Reperfusion-Induced Arrhythmias
A Role for Washout of Extracellular Protons?

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Rapid washout of extracellular H⁺ accumulated during preceding ischemia (i.e., the abrupt restoration of extracellular pH) has been implicated as an arrhythmogenic factor during reperfusion. Therefore, we hypothesized that by limiting the rate at which extracellular pH was restored during early reperfusion it should be possible to protect against reperfusion-induced arrhythmias. To test this, we used isolated rat hearts (n=12 per group) and a novel dual coronary perfusion cannula that permitted the induction of regional ischemia (10 minutes) and the selective reperfusion (8 minutes) of the ischemic zone with modified solutions. We examined the antiarrhythmic efficacy of 1) acidic (pH 6.6) reperfusion with stepwise restoration of extracellular pH to 7.4 (stepped pH) and 2) transient (2-minute) acidic (pH 7.1, 6.8, 6.6, or 6.4) reperfusion with subsequent abrupt restoration of extracellular pH to 7.4. Hearts in two contemporary control groups were reperfused with solution at pH 7.4 throughout. In all groups, 100% of hearts exhibited ventricular tachycardia (VT) on reperfusion. VT degenerated into ventricular fibrillation (VF) in 100% of hearts in the control group but in only 42% of hearts in the stepped-pH group (p<0.05). In the groups subjected to transient acidic reperfusion, there was a pH-dependent prolongation of VT cycle length (measured at 15 seconds of reperfusion), which was 47.1±3.9, 51.1±5.5, 56.0±1.9, 60.4±2.8 (p<0.05), and 68.8±5.0 (p<0.05) msec in the pH 7.4 (control), 7.1, 6.8, 6.6, and 6.4 groups, respectively. In these groups, VT degenerated into VF in 92%, 92%, 93%, 42% (p<0.05), and 33% (p<0.05) of hearts, respectively. In conclusion, limiting the rate at which extracellular pH is restored during early reperfusion does not affect the rapid induction of VT but inhibits the degeneration of VT into VF and promotes spontaneous reversion to normal sinus rhythm. This is consistent with a major arrhythmogenic role, during uncontrolled reperfusion, for the rapid washout of extracellular H⁺. (Circulation Research 1992;71:1429-1440)

KEY WORDS • regional ischemia • reperfusion-induced arrhythmias • ventricular fibrillation • protons • dual coronary perfusion • rats

Reperfusion of myocardium subjected to a transient period of ischemia rapidly induces severe ventricular arrhythmias (for review, see Manning and Hearse). Although the precise mechanisms have not been elucidated, several factors have already been implicated in reperfusion arrhythmogenesis, including the generation of free oxygen radicals, and the rapid washout of K⁺ from the extracellular space, and the accumulation of Ca²⁺ in the intracellular space. However, as recently pointed out, the fundamental question of whether the arrhythmogenic process is initiated by the washout of substances (e.g., K⁺) accumulated during ischemia or by the resupply of substances (e.g., oxygen) absent during ischemia remains to be resolved. Yamada and colleagues have reported that, whereas reestablishment of flow and readmission of oxygen were independent determinants of reperfusion-induced arrhythmias, the latter was not a prerequisite for arrhythmogenesis (since anoxic reperfusion did not significantly alter the incidence of reperfusion-induced ventricular fibrillation), indicating that multiple mechanisms may be involved. In this context, the recent study by Curtis on the effects of regional infusion and washout of high [K⁺] solutions in the isolated rabbit heart suggests that the rapid washout of extracellular K⁺ could be a significant arrhythmogenic factor.

Protons (H⁺) also accumulate in the extracellular space during ischemia and their rapid washout may be another factor. Lazdunski and colleagues suggested in 1985 that the rapid washout of extracellular H⁺ on reperfusion may create an intracellular to extracellular H⁺ gradient, resulting in an influx of Na⁺ via the Na⁺-H⁺ exchanger. Such an influx of Na⁺, in the face of Na⁺, K⁺-ATPase inhibition caused by the preceding ischemia, could result in an increase in intracellular [Na⁺], which in turn would favor an increase in intracellular [Ca²⁺] via the Na⁺-Ca²⁺ exchanger. Increase in intracellular [Ca²⁺] on reperfusion, which can be ameliorated by inhibition of Na⁺-H⁺ exchange with amiloride or by acidic reperfusion, has been demonstrated in the isolated rat heart. Such an increase in intracellular [Ca²⁺] has been proposed as a potential culprit for reperfusion arrhythmogenesis. Indeed, the

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Diagrams illustrating the perfusion apparatus and the correct position of the dual-perfusion cannula in the aorta of an isolated heart (inset). A divided lumen to the lumen of the cannula permits each hemicylinder to deliver an independent supply of perfusate. The orifices near the proximal end are designed to be opposite the left and right coronary ostia when the cannula is in position. Regional ischemia was induced by stopping flow to the left coronary bed. During reperfusion, the left coronary bed was perfused at the preischemic flow rate via a roller pump, with perfusion solution from one of a bank of four reservoirs. At the end of each experiment, disulphine blue dye was infused into one coronary artery to delineate the left and right coronary beds, thus enabling the size of the ischemic zone to be quantified.

Recent study by Dennis and colleagues, who used solutions containing various buffer concentrations and amiloride analogues to modify the activity of the Na⁺-H⁺ exchanger, provided support for an arrhythmogenic role for Na⁺-H⁺ exchange–mediated mechanisms during reperfusion.

