Calcium Oscillations in Digitalis-Induced Ventricular Fibrillation: Pathogenetic Role and Metabolic Consequences in Isolated Ferret Hearts

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The pathophysiology of the ventricular fibrillation that complicates digitalis intoxication was investigated. In this and other calcium-overload states, oscillations of the intracellular free calcium concentration ([Ca\(^{2+}\)]) have been implicated as the cause of ventricular tachyarrhythmias. We addressed two questions: 1) Are [Ca\(^{2+}\)] oscillations obligatory in the pathogenesis of ventricular fibrillation during digitalis toxicity? 2) What are the metabolic consequences of [Ca\(^{2+}\)] oscillations? Ferret hearts (n = 20) were Langendorff-perfused at constant flow with oxygenated HEPEs-buffered Tyrode's solution at 37°C. Isovolumic left ventricular pressure was measured along with the extracellular electrogram or with simultaneous phosphorus nuclear magnetic resonance spectra. When strophanthidin (20 µM) was added during pacing at 3 Hz, the positive inotropic effect soon gave way to a decrease in developed force. The decrease in force was accompanied by an increase in inorganic phosphate concentration and increased the phosphocreatine concentration (p<0.05) despite continuing exposure to strophanthidin. The results indicate that oscillations of [Ca\(^{2+}\)] are not required to sustain ventricular fibrillation, but when present, such oscillations contribute importantly to metabolic deterioration. (Circulation Research 1988;62:609-619)
jected to spectral and cross-correlation analysis. To investigate the role of Ca\(^{2+}\) cycling during ventricular fibrillation, we used ryanodine, a potent inhibitor of cellular Ca\(^{2+}\) oscillations.\(^{14}\) The results are interpreted in light of previous measurements of metabolites and of [Ca\(^{2+}\)]\(_{i}\), in calcium-overloaded heart cells.

**Materials and Methods**

The method for the perfusion of isolated hearts has already been described in detail.\(^{15,16}\) Briefly, hearts were removed from 10–14-week-old ferrets that had been anesthetized with sodium pentobarbital (80 mg/kg i.p.; Harvey Laboratories, Philadelphia, Pennsylvania) and heparinized. The aorta was cannulated rapidly, and each heart was perfused with 100% O\(_2\)-bubbled solution of the following millimolar composition: NaCl 108, KCl 5, MgCl\(_2\) 1, K-HEPES 5, Na acetate 20, glucose 10, CaCl\(_2\) 2. The pH was adjusted to 7.4 by titration with NaOH at 37°C. After 20–30 minutes of equilibration, the coronary flow rate, controlled by a peristaltic pump, was adjusted such that the mean perfusion pressure was maintained at 80 mm Hg (mean flow rate = 35 ml/min); flow was then kept constant throughout the experiment. The left ventricle was filled with a latex balloon containing 15 mM Mg trimetaphosphate as a standard for the NMR experiments, and isovolumic left ventricular pressure was recorded with a Statham P23dB pressure transducer (Hato Rey, Puerto Rico), a Brush chart recorder, and an FM instrumentation tape recorder (3964A, Hewlett-Packard, Palo Alto, California). The heart was paced at 2.8–3.2 Hz through the right ventricle with an agar wick soaked in saturated potassium chloride and encased in polyethylene tubing. Pacing was discontinued when the spontaneous heart rate surpassed the pacing rate because of frequent extrasystoles or when ventricular fibrillation was induced. The hearts were weighed at the conclusion of each experiment.

**Phosphorus Nuclear Magnetic Resonance Measurements**

The \(^{31}\)P NMR methods have been described previously.\(^{18}\) In summary, the heart was lowered into a 25-mm diameter NMR tube and placed into the bore of a 4.2 Tesla superconducting magnet. \(^{31}\)P NMR spectra were collected every 6 minutes with 45° pulses delivered at 2-second intervals with a Bruker WH-180 spectrometer (Billerica, Massachusetts) and a Nicolet 1280 computer. Exponential multiplication equivalent to 10-Hz line broadening was used to smooth the spectra.

