\) NMR spectra during control conditions at various times in the cardiac cycle (Panel A). Diastolic \([\text{Ca}^{2+}]_i\) is 100–200 nM, but \([\text{Ca}^{2+}]_i\) increases to about 800 nM during early systole. We then switched to time-averaged data acquisition (Panel B) and induced VF. Time-averaged \([\text{Ca}^{2+}]_i\) increased rapidly to levels at least three times higher than the peak systolic \([\text{Ca}^{2+}]_i\), in controls. Indicators that are calcium buffers, including 5F-BAPTA and its fluorescent relatives indo-1 and fura-2, systematically underestimate the true time-averaged \([\text{Ca}^{2+}]_i\) when \([\text{Ca}^{2+}]_i\) is varying with time\(^{48}\); thus, the properties of the indicator would tend to bias the results in the direction opposite to that observed. These results point out that the increase of \([\text{Ca}^{2+}]_i\) during VF must, at the very least, involve an increase in systolic \([\text{Ca}^{2+}]_i\) in any given cell. An additional increase in diastolic \([\text{Ca}^{2+}]_i\) would make it easier to explain the marked elevation of time-averaged \([\text{Ca}^{2+}]_i\) during VF.

Direct recordings of membrane potential during fibrillation reveal oscillations at about –60 mV, punctuated by frequent but irregular slow response action potentials.\(^{25,26,49,50}\) An increase in the depolarization rate tends to favor an increase in both diastolic and systolic \([\text{Ca}^{2+}]_i\).\(^{51}\) The irregularity of depolarization, by setting up variable intervals for repriming, would render many of the beats subject to postextrasystolic potentiation.\(^{52}\) These factors could conspire to elevate mean \([\text{Ca}^{2+}]_i\) as a direct consequence of the electrical events known to occur during fibrillation. The mechanical inhomogeneity in fibrillating myocardium may make an additional contribution: the pull of active regions on their relaxing neighbors would favor an increase in \([\text{Ca}^{2+}]_i\) by length-dependent mechanisms.\(^{53}\) For the present, the relative roles of these (or other) processes must remain speculative. Future pharmacologic studies and measurements of \([\text{Ca}^{2+}]_i\) in real time promise to provide a more complete understanding of the mechanisms that underlie the increase in \([\text{Ca}^{2+}]_i\) during fibrillation.

Postfibrillatory Contractile Dysfunction and Its Implications

The idea that transient calcium overload can produce long-lasting contractile dysfunction grew out of previous work from our laboratory on the reversible postischemic dysfunction known as
stunned myocardium. We found that stunning was attenuated by reperfusing with solutions of low calcium concentration and was mimicked by transient exposure to high [Ca], even in the absence of ischemia. The precise subcellular mechanism has not been elucidated, but at least part of the dysfunction is attributable to a decreased Ca⁺ responsiveness of the myofilaments. Given this background, we were not surprised that VF, which we have shown to be a calcium-overload state, leaves behind considerable contractile dysfunction even when there has been no compromise of coronary perfusion.

The clinical implications of this observation are obvious. After a cardiac arrest due to VF, patients often require hemodynamic support even when cardiopulmonary resuscitation has been performed optimally. As clinicians, we generally implicate inevitable ischemic damage to the myocardium during the arrest as the cause of the dysfunction. Our work here shows the inaccuracy of this assumption: fibration itself may well be the culprit. Thus, strategies directed at optimizing hemodynamic performance after cardiopulmonary resuscitation may profitably include maneuvers (such as exposure to calcium antagonists) directed at preventing the massive increase in [Ca²⁺], during fibration.

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