We hypothesized that, if the rapid washout of extracellular H⁺ (i.e., the abrupt restoration of extracellular pH) were a major arrhythmogenic factor during reperfusion, then limiting the rate of washout of extracellular H⁺ (i.e., the rate of restoration of extracellular pH) during early reperfusion should afford protection. To test this, we used isolated rat hearts and a recently developed dual perfusion cannula that permits the induction of regional ischemia and the selective reperfusion of the ischemic zone with modified solutions. We examined the antiarrhythmic efficacy of 1) acidic (pH 6.6) reperfusion with stepwise restoration of extracellular pH to 7.4 by sequential perfusion with solutions of increasing pH and 2) transient acidic (pH 7.1, 6.8, 6.6, or 6.4) reperfusion followed by an abrupt restoration of extracellular pH to 7.4.

Materials and Methods

Dual Coronary Perfusion of Isolated Rat Hearts

Independent dual perfusion of left and right coronary arteries in isolated rat hearts was performed as described in detail by Akivran and Curtis. In brief, male Wistar rats (Bantin and Kingman, Ltd., N. Humberside, UK) were anesthetized by inhalation of diethyl ether, and then 50 units sodium heparin was injected into the left femoral vein. The chest was opened, and the heart was excised and immersed in perfusion medium at 4°C. Within 30 seconds of excision, a dual perfusion cannula was inserted into the ascending aorta (Figure 1) and secured in position with a braided silk suture. The pulmonary artery was cut near its origin, and a stainless-steel needle was inserted into the left ventricle through the apex to allow adequate drainage of coronary and thebesian venous effluent. Perfusion of both coronary beds was then initiated at a perfusion pressure equivalent to 100 cm H₂O. Alignment of the coronary ostia with the orifices of the cannula was achieved using in-line monitoring of left and right coronary flow as a guide, the aorta being rotated on the cannula until flow in each perfusion bed reached a maximum.

Perfusion Apparatus

Each coronary bed was initially supplied with oxygenated perfusion solution from a temperature-regulated reservoir (37°C) at constant pressure (Figure 1). The flow to each bed was continuously monitored using two in-line flowmeters (Meterate, Jencons Scientific Ltd., Leighton Buzzard, UK) with a detection range of 0.25–12.00 ml/min. When required, the left coronary bed could be perfused at constant flow, via a roller pump (Gilson Minipuls 3), with perfusion solution from any one of a bank of reservoirs. A central reservoir held perfusion solution containing disulfine blue dye (0.016% [wt/vol]), which could be infused unilaterally to delineate the two perfusion beds. The heart was housed in a temperature-regulated chamber and maintained at 37°C. Because the atria in the rat are supplied by extracardiac arteries, the right atrium was continuously superfused with oxygenated perfusion medium (37°C) at a constant flow rate of 10 ml/min to maintain sinus rate.

Perfusion Solutions

All solutions were continuously gassed with 95% O₂–5% CO₂ and filtered (pore size, 5 μm) before use. The standard perfusion solution had a pH of 7.4 and
was of the following composition (mmol/l): NaCl 118.5, NaHCO₃ 25.0, KCl 3.2, MgSO₄ 1.2, KH₂PO₄ 1.2, CaCl₂ 1.4, and glucose 11.0. Solutions at pH 7.1, 6.8, 6.6, and 6.4 were obtained by reducing the concentration of NaHCO₃ to 12.5, 6.3, 4.0, and 2.5 mmol/l, respectively. The concentration of sodium and the osmolarity were kept constant in the solutions by substitution of NaHCO₃ with NaCl as necessary.

**Experimental Protocols**

After 15 minutes of perfusion of both coronary beds with the standard perfusion solution (pH 7.4) at constant pressure, regional ischemia was induced by clamping the perfusion line supplying the left coronary bed. The right coronary bed continued to receive the standard perfusion solution at constant pressure throughout the experiment. Regional ischemia was maintained for 10 minutes (the duration that results in a maximum incidence of reperfusion-induced ventricular fibrillation in the isolated rat heart⁷); then the heart was randomly assigned to one of the study groups (n=12 per group), and the left coronary bed was reperfused at constant flow (100% of its preischemic flow rate) via the roller pump according to the following protocols.

**Stepwise restoration of extracellular pH.** This protocol (Figure 2A) was designed to test the effects of stepwise restoration of extracellular pH in the reperfused zone on the severity of reperfusion-induced arrhythmias. During 8 minutes of reperfusion, the left coronary bed received either the standard perfusion solution at pH 7.4 throughout (control group) or solution at pH 6.6 for the first 2 minutes of reperfusion, pH 6.8 from 2 to 4 minutes, pH 7.1 from 4 to 6 minutes, and finally pH 7.4 from 6 to 8 minutes.

**Transient acidic reperfusion.** This protocol (Figure 2B) was designed to test the antiarrhythmic efficacy of transient acidic reperfusion and to determine the pH–response characteristics of any such effect. In the control group, the left coronary bed received the standard perfusion solution at pH 7.4 throughout 8 minutes of reperfusion, as above. In four transient acidic reperfusion groups, the left coronary bed received the solution at pH 7.1, 6.8, 6.6, or 6.4 for the first 2 minutes of reperfusion, followed by the standard solution at pH 7.4 from 2 to 8 minutes.

**Measured Variables**

**Arrhythmias.** Arrhythmias were diagnosed from a unipolar electrocardiogram (ECG), which was obtained using a silver electrode inserted into the free wall of the left ventricle and a reference electrode connected to the aorta. The ECG was continuously monitored on a digital storage oscilloscope (model 1421, Gould Electronics Ltd., Ilford, UK) and recorded on an ink-jet recorder (model 2200S, Gould). Chart speed was set at 50 mm/sec a few seconds before reperfusion so as to obtain a permanent high-speed recording of the changes in the ECG during early reperfusion. The ECG was retrospectively analyzed, in a blinded manner, for the incidence, time to onset, and duration of ventricular tachycardia (VT) and ventricular fibrillation (VF). All analyses were carried out in accordance with the Lamberth Conventions.⁶ VT was defined as four or more consecutive premature beats of ventricular origin, and VF was defined as a signal in which individual QRS deflections could no longer be distinguished from one another and for which the rate could not be determined.