The amounts of inorganic phosphate (Pi), phospho-
creatin (PCr), and ATP in myocardium were calculated from the areas under their respective peaks measured planimetrically using a Hewlett-Packard 9810A digitizer, relative to the known concentration and volume of the magnesium trimetaphosphate standard. The tissue contents of Pi, PCr, and ATP were divided by the measured weight of each heart to yield concentrations ([Pi], [PCr], and [ATP]) in units of micromoles per gram wet weight. The saturation in

spectra was corrected with the following T\(_1\) relaxation times: 1.9 seconds for Pi, 2.3 seconds for PCr, 0.81 seconds for ATP, and 4.2 seconds for magnesium trimetaphosphate. Intracellular pH (pH\(_i\)) was determined from the shift between the peaks for Pi and PCr, as described previously.\(^{18}\)

**Experimental Design**

Experiments were performed in a total of 20 hearts. In 16 experiments, strophanthidin (20 \(\mu\)M; Sigma Chemical, St. Louis, Missouri) was added to the perfusate after the acquisition of control data, and ventricular fibrillation resulted 12–25 minutes later. \(^{31}\)P NMR spectra were obtained consecutively in 12 of the hearts exposed to strophanthidin. In four hearts, six consecutive spectra were acquired during ventricular fibrillation in the absence of ryanodine as an isochronal control. In six hearts, three spectra were collected after the onset of ventricular fibrillation, after which ryanodine (10\(^{-7}\) M; Penick, Lyndhurst, New Jersey) was added to the perfusate, and three more spectra were obtained. In two hearts, the balloon in the left ventricle was deflated during ventricular fibrillation to determine the effect of mechanical unloading on energy metabolism.

To demonstrate the stability of pressure and metabolites in the absence of strophanthidin, four of the 20 hearts were simply paced and perfused with standard solution for 66 minutes, a period equal to the total duration of the usual protocol, during which time \(^{31}\)P NMR spectra were acquired as in the hearts exposed to strophanthidin. Extracellular electrical potential (V\(_e\)) and pressure were measured simultaneously in four hearts. Such experiments could not be performed in the magnet because of the technical difficulty of measuring electrical signals of small amplitude in a strong magnetic field. Electrical potential was measured differentially from a cadmium-plated steel electrode (model 3220, ITT Pomona Electronics, Pomona, California) in the solution bathing the heart to another electrode at the aortic cannula with a voltmeter (model F-29, World Precision Instruments, New Haven, Connecticut). The protocol for these experiments was the same as that of the experiments in which \(^{31}\)P NMR spectra were obtained before and after the addition of ryanodine.

**Frequency Analysis of Pressure and Electrical Potential Signals**

For frequency analysis, the simultaneously recorded data of pressure and electrical potential were digitized at 100 Hz (Cheshire Data Interface, Indec Systems, Sunnyvale, California) and analyzed for spectral density.\(^{19}\) The final power spectra of pressure and electrical potential were obtained by averaging six power spectra of consecutive data segments, each of which was calculated from 1,024 data points with a discrete fast Fourier transformation subroutine in BASIC-23 (Indec Systems, Sunnyvale, California). The data for 10.24 seconds were transformed into one power spectrum so that, finally, the power spectrum...
was obtained from a consecutive 61.44 seconds of data. For the calculation of power spectra, the direct current component was subtracted before the transformation to improve the resolution of higher frequency components. The perfusion pump sometimes caused a low-amplitude ripple on the pressure trace at 0.7-1.4 Hz, which remains evident in the power spectra even after the biological pressure oscillations are attenuated (e.g., Figure 5, top row, right-hand panel). This peak did not obscure the biological signals, which are much greater in amplitude and tend to occur at higher frequencies.

