Metabolic Consequences and Predictability of Ventricular Fibrillation in Hypoxia

A 31P- and 23Na-Nuclear Magnetic Resonance Study of the Isolated Rat Heart

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Background. Ventricular fibrillation has deleterious metabolic and functional consequences for the heart. This study had two purposes: first, to define the effects of ventricular fibrillation during hypoxia on energy metabolism and accumulation of intracellular Na+ and, second, to test whether the occurrence of ventricular fibrillation can be predicted from functional or metabolic parameters.

Methods and Results. Isolated isovolumic rat hearts were perfused with oxygenated Krebs-Henseleit buffer at 37°C. After a prehypoxic period, hearts were subjected to hypoxic perfusion (95% N2 - 5% CO2) for 30 minutes. High-energy phosphates and intracellular pH were determined by 31P-nuclear magnetic resonance (NMR) spectroscopy, and intracellular Na+ accumulation was followed by 23Na-NMR spectroscopy in combination with the shift reagent dysprosium triethylenetetraminehexa-acetate. Five of 10 (31P-NMR) and four of 10 (23Na-NMR) hearts developed spontaneous ventricular fibrillation at 19±2 minutes (31P-NMR) and 18±3 minutes (23Na-NMR) of hypoxia (ventricular fibrillation group), whereas other hearts (non-ventricular fibrillation group) remained beating throughout hypoxia. Cardiac function and high-energy phosphate content declined during hypoxia, and ventricular fibrillation exacerbated this decline significantly. Similarly, ventricular fibrillation exacerbated the accumulation of intracellular Na+ occurring during hypoxia. Statistical analysis showed that the event of ventricular fibrillation could be predicted from changes of end-diastolic pressure, rate–pressure product, and creatine phosphate content before ventricular fibrillation. However, the strongest predictor of ventricular fibrillation was intracellular Na+ accumulation, which occurred in ventricular fibrillation hearts throughout the hypoxic period long before ventricular fibrillation was initiated.

Conclusions. Loss of systolic and diastolic functions, creatine phosphate depletion, and, in particular, intracellular Na+ accumulation may be causally related to induction of ventricular fibrillation during hypoxia, all of which are most likely linked to concomitant intracellular Ca2+ accumulation. (Circulation 1992;86:302–310)

Key Words • calcium, intracellular • sodium, intracellular • high-energy phosphates • nuclear magnetic resonance • hypoxia

Deleterious effects of ventricular fibrillation (VF) on myocardial function and metabolism have long been recognized.1-5 Early studies indicated that VF induced as a cardioplegic intervention during open-heart surgery resulted in reduced functional recovery of the heart.2 Increased energy expenditure during fibrillation was thought to be responsible,1,2 and reduced levels of ATP and creatine phosphate due to VF have been demonstrated.4,5 Furthermore, VF was shown to induce massive release of intracellular enzymes such as creatine kinase, suggesting that some form of sarcolemmal damage also may be involved.5,6 More recently, attention has shifted to ionic consequences of VF. Using 19F-nuclear magnetic resonance (NMR) spectroscopy and the Ca2+ indicator 5-F-BAPTA, Koretsune and Marban5 demonstrated a fivefold increase of intracellular Ca2+ concentration induced by electrical stimulation leading to VF in isolated perfused ferret hearts; after VF was terminated, hearts showed reduced postfibrillatory cardiac performance. These authors postulated that VF causes intracellular Ca2+ overload, which in turn is at least partially responsible for the deleterious effects of VF.

In ischemic heart disease, sudden death is one of the major contributors to patient mortality, and in the majority of cases, sudden death is brought about by VF.7 It is thus surprising that although biochemical and functional consequences of VF are partially understood, few studies have attempted to identify the metabolic events that trigger the occurrence of VF in injured myocardium. Results from experiments with isolated cells8,9 and intact dog hearts10 suggest that an increase in intracellular Ca2+ concentration may initiate VF. This
hypothesis is supported further by the observation that ischemia, digitalis, and catecholamine intoxication increase intracellular Ca\(^{2+}\), and all predispose to the occurrence of VF.