**VT cycle length.** In hearts subjected to transient acidic reperfusion and contemporary controls, average VT cycle length was determined during reperfusion with the solution at pH 7.4, 7.1, 6.8, 6.6, or 6.4. VT cycle length was determined after 15 seconds of reperfusion, because at this time a significant number of hearts (n=6-12) were in VT in each study group. VT cycle length was calculated from the number of QRS deflections over a 2-second interval using the ECG tracing.

**Coronary flow and heart rate.** Throughout the experimental protocol, coronary flow was monitored by the in-line flowmeters, and heart rate was determined from the ECG.

**Size of ischemic zone.** At the end of each experiment, the left coronary bed was perfused for 3 minutes with a solution containing 0.016% disulfine blue dye at 100 cm H₂O perfusion pressure. The heart was then removed from the perfusion apparatus, the atria and mediastinal tissue were removed, and dye-stained tissue, representing ventricular myocardium subjected to ischemia and reperfusion, was carefully dissected away from the remainder. The stained and unstained tissues were lightly blotted and weighed. The size of the ischemic zone, expressed as a percentage of total ventricular weight, was calculated from the following equation: (weight of stained tissue/total ventricular weight) × 100.

**Exclusion Criteria**

Exclusion criteria for the present study, selected to minimize variations in heart rate and size of the ischemic zone (due to atypical coronary anatomy) among the hearts, were based on our previous experience with the model. These criteria demanded that hearts were excluded if 1) heart rate was less than 280 or more than 420 beats per minute during the preischemic
period, or 2) the size of the ischemic zone was found to be greater than 70% of total ventricular weight after the end of the experiment. Hearts were also excluded if there was cross flow between the right and left coronary ostia. To verify whether significant cross flow occurred, the perfusion line to one bed was clamped for 10 seconds at the beginning of each experiment. If flow to the contralateral bed increased by more than 10% of the preceding flow in the occluded bed, the heart was excluded from the study (because in the rat heart collateral flow alone could not have been responsible for such an increase19). In addition, hearts that exhibited ventricular arrhythmias during the final 3 seconds of ischemia before reperfusion were not included in the analysis of reperfusion-induced arrhythmias, because in those hearts it would have been impossible to differentiate arrhythmias induced by reperfusion from those induced by ischemia. Of 116 hearts entered into the study, four were excluded on the basis of heart rate, 10 were excluded on the basis of the size of the ischemic zone, nine were excluded because of cross flow, and nine were excluded because of arrhythmias during the final 3 seconds of ischemia.

Data Analysis

The general approach to statistical analysis adopted the guidelines described by Wallenstein and colleagues.19 Gaussian-distributed variables were expressed as mean±SEM and were subjected to analysis of variance. If a difference among mean values was established with one-way analysis of variance, comparison with controls was performed using Dunnett's test. Binomially distributed variables, such as the incidence of VT or VF, were compared using the χ² test for a 2×n table, followed by a sequence of 2×2 χ² tests with Yates's correction. A value of p<0.05 was considered significant.

Results

Ischemia-Induced Arrhythmias

The objective of the present study was to assess the effects of interventions applied after ischemia on the incidence and severity of reperfusion-induced arrhythmias. However, arrhythmias were also quantified in the preceding period of ischemia (during which time the composition of the perfusate was identical in all study groups) to confirm that all groups were, in fact, identical before the onset of reperfusion.

Stepwise restoration of extracellular pH. The incidence of VT during the 10-minute period of ischemia preceding reperfusion was similar in both the control (92%) and the stepped-pH (100%) groups, and all episodes of VT were nonsustained. None of the hearts in either group exhibited ischemia-induced VF. There was no significant difference between the control and stepped-pH groups in the mean time to onset of ischemia-induced VT (520±13 and 531±13 seconds, respectively) or in the mean total duration of nonsinus rhythm (consisting of ventricular premature beats and VT) during the 10-minute period of ischemia (25±6 and 24±5 seconds, respectively).

Transient acidic reperfusion. The incidences of VT during the 10-minute period of ischemia preceding reperfusion were 92%, 83%, 75%, 50%, and 67% in the control, pH 7.1, pH 6.8, pH 6.6, and pH 6.4 groups, respectively (p=NS). Again, all episodes of VT were nonsustained, and none of the hearts in the five study groups exhibited ischemia-induced VF. There were no significant differences between control, pH 7.1, pH 6.8, pH 6.6, and pH 6.4 groups in the mean time to onset of ischemia-induced VT (534±12, 470±23, 536±15, 487±15, and 536±16 seconds, respectively). The mean total duration of nonsinus rhythm (consisting of ventricular premature beats and VT) during the 10-minute period of ischemia was less than 35 seconds in all groups.

Reperfusion-Induced Arrhythmias

Reperfusion of the ischemic region resulted in the rapid (within a few beats) induction of VT (Figure 3A) regardless of the pH of the reperfusion solution. Reperfusion-induced VT was generally polymorphic in nature (Figure 3A), and episodes of reperfusion-induced VT were uninterrupted (see Figures 4 and 5) until either spontaneous reversion to normal sinus rhythm or degeneration into VF, as illustrated in Figures 3B and 3C, respectively.