To determine the temporal relation between the voltage ($V_e$) and pressure ($P$) signals, the cross-correlation function $r(kt)$ was calculated from the same 1,024 data points obtained simultaneously (not averaged) and expressed after normalization by the standard deviation of both sets of data. The discrete form of the cross-correlation function is as follows:

$$r(kt) = \frac{\sum_{i=1}^{1,024-k} (P(it) - \bar{P})(V_e(i + k)t - \bar{V}_e)}{\sqrt{\sum_{i=1}^{1,024-k} (P(it) - \bar{P})^2 \cdot \sum_{i=1}^{1,024-k} (V_e(i + k)t - \bar{V}_e)^2}}$$

(1)

where $t$ is the sampling interval (10 msec), $\bar{P}$ and $\bar{V}_e$ are the means of $P(it)$ and $V_e(it)$, respectively,

$$\bar{P} = \frac{1}{1,024-k} \sum_{i=1}^{1,024-k} P(it)$$

(2)

and

$$\bar{V}_e = \frac{1}{1,024-k} \sum_{i=1}^{1,024-k} V_e(i + k)t$$

(3)

When the electrical potential showed very slow direct current drift, $V_e$ was processed with a high-pass digital filter (cut-off frequency 0.33 Hz) before the calculation of the cross-correlation function.

Statistical Analysis

Pooled data are presented as mean ± SEM. Statistical significance was checked using paired or non-paired $t$ tests. Repeated analysis of variance was used to assess the significance of sequential changes in myocardial metabolites. Probability of null hypothesis of less than 5% ($p<0.05$) was considered significant.

Results

Figure 1 illustrates the induction and maintenance of ventricular fibrillation by digitalis intoxication. The top panel shows isovolumic left ventricular pressure at a slow time base before and during perfusion with 20 $\mu$M strophanthidin. The rhythm is initially paced, as is evident in the records in the bottom panel, in which pressure and the simultaneously recorded $V_e$ are expanded from segment a. The initial fall in pressure upon exposure to strophanthidin in this heart was not observed in all experiments; a similar phenomenon has been reported previously in both perfused hearts and in Purkinje fibers. The negative inotropic effect described in Purkinje fibers was also quite variable and was interpreted by the investigators as a manifestation of (labile) sodium-pump stimulation by cardiotonic steroids. The initial fall in pressure we observed soon gave way to an increase in force, as expected with sodium-pump inhibition. Within 6-7 minutes at this high strophanthidin concentration, however, end-diastolic pressure began to rise and systolic pressure to fall. This fall in force is not due to a decrease in $[Ca^{2+}]$, rather, the secondary decline in force has been attributed to asynchronous contractile activation throughout the myocardium due to spatiotemporal $[Ca^{2+}]$ inhomogeneity and has been termed calcium overload. Metabolic factors may also contribute: $Pi$ and $H^+$ are known to accumulate during sodium-pump inhibition, and both are potent in depressing myofilament $Ca^{2+}$ responsiveness.

After 18 minutes of exposure to strophanthidin, a rapid spontaneous rhythm developed, and the contractions no longer tracked the pacing stimuli. Four minutes later, the pattern of pressure generation...
changed abruptly from coordinate twitches to incoordinate oscillations approximately 20 mm Hg in amplitude. These oscillations are readily apparent in the records at a rapid time base (segment b, lower panel). We interpreted these oscillations in pressure as indicative of ventricular fibrillation, as confirmed in this and three other hearts by simultaneous recordings of V, (segment b, lower panel). In the 31P NMR experiments, electrical recordings were impossible, but as demonstrated in Figure 1, the onset of ventricular fibrillation was readily recognizable from the pressure recordings. The transition from twitches to incoordinate contraction occurred at 12–25 minutes (mean 16.8 minutes) in the 16 hearts exposed to strophanthidin. Once ventricular fibrillation was initiated, it reached steady state (i.e., a stable mean pressure and waveform) in less than 10 minutes.

Phosphate Compounds and pH During Digitalis Intoxication

Every 6 minutes, 31P NMR spectra were measured simultaneously with pressure in a total of 12 hearts, four of which were dedicated to the protocol illustrated in Figure 1 (i.e., no further pharmacological intervention). Figure 2 shows typical NMR spectra during perfusion with control solution (Panel A) and at two different times during ventricular fibrillation (Panels B and C). The control spectrum exhibits a small Pi peak, a large PCr peak (the highest in the spectrum), and three peaks corresponding to the phosphates of ATP. The rightmost peak (at −20.5 ppm) arises from the magnesium trimetaphosphate in the left ventricular balloon. The pH, calculated from the shift between Pi and PCr, is 7.14. During 12–18 minutes of ventricular fibrillation (Panel B), [Pi] increased considerably, with a corresponding decrease in [PCr]. There was also a decrease in [ATP] of almost 50% and slight acidosis. However, these metabolites reached steady state rapidly during ventricular fibrillation. The spectrum shown in Panel C, although taken 18 minutes later, is almost identical to that in Panel B.