In the present study, we took advantage of the fact that a certain percentage of isolated hearts develops spontaneous VF during perfusion with hypoxic buffer. Thus, it was not necessary to use interventions such as digitalis poisoning or electrical stimulation to induce VF. Our goal was, first, to define the effects of VF during hypoxia on high-energy phosphate metabolism and accumulation of intracellular Na\(^{+}\). More importantly, we tested the clinically relevant question of whether VF could be predicted from changes in cardiac performance, high-energy phosphate metabolism, or intracellular Na\(^{+}\).

**Methods**

**Isolated Rat Heart Preparation**

Male Sprague-Dawley rats (350–400 g) were anesthetized by intraperitoneal injection of 20 mg pentobarbital sodium. Hearts then were isolated as previously described. Retrograde perfusion of the heart was started in the Langendorff mode at 37°C and at a constant coronary perfusion pressure of 100 mm Hg. To allow drainage of flow from the ventricles, a small vent made from polyethylene tubing was pierced through the apex of the left ventricle. For control perfusion, phosphate-free Krebs-Henseleit buffer was used containing (mM) 118 NaCl, 4.7 KCl, 1.75 CaCl\(_2\), 1.2 MgSO\(_4\), 0.5 EDTA tetrasodium, 25.0 NaHCO\(_3\), and 11.0 glucose. Equilibration of the buffer with 95% O\(_2\)-5% CO\(_2\) yielded pH 7.4. Coronary flow was measured by collecting coronary sinus effluent in a calibrated cylinder. As previously shown, the perfusion system maintained hearts in a steady state for at least 90 minutes with changes of <5% for all mechanical and metabolic parameters.

**Cardiac Performance Measurements**

Performance was measured by means of a left ventricular balloon as previously described. Volume of the balloon was adjusted to maintain an end-diastolic pressure (EDP) of 8–11 mm Hg. Global mechanical performance was estimated as the product of heart rate and left ventricular developed pressure (LVDP; mm Hg/ min).

**NMR Spectroscopy**

The perfused hearts were placed into a 20-mm NMR sample tube and inserted into a \(^{31}\)P- or \(^{23}\)Na-NMR probe that was seated in the bore of a superconducting wide-bore (89 mm) 8.4-T magnet (Oxford Instruments). A Nicolet 1280 computer was used in the pulsed Fourier transform mode to generate \(^{31}\)P- or \(^{23}\)Na-NMR spectra using a Nicolet NT-360 spectrometer operating at 145.75 MHz for \(^{31}\)P and 95.25 MHz for \(^{23}\)Na. An 18-channel Oxford Instrumentation Shim Supply served to homogenize the magnetic field.

\(^{31}\)P-NMR spectra were accumulated over 5-minute periods and averaged 128 free induction decays obtained with a pulse angle of 45° and an interpulse delay of 2.15 seconds. The resonance areas corresponding to ATP, creatine phosphate, inorganic phosphate (P\(_i\)), monophosphate esters, and NAD, which are proportional to the total number of moles of each compound within the NMR-sensitive volume of the probe, were measured using the Nicolet Integration Program. In each \(^{31}\)P-NMR experiment, the area of the [\(\beta\)]P-ATP resonance of the first spectrum was set arbitrarily to 100% and used as the reference value for all resonances in the set of \(^{31}\)P-NMR spectra obtained for a given protocol. This corresponds to an ATP concentration of 11.2 mmol/l obtained by high-pressure liquid chromatography. Intracellular pH (pH\(_i\)) was measured by comparing the chemical shift between the inorganic phosphate and creatine phosphate resonances with values obtained from a standard curve. Because the analyses performed required knowledge only of changes in measured metabolites and not of their absolute concentrations, no corrections were made for saturation. Changes in ATP and creatine phosphate resonance areas accurately report changes in amount of metabolite assessed using standard biochemical assays.