Stepwise restoration of extracellular pH. Figure 4 shows the time course of reperfusion-induced arrhythmias in control hearts in which the left coronary bed was reperfused with solution at pH 7.4 throughout and in hearts in which the left coronary bed was subjected to a stepwise restoration of extracellular pH (stepped pH) by sequential perfusion with solutions at pH 6.6, 6.8, 7.1, and 7.4 during the reperfusion period. All hearts in both groups developed VT within the first 3 seconds of reperfusion. In all hearts in the control group, VT degenerated into VF within the first 30 seconds of reperfusion. In contrast, only 42% of hearts in the stepped-pH group exhibited VF during reperfusion (p<0.05). Among hearts that exhibited VF, the mean time to onset of VF was significantly prolonged from 14±2 seconds in the control group (n=12) to 90±23 seconds in the stepped-pH group (n=5). By the end of reperfusion, 92% of hearts in the stepped-pH group were in normal sinus rhythm compared with only 8% of hearts in the control group (p<0.05).

Transient acidic reperfusion. Figure 5 shows the time course of reperfusion-induced arrhythmias in control hearts in which the left coronary bed was reperfused with solution at pH 7.4 throughout and in hearts in which the left coronary bed was subjected to transient (2-minute) acidic reperfusion with the solution at pH 7.1, 6.8, 6.6, or 6.4. There was a 100% incidence of VT in all five groups. In the control group, VT degenerated into VF within the first 30 seconds of reperfusion in 92% of the hearts; in the remaining heart, VT converted to sinus rhythm within 2 minutes of reperfusion. Transient acidic reperfusion resulted in a pH-dependent reduction in the incidence of VF (Figure 6), with 92%, 83%, 42% (p<0.05), and 33% (p<0.05) of hearts exhibiting VF in the pH 7.1, 6.8, 6.6, and 6.4 groups, respectively. Among hearts that exhibited VF during reperfusion, the mean time to onset was significantly prolonged (Figure 6) from 14±1 seconds in the control group (n=11) to 55±22 seconds in the pH 6.8 group (n=10), 64±24 seconds in the pH 6.6 group (n=5), and 190±42 seconds in the pH 6.4 group (n=4). The mean time to onset of VF was not significantly different from the control value in the pH 7.1 group (18±2 seconds). In
VT cycle length was measured in control hearts and those subjected to transient acidic reperfusion to determine whether it was affected by the pH of the initial reperfusion solution. As illustrated in Figure 7, acidic reperfusion resulted in a pH-dependent prolongation of VT cycle length at 15 seconds of reperfusion. VT cycle length at this time point was significantly greater in the two groups reperfused with solution at pH 6.6 or 6.4 than in the control group reperfused with solution at pH 7.4. Mean VT cycle length at 15 seconds of reperfusion was also calculated for two subpopulations of hearts in which VT subsequently either reverted to normal sinus rhythm (n=20) or degenerated into VF (n=30). Mean VT cycle length was 67.5±3.2 msec in the former and 52.1±1.8 msec in the latter subpopulations (p<0.05).

Coronary Flow, Heart Rate, and Size of Ischemic Zone

Stepwise restoration of extracellular pH. Preischemic flow was similar in control and stepped-pH groups in both right (14.1±0.7 and 12.9±0.7 ml/min per gram, respectively) and left (10.9±0.5 and 10.8±0.7 ml/min per gram, respectively) coronary beds. During regional ischemia induced by cessation of flow to the left coronary bed, flow in the right coronary bed did not change significantly (12.9±0.5 and 13.0±0.7 ml/min per gram after 9 minutes of ischemia in control and stepped-pH groups, respectively). During the first minute of reperfusion of the left coronary bed (at 100% of precirculatory flow), flow in the right coronary bed increased in both control (19.5±0.7 ml/min per gram) and stepped-pH (16.5±0.8 ml/min per gram) groups. This increase in right coronary flow was maintained throughout reperfusion in the control group, with a flow of 17.6±0.9 ml/min per gram recorded after 7 minutes of reperfusion. In contrast, right coronary flow in the stepped-pH group reverted to the prerereperfusion value within 3 minutes of reperfusion (11.6±0.8 ml/min per gram).

The preischemic heart rate was similar in control and stepped-pH groups (323±7 and 324±6 beats per minute, respectively). Regional ischemia had no effect on heart rate, which was 320±11 beats per minute in the control group and 328±8 beats per minute in the stepped-pH group after 9 minutes of ischemia. Sinus heart rate could not be measured during early reper-

FIGURE 3. Representative electrocardiographic tracings illustrating the rapid induction and polymorphic nature of reperfusion-induced VT (panel A), spontaneous reversion of reperfusion-induced VT to normal sinus rhythm (panel B), and degeneration of reperfusion-induced VT into VF (panel C). Arrows indicate the moments of initiation/degeneration of arrhythmia or reversion to a normal sinus rhythm. Chart speeds are shown on the horizontal lines above each panel.
fusion because of the almost immediate onset of VT and VF.

The size of the ischemic zone was similar in control and stepped-pH groups at 56±2% and 57±3% of the total ventricular weight, respectively.

Transient acidic reperfusion. Preischemic flow was similar in control, pH 7.1, pH 6.8, pH 6.6, and pH 6.4 groups in both right (11.3±0.5, 12.9±0.8, 13.3±1.6, 13.4±0.6, and 12.6±0.9 ml/min per gram, respectively) and left (11.2±1.2, 10.2±0.7, 9.7±0.5, 10.7±0.6, and 9.9±0.8 ml/min per gram, respectively) coronary beds. The time course of the changes in right coronary flow during regional ischemia and reperfusion are illustrated in Figure 8. During the first minute of reperfusion of the left coronary bed, flow in the right coronary bed increased in all groups. This increase was maintained throughout reperfusion in the control and pH 7.1 groups. In contrast, right coronary flow returned toward prerperfusion values within 3 minutes of reperfusion in the pH 6.8, pH 6.6, and pH 6.4 groups.