The attainment of steady state during ventricular fibrillation was seen in all four hearts, the pooled results of which are shown in Figure 3, Panels A–E. Developed pressure (Panel A) and the concentrations of each of the metabolites (Panels B–E) are shown as a function of time, with each mark on the x axis representing a 6-minute window over which the individual spectra were obtained. As indicated at the bottom of the figure, strophanthidin was added at the second time mark. There is an initial positive inotropic effect, after which developed pressure tends to decrease, signifying the development of calcium overload. The fall in force is accompanied by a marked increase in [Pi] and a fall in [PCr], both of which then reach a plateau; [ATP] and pH decrease more gradually. Ventricular fibrillation occurred between 18 and 24 minutes of exposure to strophanthidin in all four of these hearts, after which developed pressure is undefined and is therefore not plotted. The transition from coordinate contraction to ventricular fibrillation is initially associated with a further increase in [Pi] and decrease in [PCr], although both of these metabolites, as well as [ATP] and pH, reach steady state within 12–18 minutes of ventricular fibrillation, consistent with the single experiment shown in Figure 2. The attainment of steady state in this protocol makes it possible to test pharmacological interventions without superimposed time-dependent changes.

We also confirmed that the changes observed in the myocardial metabolic concentrations during ventricular fibrillation are not due to (or exacerbated by) the loading conditions of the ventricle. In two hearts, the intraventricular balloon was deflated completely after the spectra had reached steady state in ventricular fibrillation. There were no clear-cut changes in [Pi], [PCr], [ATP], or pH after deflation as compared with the previous spectra, indicating that ventricular loading is not required for manifestation of the metabolic behavior summarized in Figure 3. This result also suggests that subendocardial ischemia does not account
FIGURE 3. Pooled results from four hearts exposed to strophanthidin with no further intervention (Panels A–E) and from four other hearts perfused for the same duration with drug-free solution (Panels F–J). Means ± SEM of developed pressure (Panels A and F) and concentrations of myocardial metabolites (Panels B and G, [Pi]; Panels C and H, [PCr]; Panels D and I, [ATP], in micromoles per gram wet weight; Panels E and J, pH) are plotted as a function of time. Each point on the x axis represents one 6-minute period during which an NMR spectrum was acquired. Panels A–E, strophanthidin (20 μM) was started at the first vertical dashed line and maintained thereafter, as indicated on the x axis; ventricular fibrillation began at the time indicated by the second vertical dashed line. Developed pressure is undefined during the incoordinate contraction that characterizes ventricular fibrillation, accounting for the termination of the data displayed in Panel A after the first four points. Panels F–J, hearts were perfused with drug-free solution throughout. As in Panels A–E, each point represents one 6-minute period. DP, developed pressure; [Pi], concentration of inorganic phosphate; [PCr], concentration of phosphocreatine; [ATP], concentration of adenosine triphosphate; pH, intracellular pH.
for the metabolic changes in this model since the transmural coronary pressure gradient is minimized by deflation of the intraventricular balloon.

To ensure that the changes in pressure and metabolites were due solely to strophanthidin and not to time-dependent rundown, we perfused four hearts with drug-free solution and observed pressure and $^{31}$P NMR spectra. The results, summarized in Panels F–J, confirm that developed pressure and intracellular metabolites remain quite stable for more than 1 hour in the absence of digitalis.

Spectral Analysis of Electrical and Mechanical Signals: Evidence for Periodic Oscillations

The extracellular electrogram ($V_e$) and left ventricular pressure were recorded simultaneously in four hearts. Such experiments allowed us to address the still controversial question of whether ventricular fibrillation is a chaotic, random process\(^7\) or, alternatively, whether there is an underlying periodicity to the rhythm.\(^10\) We checked for periodicity by subjecting the pressure and $V_e$ records to fast Fourier transformation. The resulting power spectra from one experiment are shown in Figure 4 (top row). There is a clear-cut peak at 6–8 Hz in the spectra of both pressure (left-hand panel) and $V_e$ (right-hand panel), indicating the presence of periodic oscillations that are greatest in amplitude at this frequency. Such oscillations are readily evident in the raw records shown above the corresponding spectra. We consistently observed such periodic behavior in both signals, indicating that digitalis-induced ventricular fibrillation is not a random process.