\(^{23}\)Na-NMR spectra were accumulated over 4-minute periods, averaging data from 960 free induction decays that were obtained using a pulse angle of 90° and a recycle time of 256 μsec. Details of \(^{23}\)Na-NMR spectroscopy using shift-reagent perfused, isolated hearts have been described previously. In our model, resolution of the intracellular Na\(^{+}\) resonance during control perfusion was unsatisfactory. To solve this problem, we increased the y scale of the first \(^{23}\)Na spectrum with shift reagent recorded in each experiment. This allowed the inflection point of the spectral curve marking the border between the intracellular and extracellular Na\(^{+}\) resonances to be identified. We then defined the frequency range over which the intracellular Na\(^{+}\) resonance was integrated in the entire set of spectra from each experiment. With this strategy, we obtained reproducible integration results defining the change in intracellular Na\(^{+}\) signal intensity with time. Intracellular Na\(^{+}\) concentration in control hearts was set to 17 mmol/l, the value obtained by Shatock and Bers for rat ventricular tissue using Na-selective microelectrodes. Intracellular volume was assumed to be constant throughout the protocol. Importantly, the ratios of tissue wet to dry weights of control and fibrillating hearts measured at the end of 28 minutes of hypoxia were indistinguishable, suggesting that there are no large differences between intracellular volumes in the two groups of hearts.

**Experimental Protocol**

For \(^{31}\)P-NMR experiments, hearts were perfused with oxygenated Krebs-Henseleit buffer (P\(_{O_2}\) = 600 mm Hg) for =15 minutes, after which left ventricular balloon volume was adjusted to yield an end-diastolic pressure of 10 mm Hg. The experimental protocol was then started. After a 30-minute prehypoxic period, the perfusate was switched to buffer made hypoxic by equilibration with 95% N\(_2\)-5% CO\(_2\) (P\(_{O_2}\) = 20 mm Hg). Hypoxia was maintained for 30 minutes, after which hearts were frozen for determination of wet weight-to-dry weight ratio. Cardiac performance and \(^{31}\)P-NMR spectra were recorded throughout this protocol, yielding six spectra during prehypoxia and six spectra during hypoxia. Volume of the left ventricular balloon was not adjusted during hypoxia so hearts could develop high
end-diastolic pressures. In 31P-NMR experiments, five hearts developed spontaneous VF at 19±2 minutes of hypoxia (VF), whereas five hearts (control) remained beating throughout hypoxia.

23Na-NMR experiments were performed in an analogous fashion, except that at 10 minutes during the prehypoxic period, perfusate was changed to buffer containing 10 mmol/l shift reagent Dy (TTHA)3+; this perfusate was used for the remainder of the protocol. As reported previously,17 perfusing the heart with shift reagent had only small effects on cardiac performance. LVDP was increased slightly (11%), but heart rate was decreased slightly (9%), yielding the same product of heart rate multiplied by LVDP. Peak LVDP was unchanged. Hemodynamic parameters measured in the living rat before and after infusion with shift reagent (maximum concentration, 8.8 mmol/l) also were unchanged.19 23Na spectra were recorded at 4-minute intervals. One spectrum was obtained before switching to shift reagent—containing buffer, and five spectra were recorded in the remaining 20 minutes of prehypoxia. During hypoxia, seven consecutive spectra were accumulated. In 23Na-NMR experiments, four hearts developed spontaneous VF at 18±3 minutes (p=NS versus 31P-NMR experiments) of hypoxia (VF group), whereas six hearts (control) remained beating throughout hypoxia.

Statistical Analysis

The event of VF at 18–19 minutes of hypoxia separated the 20 hearts used for this study into two groups: 11 non-VF and nine VF hearts. Characteristics of the two groups of hearts were analyzed separately for prehypoxic and hypoxic periods.

Standard ANCOVA, where time was the covariate, was performed using the BMDP statistical package. Paired analyses were performed to compare data for VF hearts before and after the event. Unpaired analyses were performed to compare non-VF and VF hearts. This analysis assumes that the monotonic relations are linear. However, all NMR and cardiac performance variables were observed to be strongly nonlinear functions of time (see Figure 7 for examples). To fit the data for both non-VF and VF hearts up to the nominal onset of VF, three types of nonlinear functions were required. One of these three functions was chosen for each of the NMR (intracellular Na+, ATP, creatine phosphate, P, pH) and cardiac performance (end-diastolic pressure, rate–pressure product) variables according to the nature of the change over time for that variable. The first type of nonlinear function of time was

\[ F_1(t) = a_0[1 - e^{-A(t-t_0)}] \]  

This function increases exponentially to asymptote \( a_0 \) at a rate \( A \) min\(^{-1}\) beginning from 0 at time, \( t_0 \) (minutes).