The preischemic heart rate was similar in control, pH 7.1, pH 6.8, pH 6.6, and pH 6.4 groups, at 323±10, 334±10, 326±12, 330±8, and 325±10 beats per minute, respectively. Regional ischemia had no effect on heart rate, and the corresponding values after 9 minutes of ischemia were 335±8, 333±10, 326±9, 320±12, and 327±7 beats per minute, respectively. Sinus heart rate could not again be measured during early reperfusion because of the almost immediate onset of VT and VF.

The size of the ischemic zone was also similar in the control, pH 7.1, pH 6.8, pH 6.6, and pH 6.4 groups at 55±3%, 53±2%, 53±3%, 55±3%, and 51±2% of total ventricular weight, respectively.

Discussion

The results of the present study indicate that acidic reperfusion of the ischemic rat myocardium does not affect the incidence of reperfusion-induced VT but inhibits the degeneration of VT into VF and promotes spontaneous reversion to normal sinus rhythm. Within the pH range of 7.4–6.4, the protective effect appeared to be pH dependent, with the most significant protection obtained by initial reperfusion with solution at pH 6.6 or 6.4. In these two groups, VT cycle length during early reperfusion was significantly prolonged relative to the control group. The protection afforded by acidic reperfusion was not associated with changes in antecedent variables such as heart rate, the size of the ischemic/reperfused zone, and the rate of reflow, because there were no differences between the groups in the former two variables and reperfusion of the left coronary bed was performed at 100% of preischemic flow in all cases.

Interestingly, initial acidic reperfusion with solution at pH 6.6 was equally protective regardless of whether the subsequent restoration of extracellular pH to 7.4 occurred in an abrupt or a stepwise manner. Thus, although the results of the present study support a profibrillatory role for the rapid restoration of extracellular pH, they also suggest that this effect is evident only when such restoration occurs within the first 2 minutes of reperfusion.
FIGURE 5. Time course of reperfusion-induced ventricular tachycardia and ventricular fibrillation in individual hearts in the control group and in the groups in which the left coronary bed underwent transient acidic reperfusion with solution at pH 7.1, 6.8, 6.6, or 6.4. All hearts (n=12 per group) were subjected to 10 minutes of regional ischemia. Reperfusion began at time zero at the preischemic flow rate and was continued for 8 minutes. All hearts were in sinus rhythm at the moment of reperfusion. Open bars represent normal sinus rhythm, hatched bars represent ventricular tachycardia, and filled bars represent ventricular fibrillation. The hearts in each group are arranged in the order in which the experiments were carried out (the study was randomized).
Possible Mechanisms of the Protective Effect of Acids Reperfusion

Role of Na⁺-H⁺ exchange. As noted earlier, the rapid washout during reperfusion of H⁺, accumulated in the extracellular space during the preceding period of ischemia, has been thought to result in Na⁺ influx via Na⁺-H⁺ exchange and consequently an increase in intracellular [Ca²⁺] via Na⁺-Ca²⁺ exchange. Increased intracellular [Ca²⁺] ("Ca²⁺ overload") on reperfusion has been implicated as a causal factor in several manifestations of reperfusion injury (for review, see Hearse and Bolli). These include myocardial stunning and ventricular arrhythmias, the latter possibly occurring via the induction of oscillatory release of Ca²⁺ from the sarcoplasmic reticulum and the subsequent activation of the Ca²⁺-induced transient inward current. However, beat-to-beat measurements of calcium transients by Lee et al. and Kihara et al. in isolated hearts (using iodo 1 or aequorin, respectively) have not shown an increase in intracellular [Ca²⁺] during reperfusion. Instead, intracellular [Ca²⁺] was shown to increase dur-

Figure 6. Bar graphs showing the incidence of reperfusion-induced ventricular fibrillation (VF) (n=12 per group, panel A) and mean time to onset of VF among those hearts that exhibited reperfusion-induced VF (n=4-11 per group, panel B) in the control group and in the groups in which the left coronary bed underwent transient acidic reperfusion with solution at pH 7.1, 6.8, 6.6, or 6.4. *p<0.05 compared with the control group.

Figure 7. Bar graph showing the mean ventricular tachycardia (VT) cycle length at 15 seconds of reperfusion (in those hearts that were in VT at that time, n=6-12 per group) in the control group and in the groups in which the left coronary bed underwent transient acidic reperfusion with solution at pH 7.1, 6.8, 6.6, or 6.4. *p<0.05 compared with the control group.

Figure 8. Bar graphs showing the right coronary flow rate before regional ischemia (C), during regional ischemia induced by cessation of flow in the left coronary bed, and during reperfusion of the left coronary bed at the preischemic flow rate in the control group and in the groups in which the left coronary bed underwent transient acidic reperfusion with solution at pH 7.1, 6.8, 6.6, or 6.4. *p<0.05 compared with the value after 9 minutes of ischemia (n=12 per group).
ing ischemia but normalize on reperfusion. Although this appears to contradict a major role for Ca\(^{2+}\)
overload in reperfusion-induced cardiac dysfunction, it should be noted that such dysfunction would not be
expected to occur after the short durations of ischemia (up to 3 minutes) used in the studies of Lee et al.\(^{27}\) and
Kihara et al.\(^{25}\) Indeed, a more recent study by Kihara and Morgan,\(^{26}\) in a model identical to that used in
their earlier study,\(^ {25}\) has shown that reperfusion after 20 minutes of ischemia results in a further increase in
intracellular [Ca\(^{2+}\)] and that this is associated with transition into VF.