Although we know from the top and middle rows that pressure and $V_e$ have similar frequency profiles, it is not clear whether the signals are truly correlated in time and, if so, whether or not they are in phase with each other. The temporal relation between the electrical and mechanical signals was examined by cross-correlation analysis. The bottom row shows the cross-correlogram $r(kt)$ of pressure ($P$) and $V_e$, as defined by Equation 1. If the peaks of pressure and $V_e$ were exactly synchronous, $r(kt)$ would show a maximum at $kt = 0$; in fact, a phase shift of 110 msec is apparent in the cross-correlogram. Similar cross-correlation behavior characterizes the relation between membrane current and tension in digitalis-intoxicated Purkinje fibers.\(^30\) The phase shift of 80 msec reported in that study was interpreted as indicating a fundamental delay in the response time between two homologous signals. In contrast, we cannot attribute unique significance to the value of the phase shift measured in our present experiment. Unlike membrane current under voltage clamp, $V_e$ is the one-dimensional projection of a three-dimensional vector, and thus, its timing will vary with the orientation of the extracellular recording electrodes. Nevertheless, the cross-correlation function confirms the presence of a periodic correlation between the two signals.

Are Oscillations of $[Ca^{2+}]$, Obligatory to Sustain Ventricular Fibrillation?

The observation that $V_e$ and pressure exhibit periodic oscillations at an identical frequency suggests that the two signals may share a common origin, namely, periodic oscillations of $[Ca^{2+}]$. Oscillations of $[Ca^{2+}]$ occur spontaneously during calcium overload in mammalian cardiac tissue with strikingly similar frequency behavior to that observed here.\(^28\)\(^31\) Spontaneous periodic oscillations of $[Ca^{2+}]$ can produce corresponding mechanical oscillations as well as oscillations in membrane potential.\(^28\)\(^31\) Alternatively, the voltage changes may be primary. In this case, periodic reentry (or another voltage-dependent mechanism) would perpetuate the arrhythmia, and the resultant changes in membrane potential would in turn produce periodic activation of the contractile machinery. The important distinction to be made is between internal and surface membrane oscillators.\(^32\) Either type of oscillator could produce cross-correlated electrical and mechanical fluctuations. In their digitalis-intoxicated Purkinje fibers, Tsien and coworkers\(^32\) resolved the controversy by finding oscillatory ionic current and tension signals even when the surface membrane potential was controlled by voltage clamp, thereby eliminating the possibility of a primary voltage oscillation.

Because it is impossible to voltage clamp the whole heart, we used a maneuver targeted specifically at the putative internal oscillator. We reasoned that if the oscillations of $[Ca^{2+}]$ are primary, then suppressing these oscillations would eliminate the arrhythmia. We therefore determined the effect of ryanodine, an alkaloid that interferes with calcium release from the sarcoplasmic reticulum,\(^14\) on the electrical and me-
Mechanical oscillations. Ryanodine is a potent antagonist of spontaneous and triggered \([Ca^{2+}]_i\) oscillations. If the oscillations in pressure and voltage both arise from primary oscillations of \([Ca^{2+}]_i\), then ryanodine should suppress both. On the other hand, if the electrical signal can be sustained by reentry without the requirement for an ongoing calcium trigger, then ryanodine might suppress the mechanical, but not the electrical, oscillations.

The effects of ryanodine on the electrical and mechanical fluctuations are illustrated in Figure 5. The left-hand panels show raw records and power spectra of pressure (top row) and \(V_c\) (bottom row) during ventricular fibrillation before exposure to ryanodine. The signals show characteristic oscillations with a frequency peak at 6–8 Hz. Ryanodine (10\(^{-7}\) M) was then added to the perfusate in the continuing presence of strophanthidin. A steady-state effect was reached within 6 minutes, and the results are shown in the right-hand panels. The oscillations of pressure (top row) were abolished, as is evident from both the raw record and the power spectrum. In contrast, the oscillations in \(V_c\) (bottom row) were only slightly decreased in amplitude. The striking dissociation between the mechanical and electrical processes observed here, confirmed in three other analyses, indicates that oscillations of \([Ca^{2+}]_i\) are not required to sustain digitalis-induced ventricular fibrillation. This does not necessarily imply that calcium oscillations are not important in initiating the arrhythmia, but it does establish that the ongoing ventricular fibrillation is not a continuously triggered arrhythmia.