The second type of nonlinear function of time was

\[ F_2(t) = a_1 + a_0 \left( \frac{e^{A(t-t_0)}}{1+e^{A(t-t_0)}} \right) \]  

This antilogit function moves sigmoidally from asymptote \( a_0 \) in the remote past to asymptote \( a_1 + a_0 \) in the remote future and has undergone half this change at time \( t_0 \) (minutes). The rate of this sigmoidal change is expressed by rate parameter \( A \) min\(^{-1}\). The third type of nonlinear function used to describe some of the variables was

\[ F_3(t) = a_0 + a_1 e^{-A t} \]  

This exponentially decreasing (or increasing, if \( a_1 < 0 \)) function has a maximum value of \( a_0 + a_1 \) at time \( t=0 \) and a minimum asymptotic value of \( a_0 \) at late times. Again, the rate of change is expressed in the parameter \( A \) min\(^{-1}\).

The analysis was performed as follows. First, all non-VF hearts were fit simultaneously with the appropriate function; then, all VF hearts were fit with the same function. The fitting was performed with the RS/1 FIT FUNCTION program.20 Any parameter of the function that was found to not differ significantly between the two groups of hearts then was fixed at the mean value fitted for the two groups, and the fitting was reperformed for each of the two groups until a maximum of two fitting parameters remained free for the chosen function. After the parameters to be held fixed for each variable were calculated in this way, the function with these fixed parameters always held fixed were fit to data for individual hearts. Thus, this analysis established both the form of the function needed to describe the nonlinear time dependence and one or two of the parameters of the function. The remaining free parameters then were held to characterize the time course of the variable for each heart. In this way, for each heart, a maximum of two numbers for each variable were calculated to characterize the entire time course of that variable for that heart. As shown in Table 1, usually the parameters \( A \) and \( t_0 \) could be held fixed while one or both of the two amplitudes \( a_0 \) and \( a_1 \) were estimated for each heart.

After each variable was characterized by a maximum of two numbers for each heart with the nonlinear fitting procedure, all parameters of all variables were entered into a MANOVA to test for differences between the hearts that went into VF after 17.5 minutes (VF group of five hearts) and those that never went into VF (the non-VF group of five hearts). The BMDP statistical package was used to perform the overall multivariate Hotelling’s \( T^2 \) test and the individual \( t \) tests for differences between groups for each variable (as characterized by one or two parameters).21 Hearts used for 23Na-NMR experiments were analyzed separately because the six non-VF hearts and the four VF hearts used in the Na+ studies were separate hearts. Those variables found to differ between the VF and non-VF groups then were entered into a logistic regression analysis to determine which variables could best predict VF in an independent fashion, i.e., highly correlated variables were eliminated by the stepwise logistic regression.22

Results

Cardiac Performance

Heart rate (298±9 min\(^{-1}\)), left ventricular systolic (127±6 mm Hg) and developed (118±5 mm Hg) pressures, end-diastolic pressure (set to 10 mm Hg), rate–pressure product (34,600±1,800 mm Hg·min\(^{-1}\)), and coronary flow (21±2 ml·min\(^{-1}\)) were indistinguishable in the non-VF and VF hearts during prehypoxic baseline perfusion. None of the measured variables showed any significant change throughout the prehypoxic period. Thus, only one prehypoxic value (the value ob-
Coronary flow changes in hypoxia were as previously observed\(^4\) with an early vasodilator response followed by a gradual return of coronary flow toward prehypoxic levels. Prehypoxic values were 21±2 and 23±2 ml·min\(^{-1}\) in non-VF and VF hearts, respectively. At 5 minutes of hypoxia, flow increased to 34±2 and 31±2 ml·min\(^{-1}\); at 28 minutes, flow was 27±2 and 22±2 ml·min\(^{-1}\) for non-VF and VF hearts, respectively. There were no significant differences among hearts at any point in time during the protocol.

**\(31^P\)-NMR Spectroscopy**

Representative \(31^P\)-NMR spectra for prehypoxic and end hypoxia for both non-VF and VF hearts are shown in Figure 2. These spectra show that ATP and creatine phosphate contents of fibrillating hypoxic hearts are lower and \(P_i\) is correspondingly higher than for non-fibrillating hypoxic hearts. Mean time-dependent values for these metabolites during hypoxia for both VF and non-VF hearts are shown in Figure 3; changes in pH are shown in Figure 4. As was true for indexes of cardiac performance, none of the metabolic parameters showed any significant change throughout the prehypoxic period, and, thus, only one prehypoxic value is given in the figures. Data shown in Figures 3 and 4 demonstrate that contents of ATP, creatine phosphate, \(P_i\) and pH were similar for non-VF and VF groups during prehypoxic baseline perfusion.