Other recent studies have supported a role for Na\(^{+}\)-H\(^{+}\) exchange-mediated mechanisms in postreper-
fusion Ca\(^{2+}\) overload and contractile dysfunction. Thus, inhibition of Na\(^{+}\)-H\(^{+}\) exchange by amiloride or its
analogues has been shown to attenuate reperfusion-induced Ca\(^{2+}\) overload\(^ {11}\) and to improve functional
recovery.\(^ {11,27,29}\) Within the context of the present discussion, amiloride analogues have also been shown to
inhibit reperfusion-induced arrhythmias in both rat\(^ {13}\) and guinea pig\(^ {29}\) hearts. Although acidic reperfusion has
previously been shown to attenuate reperfusion-induced Ca\(^{2+}\) overload\(^ {22}\) and to improve postischemic
recovery of function,\(^ {30-32}\) the present study is the first to report the antifibrillatory efficacy of selective acidic
reperfusion of the ischemic zone after a period of regional ischemia.

Vaughan-Jones and Wu\(^ {34}\) have shown, in sheep Pur-
kinje fibers, that H\(^{+}\) extrusion and Na\(^{+}\) influx via Na\(^{+}\)-H\(^{+}\) exchange during recovery from any given
intracellular acid load is inhibited by lowering extracellular pH. Assuming that in a preparation perfused with
CO\(_2\)/HCO\(_3\)^{−}/buffered solution, as in the present study, intracellular pH would be reduced to approximately 6.6
after 10 minutes of ischemia,\(^ {35}\) certain estimates may be made on the basis of the findings of Vaughan-Jones and
Wu.\(^ {34}\) Thus, at an extracellular pH of 6.4–6.6 (i.e., the
pH of the acidic solutions shown to exhibit the most significant antiarrhythmic efficacy in the present study),
H\(^{+}\) extrusion and Na\(^{+}\) influx via Na\(^{+}\)-H\(^{+}\) exchange would be expected to be inhibited to approximately 20–25% of that at an extracellular pH of 7.4. Similar estimates may also be obtained on the basis of the reported sensitivity of the Na\(^{+}\)-H\(^{+}\) exchanger to extracellu-
lar pH in isolated rat myocytes.\(^ {36}\)

It should also be noted that Na\(^{+}\)-Ca\(^{2+}\) exchange has been shown\(^ {37}\) to be inhibited by low intracellular pH,
with 50% inhibition at pH 6.7. Inhibition of Na\(^{+}\)-H\(^{+}\) exchange by acidic reperfusion would be expected not
only to inhibit Na\(^{+}\) influx but also to maintain intracel-
ular acidosis for a longer period during reperfusion, which may directly inhibit Na\(^{+}\)-Ca\(^{2+}\) exchange. Al-
though Na\(^{+}\)-Ca\(^{2+}\) exchange largely mediates Ca\(^{2+}\) efflux
under normal conditions, its reversal potential is readily attainable even under physiological conditions and is
significantly affected by small changes in intracellular [Na\(^{+}\)].\(^ {38}\) Therefore, under conditions of increased intra-
cellular [Na\(^{+}\)] and membrane depolarization, as may be prevalent during the early moments of reperfusion,
Na\(^{+}\)-Ca\(^{2+}\) exchange may primarily mediate Ca\(^{2+}\) influx\(^ {39,40}\) and its inhibition by maintained intracellular
acidosis during early reperfusion may contribute to an
attenuation of Ca\(^{2+}\) overload.

In light of these observations, it is feasible to propose
that the protective effect of acidic reperfusion reported
in the present study may result from an attenuation of
Ca\(^{2+}\) overload via Na\(^{+}\)-Ca\(^{2+}\) exchange that is due to
reduced Na\(^{+}\) influx and maintained intracellular acidosis
by inhibition of Na\(^{+}\)-H\(^{+}\) exchange. If, however, the rapid washout of extracellular H\(^{+}\) and the subsequent
influx of Na\(^{+}\) (with concomitant recovery from intracel-
ular acidosis) is a major arrhythmogenic factor during
reperfusion, two questions remain to be answered: 1) Why did acidic reperfusion suppress the incidence of reperfusion-induced VF but not that of reperfusion-
induced VT? 2) Why did the abrupt change to pH 7.4
after transient (2-minute) acidic (pH 6.6 or 6.4) reper-
fusion seldom initiate de novo arrhythmias in those
hearts that were in normal sinus rhythm at that time?

In relation to the first question, the recent studies of
Kihara and Morgan\(^ {26}\) in the intact ferret heart have shown
that spontaneous transitions to VF do not occur unless a state of Ca\(^{2+}\) overload is present and that
diastolic Ca\(^{2+}\) oscillations temporally precede such transitions. Kihara and Morgan concluded that impaired
Ca\(^{2+}\) homeostasis might be a crucial factor for the
initiation of VF. In support of this, the recent studies of
Thadroyen and colleagues\(^ {41}\) in isolated spontaneously
beating ventricular myocytes have led them to suggest
that increased intracellular [Ca\(^{2+}\)] may be a causal
factor in the degeneration of VT into VF. Therefore, it is possible that acidic reperfusion may preferentially
inhibit the transition to VF by inhibition of Ca\(^{2+}\)
overload and consequent Ca\(^{2+}\) oscillations.