Metabolic Consequences of \([Ca^{2+}]_i\) Oscillations

Ventricular fibrillation per se leads to an increase in myocardial energy demand because of the rapid fluctuation of the membrane potential at the expense of transmembrane ionic gradients and because of the repetitive asynchronous contractile activation. The metabolic demands of digitalis-induced ventricular fibrillation are even greater. Digitalis toxicity is characterized by an elevation of \([Ca^{2+}]_i\). There are several reasons to expect that elevated \([Ca^{2+}]_i\), in general, and oscillations of \([Ca^{2+}]_i\), in particular, would tend to increase energy demand during ventricular fibrillation, even in the absence of ischemia. First, activation of actomyosin by \(Ca^{2+}\) consumes ATP. Second, \(Ca^{2+}\) reuptake by the sarcoplasmic reticulum, which would be occurring actively throughout the muscle, also requires ATP. Third, elevated \([Ca^{2+}]_i\), itself could potentiate the oscillations of membrane potential, and hence the dissipation of transsarcolemmal ionic gradients, by various modulatory effects on ionic charge carriers. An elevation of \([Ca^{2+}]_i\) would also decrease energy supply by allowing mitochondrial \(Ca^{2+}\) accumulation to occur in competition with oxidative phosphorylation. The net result would be a considerable supply/demand imbalance, even if perfusion of the myocardium is kept constant.

We tested the contribution of calcium cycling to the metabolic deterioration by comparing \(^3\)P NMR spectra obtained before and during exposure to ryanodine. Metabolites were allowed to reach steady state in ventricular fibrillation, and ryanodine (10\(^{-7}\) M) was then added during continued exposure to strophanthidin. The results of such an experiment are shown in Figure 6. Panels A and B show NMR spectra before and after, respectively, the addition of ryanodine to the perfusate. There is a marked increase in [PCR] and a concomitant fall in [P] during exposure to ryanodine. [ATP] and pH are little affected. In Panel C, the changes in [P] and [PCR] are plotted as a function of time. As can be seen, [P] and [PCR] reach a new steady state in the presence of ryanodine, with partial restoration toward their control levels.

As shown in Figure 7, the improvement in [P] and [PCR] (and in their ratio, [P]/[PCR]) with ryanodine was a consistent finding in all six experiments with this protocol. Means \pm SEM are shown during control, ventricular fibrillation without ryanodine, and ventricular fibrillation during exposure to ryanodine. Data for individual hearts are also shown for the points before and during exposure to ryanodine. The decreases in [P] and in the [P]/[PCR] ratio, as well as the increase in [PCR], are statistically significant (p < 0.05). Although the restoration toward control values is incomplete, the effect is nevertheless notable given the...
Figure 6. Effect of ryanodine on myocardial metabolites in one heart. Individual 31P-NMR spectra are shown before (Panel A) and during 12–18 minutes with addition of ryanodine (10^{-7} M) to the perfusate (Panel B). Panel C, [Pi] and [PCr] each plotted as a function of time. Each point represents data acquired over 6 minutes. Ryanodine was present at the times indicated by the bar below the x axis. Pi, inorganic phosphate; PCr, phosphocreatine; ATP, adenosine triphosphate; VF, ventricular fibrillation.

lack of pretreatment with ryanodine in this protocol.

Our data suggest that the beneficial effect of ryanodine is not due to mechanical unloading. After ryanodine, the hearts actually exhibited a slightly higher mean pressure. In Figure 8, mean pressure is plotted as a function of [Pi]/[PCr] before and during exposure to ryanodine. If the energy-sparing effect had simply been due to a decrease in generated tension, the apparent correlation would have been in the opposite direction.