In non-VF hearts, ATP and creatine phosphate decreased, and \(P_i\) increased monotonically throughout hypoxia. After VF occurred, ATP and creatine phosphate depletion and \(P_i\) accumulation were markedly accelerated. By the end of hypoxia, \(P_i\) increased 3.9-fold in non-VF hearts and 7.0-fold in VF hearts. In these cases, ATP was maintained at 62±5 mm Hg compared with 62±5 mm Hg in non-VF hypoxic hearts. Consequently, LVDP decreased rapidly from 119±2 mm Hg during prehypoxic perfusion to 48 mm Hg within the first 2.5 minutes of hypoxia. For non-VF hearts, LVDP was maintained at 45 mm Hg throughout the remainder of the hypoxic period. In hearts that fibrillated at 19±2 minutes, LVDP decreased monotonically to the time of fibrillation. Thus, hypoxia led to severe diastolic but only moderate systolic dysfunction.

<table>
<thead>
<tr>
<th>Variable (fitting parameter)</th>
<th>Function type</th>
<th>(\lambda) (min(^{-1}))</th>
<th>(t_n) (min)</th>
<th>No VF (mean±SEM)</th>
<th>VF (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP (a(_0))</td>
<td>2</td>
<td>0.22</td>
<td>11</td>
<td>62±5</td>
<td>86±11</td>
</tr>
<tr>
<td>ATP (a(_i))</td>
<td>2</td>
<td>38±5</td>
<td></td>
<td>18±9</td>
<td></td>
</tr>
<tr>
<td>CP (a(_0))</td>
<td>3</td>
<td>0.11</td>
<td></td>
<td>32±3</td>
<td>19±4.5†</td>
</tr>
<tr>
<td>CP (a(_i))</td>
<td>3</td>
<td>65±5</td>
<td></td>
<td>76±2.6</td>
<td></td>
</tr>
<tr>
<td>EDP (a(_0))</td>
<td>1</td>
<td>0.05</td>
<td>-4.3</td>
<td>92±8</td>
<td>123±9†</td>
</tr>
<tr>
<td>N(_a) ((a_0))*</td>
<td>2</td>
<td>0.50</td>
<td>11</td>
<td>1.5±1.1</td>
<td>23±7§‡</td>
</tr>
<tr>
<td>pH (a(_0))</td>
<td>2</td>
<td>1.00</td>
<td>4.5</td>
<td>0.08±0.01</td>
<td>0.08±0.009</td>
</tr>
<tr>
<td>pH (a(_i))</td>
<td>2</td>
<td>7.06±0.003</td>
<td></td>
<td>7.07±0.01</td>
<td></td>
</tr>
<tr>
<td>(P_i) (a(_0))</td>
<td>1</td>
<td>0.02</td>
<td>-7.1</td>
<td>367±8</td>
<td>383±11</td>
</tr>
<tr>
<td>RPP (a(_0))†</td>
<td>3</td>
<td>3.0±1.1</td>
<td></td>
<td>2.7±1.6</td>
<td></td>
</tr>
<tr>
<td>RPP ((\lambda))</td>
<td>3</td>
<td>0.3±0.01</td>
<td></td>
<td>0.25±0.01‡</td>
<td></td>
</tr>
</tbody>
</table>

VF, ventricular fibrillation; CP, creatine phosphate; EDP, end-diastolic pressure; \(P_i\), inorganic phosphate; RPP, rate-pressure product.

Function types:

\[
F_1(t) = a_0(1 - e^{-\lambda(t-t_0)})
\]

\[
F_2(t) = a_1 + a_0 \left( \frac{e^{\lambda(t-t_0)}}{1 + e^{\lambda(t-t_0)}} \right)
\]

\[
F_3(t) = a_0 + a_1 e^{-\lambda t}
\]

\(n=6\) separate non-VF and four separate VF hearts.