The answer to the second question is likely to be
found in the extent of inhibition of sarcolemmal Na\(^{+}\),K\(^{+}\)-ATPase activity by ischemia and the rate of its
recovery during reperfusion. Components of ischemia
such as acidosis, depletion of ATP, and accumulation of
inorganic phosphate are known to inhibit Na\(^{+}\),K\(^{+}\)-ATPase activity,\(^ {42}\) as can free oxygen radicals.\(^ {43}\) It has
been proposed that, during the early moments of un-
controlled reperfusion, any Na\(^{+}\) entering the myocyte
via Na\(^{+}\)-H\(^{+}\) exchange cannot be extruded by Na\(^{+}\),K\(^{+}\)-ATPase and exits via Na\(^{+}\)-Ca\(^{2+}\) exchange, with a con-
comitant rise in intracellular Ca\(^{2+}\).\(^ {44}\) Indeed, a recent
study\(^ {45}\) in isolated rabbit myocytes has shown that,
during recovery from intracellular acidosis in the pres-
ence of partial inhibition of Na\(^{+}\),K\(^{+}\)-ATPase, a large
increase in intracellular Ca\(^{2+}\) occurs and that this can
be inhibited by inhibition of Na\(^{+}\)-H\(^{+}\) exchange with an amiloride analogue. Other recent studies using pharma-
ocological inhibition of Na\(^{+}\),K\(^{+}\)-ATPase support a key
role for the activity of this enzyme during reperfusion in
determining not only the severity of postischemic con-
tracile dysfunction but also the incidence of reper-
fusion-induced VF.\(^ {47}\) The period of acidic reperfusion with oxygenated perfusate used in the present study
may allow sufficient recovery of Na\(^{+}\),K\(^{+}\)-ATPase activity
to enable the Na\(^{+}\) entering via Na\(^{+}\)-H\(^{+}\) exchange
during the subsequent abrupt return to pH 7.4 to be
extruded by Na\(^{+}\),K\(^{+}\)-ATPase rather than via Na\(^{+}\)-Ca\(^{2+}\)
exchange. Although Na\(^{+}\),K\(^{+}\)-ATPase is inhibited by low
pH, in the presence of optimal ATP and inorganic
phosphate concentrations it retains approximately 82%
of its maximal activity even at pH 6.5.\(^ {42}\) Therefore, it is
reasonable to propose that the absence of additional
arrhythmias on switching the perfusion solution from
pH 6.6 or 6.4 to pH 7.4 after 2 minutes of reperfusion may reflect significant recovery of Na\(^+\),K\(^+\)-ATPase activity by that time.

**Role of prolongation of VT cycle length.** In the present study, the pH of the initial reperfusion solution did not influence the rapid induction of VT but had a significant effect on VT cycle length; this was prolonged with decreasing pH such that it was significantly greater at pH 6.6 and 6.4 than at pH 7.4. As noted earlier, the greatest reductions in the incidence of degeneration from VT into VF were also observed in the two groups initially reperfused with pH 6.6 or 6.4 solution. This may suggest a triggering role for rapid VT in the initiation of VF, which is suppressed by prolongation of VT cycle length. Indeed, in support of an important role for VT cycle length in determining vulnerability to VF, VT cycle length during early reperfusion was found to be significantly shorter in hearts in which VT subsequently degenerated into VF than in those in which VT subsequently reverted to normal sinus rhythm.

It is known\(^{46}\) that acidosis causes reductions in the maximum rate of rise of the action potential and conduction velocity and may also prolong action potential duration. Such effects on action potential morphology and propagation probably underlie the prolongation of VT cycle length by acidic reperfusion observed in the present study. In addition to imposing a further burden on the metabolic deficit from antecedent ischemia, rapid VT during early reperfusion may also result in a further increase in intracellular [Ca\(^{2+}\)], thereby increasing the probability of degeneration into VF.\(^{41}\) Therefore, it follows that slower VT during reperfusion with acidic solutions may allow enhanced metabolic and electrophysiological recovery and that this mechanism may contribute, at least in part, to the antifibrillatory efficacy of acidic reperfusion.

**Role of differences in coronary flow.** Although there were no significant differences between the groups in left coronary flow rate at any point during the experimental protocol, there were differences in the profiles of right coronary flow rate during reperfusion of the left coronary bed at 100% of its preischemic flow rate (Figure 8). However, the intergroup differences in right coronary flow rate are unlikely to be causally related to the differences in the severity of reperfusion-induced arrhythmias. Rather, the differences in right coronary flow rate probably reflect the differences in the severity and duration of reperfusion-induced arrhythmias (Figure 5). Severe ventricular arrhythmias result in reduced extravascular compression (due to loss of coordinated contractile activity), which in turn may result in increased flow in the zone not subjected to ischemia and reperfusion.\(^{14}\) Thus, although the right coronary flow rate was significantly elevated in all groups during the first minute of reperfusion, during which all hearts exhibited episodes of VT or VF (or both), by 3 minutes of reperfusion, flow had returned toward the prerefusion value in those groups (pH 6.8, 6.6, and 6.4) in which a significant proportion of the hearts had reverted to normal sinus rhythm.

**Other possible mechanisms.** There are a number of other possible mechanisms by which the acidic reperfusion procedure used in the present study may suppress reperfusion-induced VF. The acidic solutions used were obtained by lowering the HCO\(_{3}^{-}\) concentration of the standard perfusion solution. It has recently been shown that recovery from an intracellular acid load in cardiac myocytes is mediated not only by Na\(^{-}\)-H\(^{+}\) exchange but also by Na\(^{-}\)-HCO\(_{3}^{-}\) cotransport,\(^{46}\) a process that may also result in an elevated intracellular [Na\(^{+}\)] in the presence of inhibited Na\(^{-}\),K\(^{-}\)-ATPase activity. Therefore, the protective effect observed in the present study may have been a property of the low [HCO\(_{3}^{-}\)] rather than the high [H\(^{+}\)] of the acidic reperfusion solution. However, our recent studies\(^{46}\) that showed acidic reperfusion to be equally protective in the absence of HCO\(_{3}^{-}\) would argue strongly against such a possibility. These studies also showed that reperfusion with solution at pH 7.4 was equally arrhythmogenic in the presence of either 25 mmol/l HCO\(_{3}^{-}\) (gassed with 5\% CO\(_{2}\)) or 5 mmol/l HEPES as buffer, despite a much reduced buffering capacity in the latter case. Therefore, it is unlikely that the protective effect of acidic reperfusion (with low [HCO\(_{3}^{-}\]) solutions) observed in the present study was due to the reduced buffering capacity of the reperfusion solutions.