Discussion

Although fibrillation has been recognized and studied in isolated tissue for more than 60 years, little is known about its pathophysiology from a cellular viewpoint. Part of the difficulty in characterizing ventricular fibrillation has been the lack of a model in which the arrhythmia can be reproduced without recourse to ischemia and/or reperfusion. This is particularly true in small hearts suitable for NMR spectroscopy; the myocardial mass of such hearts (<5 g) is insufficient to sustain ventricular fibrillation induced by alternating current. We have used digitalis intoxication to induce and sustain ventricular fibrillation, an approach that necessitates careful consideration of the superimposed effects of digitalis intoxication but has the advantages that it results in ventricular fibrillation quite reproducibly without interruption of myocardial perfusion and that the ventricular fibrillation is quite stable. The hearts quickly reach steady state in pressure and in the extracellular electrical potential.

Frequency Analysis of Ventricular Fibrillation

Our frequency analysis of the electrical and mechanical signals during ventricular fibrillation reveals an underlying periodicity in this seemingly chaotic process. A similar result was obtained with fast Fourier transformation analysis of ventricular monophasic action potential recordings from dogs in which ventricular fibrillation had been induced by programmed stimulation. It remains to be seen whether this periodicity is a general finding applicable to ventricular fibrillation induced by other means, such as ischemia.

[Ca^{2+}], in Pathogenesis of Ventricular Fibrillation

It has been proposed that an elevation of [Ca^{2+}] during ischemia may initiate ventricular fibrillation by triggering membrane depolarization, but experimental evidence for this hypothesis is still circumstantial. Whether or not elevated [Ca^{2+}] initiates ventricular fibrillation, the direct visual observation of contractile oscillations during ventricular fibrillation hints that [Ca^{2+}] is elevated and fluctuating throughout the myocardium. Nevertheless, in our model, ventricular
fibrillation does not arise from a primary oscillation of 
\([\text{Ca}^{2+}]\) since it persists even after calcium cycling has 
been inhibited by ryanodine. As pointed out earlier, this 
observation sets limits on the mechanisms for sustain-
ing ventricular fibrillation, although it is still possible 
that ventricular fibrillation may be initiated by calcium 
overload either in this setting or during myocardial ischemia. The arrhythmia might then be sustained by 
reentry or by abnormal automaticity unrelated to 
elevated \([\text{Ca}^{2+}]\). Future experiments designed to test 
this idea might include exposure to ryanodine before 
exposure to strophanthidin to see whether such 
treatment prevents the occurrence of ventricular fibrillation.

**Metabolic Consequences of Calcium Cycling**

Ryanodine has previously been shown to have 
beneficial effects on myocardial metabolism during 
calcium overload induced by lowering extracellular 
sodium concentration, an effect attributed to inhibi-
tion of intracellular calcium oscillations. The previous 
study differs from ours in that it was performed in 
verapamil-arrested hearts, rhythm was not determined, 
pretreatment was employed, and the concentration of 
ryanodine used \((10^{-5} \text{ M})\) was very high. Nevertheless, 
we have found that ryanodine ameliorates the meta-
biologic deterioration that characterizes digitalis-induced 
ventricular fibrillation, which is consistent with the 
previous findings.

We will consider the various mechanisms whereby 
calcium oscillations are believed to increase energy 
usage (or decrease energy production) to try to deduce 
the level at which ryanodine is effective in partially 
reversing the metabolic deterioration. First, actomyo-
sin ATPase is still activated during exposure to 
ryanodine, as indicated by the elevated mean pressure 
(Figure 8). Nevertheless, the cyclical activation of the 
contractile proteins is inhibited by ryanodine. Because 
such cyclical activation occurs inhomogeneously 
throughout the preparation during ventricular fibril-
lation, little net pressure is generated since active 
regions will be pulling against the series elastance of 
their relaxed neighbors. These scattered active regions 
may still account for a great deal of energy consump-
tion. Ryanodine would decrease energy usage by 
inhibiting such cyclical activation of the contractile 
proteins.