\(t_{a_1}=15.3\).

\(\dagger p<0.04\) vs. non-VF.

\(\ddagger p=0.002\) vs. non-VF.
FiguRE 1. Plots of changes of left ventricular developed pressure (LVDP; mm Hg; upper panel), heart rate (HR; min⁻¹; middle panel), and end-diastolic pressure (EDP; mm Hg; lower panel) during control and hypoxia in non-ventricular fibrillation (VF) (□) and VF (○) hearts. At time "0," hypoxia is initiated. Arrow, onset of VF. In the upper and middle panels, there are no data points for VF hearts after the occurrence of VF.

experiments, pHᵢ could not be used to discriminate between control and VF hearts (Figure 4). In all hearts, pHᵢ decreased by ≈0.09 pH units within 12.5 minutes of hypoxia and then gradually returned toward prehypoxic values. These changes are typical of pH changes occurring during hypoxia.¹⁴

Thus, increased work of fibrillation during hypoxia results in faster depletion of ATP and creatine phosphate and a concomitant increase in Pᵢ without differences in pHᵢ.

²³Na-NMR Spectroscopy

In Figure 5, plots of ²³Na-NMR spectra obtained at 4-minute intervals during hypoxia are shown for a representative non-VF heart and a heart that fibrillated during hypoxia. In the non-VF heart, the Naᵢ resonance area increases sharply with hypoxia in concert with increased coronary flow during hypoxia, but the change in the intracellular Na⁺ resonance area was barely detectable. In contrast, in the VF heart, an increase of the intracellular Na⁺ resonance can be detected even before onset of fibrillation.

In Figure 6, changes of the intracellular Na⁺ resonance area (where area for control heart is set to 17 mmol/l) are shown during hypoxic perfusion for the non-VF and VF hearts. In non-VF hearts, there was little change throughout the hypoxic period. The intracellular Na⁺ area was only 36% higher at the end of hypoxia. In contrast, hearts that fibrillated displayed an approximately linear increase of intracellular Na⁺ throughout hypoxia. Just before VF, intracellular Na⁺ had increased by approximately threefold. After VF, intracellular Na⁺ continued to increase.
Predictability of VF

To determine whether any of the functional or metabolic parameters we measured predict VF, we analyzed all data obtained for both VF and non-VF hearts to the time of fibrillation using the statistical approach described in "Methods." For each heart, nonlinear fits for each of the parameters listed in Table 1 were obtained. The nonlinear fits were good. Of a total of 90 fits, only two had a Pearson correlation coefficient of <0.95. Examples are presented in Figure 7, where panels 1–3 show two individual fits each (one VF heart and one non-VF heart per panel) for each of the three different types of nonlinear functions needed to describe the data. This makes plausible the assumption that Gaussian errors in the parameters derived from each measured variable to characterize the entire time course for each heart. The results of these fits are summarized in Table 1 where the parameters that were determined from all hearts together then held fixed during fitting for individual hearts are shown in columns 3 and 4. These fixed parameters were the rate and time-origin parameters λ and t₀. In the three cases where other or different parameters were held fixed after group fitting, these exceptions are shown in footnotes to the table.

Columns 5 and 6 of Table 1 show mean values and SEM for each group of hearts as determined by MANOVA. Only the a₀ parameters of creatine phosphate content and end-diastolic pressure and the λ parameter of rate-pressure product differed significantly between the VF and non-VF hearts used for 31P-NMR and cardiac performance measurements. For the 10 separate hearts used for 23Na-NMR, the a₀ parameter was dramatically (=15-fold) higher (p<0.002) in the VF hearts than in the non-VF hearts. No other functional or metabolic parameter measured was predictive.

The creatine phosphate a₀ parameter for the VF group was significantly (almost twofold) lower than that for the non-VF group. This means that the VF hearts lost more creatine phosphate before fibrillation than did the non-VF hearts, even though creatine phosphate contents began at about the same level.

End-diastolic pressure increased to a value that was ~40% higher in the VF hearts than in the non-VF hearts. This is expressed in the a₀ parameter of end-diastolic pressure. The rate–constant λ for decay of rate–pressure product was significantly lower for VF hearts than for non-VF.