Acidosis inhibits a number of membrane currents, including the slow inward Ca\(^{2+}\) current\(^{51}\) and the inwardly rectifying potassium current (IK)\(_{\text{ATP}}\).\(^{52}\) The inhibition of the calcium current by acidic reperfusion, however, is unlikely to account for the antifibrillatory effect observed in the present study, because pharmacological inhibition of this current, when applied only during reperfusion, has been shown to be ineffective against reperfusion-induced arrhythmias.\(^{53,54}\) In contrast, inhibition of the potassium current may well play a role in the protective effect of acidic reperfusion, because selective inhibition of this current by a novel pharmacological agent has recently been shown to abolish reperfusion-induced VF in the isolated rat heart.\(^{55}\) Acidosis also inhibits the release of Ca\(^{2+}\) from the sarcoplasmic reticulum.\(^{56}\) As discussed earlier, oscillatory release of Ca\(^{2+}\) from the sarcoplasmic reticulum has been implicated in reperfusion-induced arrhythmogenesis, and the inhibition of this process by acidic reperfusion may be expected to afford protection. In this context, it is worth noting that ryanodine, which also inhibits the release of Ca\(^{2+}\) from the sarcoplasmic reticulum, has been shown to prevent the degeneration of VT into VF during reperfusion of the regionally ischemic rat heart.\(^{57}\)

**Relevance to the Mechanism of Reperfusion-Induced Arrhythmias**

Whereas the results of the present study indicate the rapid washout of extracellular H\(^{+}\) to be an important arrhythmogenic factor during early reperfusion, they do not preclude a significant role for other factors in reperfusion-induced arrhythmogenesis. Indeed, whereas acidic reperfusion significantly inhibited reperfusion-induced VF, the incidence of reperfusion-induced VT remained at 100%, thus supporting the argument that multiple factors are involved. The recent study by Curtis\(^{5}\) has demonstrated that the rapid washout of extracellular K\(^{+}\), in the absence of ischemia and reperfusion, is sufficient to produce ventricular arrhythmias. In addition, studies with free oxygen radical-generating and scavenging systems\(^{5,6}\) suggest a causal role for free radicals in reperfusion-induced arrhythmias. In support of this, a burst of free radical production during early
reperfusion has been demonstrated,2 and other studies have shown that reactive oxygen intermediates are sufficient to generate ventricular arrhythmias in the absence of ischemia and reperfusion58,59 and to exacerbate reperfusion-induced arrhythmias.60

It is indeed possible that the induction of oxidant stress and the rapid washout of extracellular H+ on reperfusion may act in a synergistic manner to disrupt Ca2+ homeostasis,61 with arrhythmogenic consequences. In this context, oxidant stress has been shown to inhibit Na+ K+ ATPase activity,49 stimulate sarcolemmal Na+-Ca2+ exchange,65 and increase the open probability of the sarcoplasmic reticular Ca2+ release channel.66 In addition, the recent studies of Shattuck and Matsuura64,65 have shown that reactive oxygen intermediates can induce arrhythmogenic oscillations in membrane potential, indicative of intracellular Ca2+ overload, in both isolated ventricular muscles64 and isolated myocytes.65

Limitations of the Study

Although the present study supports a major role for the rapid washout of H+ in reperfusion arrhythmogenesis, it does not permit the delineation of the precise mechanism(s) by which maintained extracellular acidosis during early reperfusion exerts its protective effect. As discussed above, several possible mechanisms may be involved, and the role of each must be methodically examined before novel therapeutic approaches can be formulated. In addition, although the model used in the present study offers many advantages over conventional models14,16 and enables the use of groups of adequate size (because of the low cost of the preparation), the use of the rat heart (which has unusual electrophysiological characteristics, such as a short action potential duration and a high heart rate) may limit the applicability of the present findings to other species. While acknowledging this, it should be noted that the response of the rat heart to ischemia and reperfusion and to many protective interventions resembles that of other mammalian species.66 Indeed, in preliminary studies using isolated rabbit hearts with dual coronary perfusion, we have made observations similar to those reported here in that transient acidic (pH 6.6) reperfusion of the ischemic bed did not significantly reduce the incidence of reperfusion-induced VT (six of eight versus eight of eight in control hearts) but reduced the incidence of reperfusion-induced VF from eight of eight in control hearts to only one of eight (authors’ unpublished observations).

Concluding Comments

The present study has shown that the selective reperfusion with acidic solutions of the ischemic rat myocardium, after 10 minutes of regional ischemia, does not affect the rapid induction of VT but significantly suppresses the degeneration of VT into VF and promotes spontaneous reversion to normal sinus rhythm. This protective effect is observed in the absence of intergroup differences in the size of the ischemic zone, the rate of reflow, and oxygen tension of the perfusate. These findings are consistent with a major arrhythmogenic role, during uncontrolled reperfusion, for the rapid washout of extracellular H+.

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References


Reperfusion-induced arrhythmias. A role for washout of extracellular protons?

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