It is difficult to predict the effect of ryanodine on
another source of energy usage, the Ca\textsuperscript{2+}-ATPase of the sarcoplasmic reticulum. Because calcium release is inhibited, there may be less calcium reuptake at steady state and therefore less high-energy phosphate consumption. This simple expectation needs to be balanced against recent indirect evidence that ryanodine may induce a "leak" of calcium from the sarcoplasmic reticulum.\textsuperscript{30,39} If this were true, the sarcoplasmic reticulum might actually continue to transport calcium avidly in the presence of ryanodine. More experiments will be required to resolve this uncertainty.

Calcium oscillations are believed to activate and inactivate a number of sarcolemmal ionic pathways.\textsuperscript{35} The inhibition of Ca\textsuperscript{2+} cycling by ryanodine may therefore result in less dissipation of transmembrane ionic gradients. The magnitude of this effect is likely to be small, as hinted by the results in Figure 5. Extracellular electrical potential changes were reduced in amplitude by ryanodine, but only by a factor of approximately 20%.

Finally, cellular calcium overload could directly reduce the rate of oxidative phosphorylation through competition for mitochondrial respiratory energy (see Figure 12 in Hoerter et al\textsuperscript{19} for a schematic model). The inner membrane proton electrochemical potential (\(\Delta \mu_{H+}\)) of approximately 230 mV represents the summation of two components, a transmembrane pH gradient, \(\Delta p\), and a 150–180-mV membrane potential, \(\Delta \Psi\).\textsuperscript{40} These two components supply the driving energy for numerous mitochondrial transport processes, with specific reactions energized by either one or the other. For example, during phosphate-dependent Ca\textsuperscript{2+} transport, the electrophoretic uniport of calcium is energized by \(\Delta \Psi\), while Pi transport responds to \(\Delta p\).\textsuperscript{41} In heart mitochondria, a second Ca\textsuperscript{2+}-linked mechanism exists for the consumption of respiratory energy, namely, Na-Ca exchange.\textsuperscript{42} Under these conditions, Ca\textsuperscript{2+} transport is still driven by \(\Delta \Psi\) respiratory energy. Since the transport of adenosine diphosphate (ADP) into the mitochondrial matrix through the adenine nucleotide translocase is also energy dependent and uses the \(\Delta \Psi\) component, an increase in mitochondrial calcium transport naturally leads to a decrease in the rate of ADP transport and, thus, ATP production. The competition between ADP and Ca\textsuperscript{2+} transport has been documented,\textsuperscript{43} and under certain conditions, Ca\textsuperscript{2+} transport even appears to take priority over oxidative phosphorylation.\textsuperscript{33} Therefore, during calcium overload, the activation of mitochondrial Ca\textsuperscript{2+} transport would lead to a steady-state reduction in ATP formation. The decrease in mean cytoplasmic calcium with ryanodine\textsuperscript{30} will thus improve the ability of the cell to maintain the high-energy phosphates (Figure 6).

Does subendocardial ischemia exacerbate the metabolic changes in this model? Evidence previously reported from our NMR laboratory suggests otherwise. In rat hearts perfused with potassium-free solution as a means of inhibiting the Na,K-ATPase, oxygen consumption increased appropriately during pump inhibition, indicating that oxygen supply was not flow-limited.\textsuperscript{13} Our own experiments with deflation of the intraventricular balloon, designed to prevent subendocardial ischemia, revealed no significant differences in myocardial metabolites between these hearts and those normally loaded. We also looked for a change in coronary resistance during ventricular fibrillation as compared with control by monitoring coronary pressure and intraventricular pressure continuously in four experiments. The difference between the means of these two parameters did not change during the experiments; since flow was constant, this indicates that there was no change in overall coronary resistance with ventricular fibrillation. Taken together, the evidence suggests that subendocardial ischemia does not account for the observed metabolic changes.

After considering the individual steps at which ryanodine might be acting to ameliorate myocardial metabolism, it is not surprising that the return of \([Pi]\) and \([PCr]\) toward normal levels is incomplete. Some of the energy-consuming processes, such as actomyosin ATPase activation, are only partially suppressed, and others, such as the dissipation of ionic gradients, are hardly affected. Nevertheless, the significant changes observed with ryanodine indicate that cellular Ca\textsuperscript{2+} cycling contributes importantly to myocardial metabolism during digitalis-induced ventricular fibrillation.

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