Clearly, the 15-fold greater accumulation of intracellular Na by the VF group was the greatest predictor of VF. This is expressed mathematically by the 15-fold increase in the a₀ parameter. This firm finding could not be combined in a multivariate way with the results from 31P-NMR because it was obtained from separate hearts. Nevertheless, in our sample of 10 23Na-NMR hearts, the asymptotic level of increase of intracellular Na⁺ (a₀) was a perfect predictor of VF: Hearts with a₀ > 6.0 went on to VF, whereas those with a lower level of asymptotic increase did not.
When the multivariate data from the 31P-NMR hearts were combined in a stepwise logistic regression, it was found that the λ parameter of rate-pressure product was the best predictor of VF followed by the $a_0$ parameter of creatine phosphate as a predictor that added significantly ($p<0.04$) to the logistic model even after λ of rate-pressure product was already in the logistic model. In contrast, $a_0$ of end-diastolic pressure did not contain independent predictive information beyond these two “best” predictors of VF.

**Discussion**

The purpose of the present study was twofold. First, we examined the functional and metabolic consequences of VF in hypoxic myocardium. Second, we sought to define physiological and metabolic parameters that would allow us to predict which hearts would fibrillate during hypoxia.

**Definition of the Model**

We chose to use global hypoxia of the isolated heart as our model for the study of VF. Although in the clinical setting ischemia is a much more common form of metabolic stress than hypoxia (where oxygen supply is reduced but flow and substrate delivery are maintained), there are a number of advantages to this design. Because VF did not occur until hearts were hypoxic for ≥18 minutes, it was possible to analyze whether any functional and metabolic parameter predicts VF. Inducing VF in the isolated heart with rapid electrical stimulation5,23 would not allow an assessment of predictors of VF because ventricular pacing induces VF within seconds. Depending on its concentration, digitalis can induce VF in isolated hearts within minutes or hours,4,6 but clinical VF caused by digitalis intoxication is rare. By studying the hypoxic isovolumic perfused heart with 31P- and 23Na-NMR spectroscopy, we were able to examine the functional, metabolic, and ionic (Na+ and H+) predictors and correlates of VF.

**Functional and Metabolic Consequences of VF**

In this study, we demonstrated that when VF occurs spontaneously during a hypoxic insult, diastolic dysfunction is aggravated markedly, and high-energy phosphate metabolism is deranged further. The rates of both ATP and creatine phosphate depletion increased after VF. VF could cause exacerbated deterioration of high-energy phosphate metabolism by either or both of two mechanisms: reduced ATP synthesis or increased ATP utilization. Our data do not allow discrimination of these two mechanisms.

Furthermore, we showed that VF during hypoxia markedly aggravated accumulation of intracellular Na+. After the occurrence of VF, intracellular Na+ continued to increase until hypoxia was terminated, whereas in non-fibrillating hearts, an increase of intracellular Na+ was
barely detectable. The concentrations of intracellular Na\(^+\) and Ca\(^{2+}\) are closely coupled via Na\(^+\)--Ca\(^{2+}\) exchange. This suggests that Ca\(^{2+}\) accumulation, and thus intracellular Ca\(^{2+}\) overload, in hypoxia is greatly accelerated after VF, which then would be responsible, at least in part, for the observed deterioration of high-energy phosphate metabolism. Thus, our results are consistent with the decreased high-energy phosphate content following VF caused by electrical stimulation reported by Koretsune et al.\(^5\)

Mechanisms Responsible for Initiation and Predictability of VF

Although numerous studies have examined the consequences of VF,\(^1\)\(^–\)\(^6\) little is known about the mechanisms responsible for initiating VF in injured myocardium. Our data show that diastolic dysfunction (end-diastolic pressure increase) induced by hypoxia was more pronounced and developed more rapidly in VF hearts before occurrence of VF. The \(a_0\) parameter of the equation describing the exponential increase of end-diastolic pressure during hypoxia was significantly different for VF and non-VF hearts \((p<0.04)\). Furthermore, systolic dysfunction also was more pronounced in VF hearts with the \(\lambda\) parameter of rate-pressure product being a significant predictor of VF \((p<0.04)\). Although the event of VF can be predicted based on measurements of systolic and diastolic functions, our data do not allow us to discriminate whether altered systolic or diastolic performance was a reason for or a consequence of metabolic and ionic alterations that occurred in the VF group.

Impaired high-energy phosphate metabolism has been proposed to contribute to the pathogenesis of VF. In intact dogs,\(^24\)\(^,\)\(^25\) occurrence of VF upon reperfusion after regional ischemia correlated with end-ischemic levels of ATP and creatine phosphate. Our own data show that changes of ATP, \(P_e\), and pH during hypoxia were similar for VF and non-VF hearts. However, the \(a_0\) parameter describing the exponential decline of creatine phosphate was a significant predictor of the occurrence of VF \((p<0.04)\). Thus, in VF hearts, creatine phosphate depletion proceeded at a greater rate and to a greater extent than in non-VF hearts. However, these data do not demonstrate a direct causal link of creatine phosphate depletion and induction of VF.

Our measurements of intracellular Na\(^+\) concentration clearly showed that from the beginning of hypoxia, hearts fell into one of two populations: one that monotonically accumulated intracellular Na\(^+\), and one that did not. All hearts that had accumulated intracellular Na\(^+\) (approximately threefold) fibrillated during hypoxia, whereas none of the hearts that did not accumulate intracellular Na\(^+\) developed VF. Statistical analysis showed that the increase of intracellular Na\(^+\) was by far the strongest predictor of VF with the \(a_0\) parameter of the equation describing intracellular Na\(^+\) accumulation being highly significantly different between VF and non-VF hearts \((p=0.002)\).

As stated above, the concentrations of intracellular Na\(^+\) and Ca\(^{2+}\) are closely linked via the Na\(^+\)--Ca\(^{2+}\) exchanger. Therefore, intracellular Ca\(^{2+}\) overload may not be responsible for only the deleterious effects of VF but also for initiation of VF. Some indirect evidence also points to the crucial role of Ca\(^{2+}\). In isolated ferret hearts, transient intracellular Ca\(^{2+}\) overload induced by high [Ca\(^{2+}\)]\(_1\) perfusion caused reduced mechanical performance and augmented [Ca\(^{2+}\)]--ATP depletion,\(^26\) changes similar to those evoked by transient VF in the same model.\(^3\) In the perfused rabbit heart, Ca\(^{2+}\) channel blockers as well as reduction of buffer [Ca\(^{2+}\)], inhibited initiation of VF by alternating current stimulation.\(^23\) Fibrillatory electrical and mechanical activities could be observed after Ca\(^{2+}\) overload was produced in small myocardial cell clusters.\(^8\) Similarly, an increase of intracellular Ca\(^{2+}\) from the nanomolar to the micromolar range caused premature and fibrillatory beating in isolated neonatal rat ventricular cells with cell-to-cell contact.\(^9\) In a dog model in which VF was induced by simultaneously occluding two coronary vessels, time to onset of VF was delayed both by the Ca\(^{2+}\) channel blocker diltiazem and by a reduction of serum Ca\(^{2+}\) concentration, suggesting that this was
achieved by limiting the rate or extent of intracellular Ca\(^{2+}\) accumulation.\(^{10}\)

**Study Limitations**

Although our direct measurements of Na\(^{+}\) changes support a role for intracellular Ca\(^{2+}\) in initiating VF, we did not directly measure Ca\(^{2+}\). Furthermore, none of the prehypoxic functional or metabolic variables were indicative of the occurrence of VF during hypoxia. Likewise, age, body weight, or heart weight of control and VF rats were similar. Thus, our data do not explain why there are two populations of hearts: one that does not and one that does accumulate intracellular Na\(^{+}\) and develop VF during hypoxia.

**Clinical Implications**

The clinical implications of this study are at least twofold. First, we provide further evidence that VF by itself, i.e., independent of ischemia, has deleterious effects on the energy balance of the heart. The responsible mechanism probably is Ca\(^{2+}\) overload. Thus, when VF has led to cardiopulmonary arrest, myocardial viability may be preserved by implementing strategies that reduce Ca\(^{2+}\) overload. Second, we provide evidence that cation imbalance may initiate VF. Thus, strategies for reducing intracellular Ca\(^{2+}\) concentration of cardiomyocytes in patients with ischemic heart disease, such as treatment with Ca\(^{2+}\) channel blockers, may help to prevent or at least delay the initiation of VF during episodes of acute myocardial ischemia.

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