Arrhythmic Activity in Reoxygenated Guinea Pig Papillary Muscles and Ventricular Cells

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Aftercontractions, delayed afterdepolarizations, and automaticity occurred in guinea pig papillary muscles that were reoxygenated after hypoxic conditioning. The emergence of dysfunction was dependent on the severity of hypoxic conditioning and on stimulation during reoxygenation. After 60 minutes of substrate-free hypoxia, reoxygenation induced automaticity in a high proportion of stimulated muscles; the automaticity appeared within 1 minute and lasted for 10–20 minutes. After similar conditioning, muscles reoxygenated for 7–15 minutes were stimulated at various cycle lengths. The incidence of automaticity and the amplitudes of delayed events had W-shaped dependencies on cycle length (200–1,000 msec), whereas coupling intervals had M-shaped dependencies. In ventricular myocytes that displayed automaticity after reoxygenation, extrasystolic upstrokes arose smoothly from delayed afterdepolarizations that reached threshold. In tissue, extrasystolic upstrokes usually rose sharply from delayed afterdepolarizations that were distinctly subthreshold. Thus, threshold was reached elsewhere in the tissue. Further evidence of electrical heterogeneity was obtained from surface mapping of delayed-afterdepolarization amplitude in reoxygenated muscle. There were no detectable aftercontractions, delayed afterdepolarizations, or signs of automaticity in quiescent reoxygenated muscles or in stimulated reoxygenated muscles that were treated with 1 μM ryanodine. We conclude that the dysfunction precipitated by reoxygenation is due to synchronized spontaneous releases of calcium from overloaded sarcoplasmic reticulum. (Circulation Research 1987;61:124–133)

The reperfusion of ischemic heart can quickly lead to damage in excess of that ascribable to the ischemia alone. Among the first to recognize this were Tennant and Wiggers, who observed that reperfusion can provoke ventricular arrhythmia. More recently, Hearse et al. measured a large release of intracellular enzymes in isolated hypoxic hearts that were abruptly reoxygenated. They described the injury during reoxygenation as the "oxygen paradox," and further investigation has indicated that there is a striking similarity between injuries provoked by reperfusion and those provoked by reoxygenation. In both cases, the injury appears to be related to a calcium overload in the cells. Ischemia/hypoxia causes a de-rangement of intracellular calcium, depletion of ATP, and a contracture; reperfusion/reoxygenation may then trigger a massive increase in calcium influx, a large uptake of calcium by the mitochondria and sarcoplasmic reticulum (SR), sarcolemmal damage with loss of cell enzymes, etc.

There are many facets to the injury itself and therefore many ways of identifying its occurrence and sometimes its degree. The aspects commonly examined include myocardial enzyme release, tissue morphology, calcium fluxes, contractility, mitochondrial function, and electrical activity. Each of these is important, and none more so than electrical activity, since serious ventricular arrhythmias are the outcome.

Abnormal automaticity in Purkinje fibers provides one suitable explanation for ventricular arrhythmias triggered by reperfusion/reoxygenation. In fact, Ferrer et al. recently documented arrhythmic activity in reoxygenated Purkinje fibers. Nevertheless, aberrant electrical activity in ventricular muscle cells also may be involved in the genesis of reperfusion/reoxygenation arrhythmias, especially since other signs of injury in the heart (e.g., massive enzyme release) cannot be solely ascribed to the small mass of the Purkinje fiber system.

The hypothesis that reoxygenation can provoke arrhythmogenesis in working myocardial tissue was investigated in isolated guinea pig papillary muscles. The intracellular electrical activity and contractile activity were monitored during 30–60 minutes of hypoxic conditioning and 30 minutes of reoxygenation. Arrhythmic activity related to the occurrence of aftercontractions and delayed afterdepolarizations was observed within minutes of reoxygenation. As expected from the literature on reoxygenation injury, the incidence of arrhythmic activity was dependent on the experimental conditions during hypoxia and reoxygenation. In parallel experiments, we found evidence of electrical dysfunction in guinea pig ventricular myocytes that were reoxygenated after 60 minutes of cyanide hypoxia.

Materials and Methods

Papillary Muscle Preparations

Papillary muscles were obtained from the right ventricles of guinea pig (250–300 g) heart. Animals were
killed by cervical dislocation, and the heart was quickly removed. Muscles were dissected in oxygenated (95% O₂-5% CO₂) Krebs solution (pH 7.4) of the following composition in mM: NaCl 113.1, KCl 4.6, CaCl₂ 2.45, MgCl₂ 1.2, NaH₂PO₄ 3.5, NaHCO₃ 21.9, and glucose 5. Papillary muscles were usually selected from the same position within the right ventricle and were about 3 mm in length and 0.5 mm in diameter. The muscles were mounted in a Perspex bath (1-ml volume) perfused with oxygenated Krebs solution at 37 ± 0.2°C. The mural ends of the papillary muscles were clamped, and the tendinous ends were tied by a short length of silk thread to a stainless steel rod to measure tension. The length of the muscle was adjusted by movement of the transducer (Statham UC2, Oxnard, Calif) until the resting tension was 50–100 mg. At similar resting tensions, muscles in control resting tension-developed tension experiments developed about 75% maximum response. Stimuli (2-msec duration, twice threshold intensity) were applied to the basal part of the preparation through a bipolar Ag-AgCl electrode. The action potentials were recorded with 3 M KCl-filled microelectrodes (8–10 MΩ) connected to a high input impedance amplifier (M-707, WPI, New Haven, Conn.) via an Ag-AgCl pellet; the reference electrode was a 3 M KCl/Ag-AgCl unit. Action potentials and tension were displayed on a storage oscilloscope (model 5113, Tektronix, Beaverton, Ore.) and a pen recorder (Gould, Cleveland, Ohio).

All muscles were equilibrated for 60 minutes in oxygenated 5 mM glucose medium prior to an experimental procedure. Hypoxic conditions were achieved by gassing solutions with 95% N₂-5% CO₂; this resulted in Po₂<5 mm Hg at the bath inflow port. The action potential duration was measured at 25 and 75% repolarization, and the upstroke was differentiated with an electronic device. Stimuli were applied at a basic cycle length (BCL) of 1,000 msec, unless otherwise indicated in "Results."

**Isolated Myocytes**

Ventricular myocytes were isolated from male guinea pigs (250–300 g) following the method described by Watanabe et al.⁷ The hearts were excised, attached to the bottom of a Langendorff column (60-cm height), and perfused with solutions gassed with 95% O₂-5% CO₂ and maintained at 36 ± 0.5°C and pH 7.4. The first perfusate was calcium-free Krebs solution (modified Krebs solution without CaCl₂) to wash out the blood remaining in the heart cavities and coronary arteries. After 3–4 minutes of the initial perfusion, 50 ml of low CaCl₂ (25–50 μM) Krebs solution containing enzymes (500 U/ml collagenase type V and 200 U/ml trypsin type III, Sigma Chemical Co., St. Louis, Mo) were added to the column and perfused for 3–5 minutes. Finally, calcium-free Krebs solution was introduced to wash out the residual enzyme solution in the heart. The ventricles were cut into small fragments with iris scissors, and myocytes were dispersed by gentle agitation in oxygenated calcium-free Krebs solution. Finally, the calcium concentration in the cell suspension was raised to 2.45 mM by the addition of CaCl₂.

A Perspex bath (volume 1 ml) with a bottom formed by a microscope coverslip was attached to the stage of a Zeiss Type D inverted microscope. A few drops of freshly isolated myocytes were placed in the bath, and the myocytes were allowed to settle to the bottom (5 minutes). The bath was then perfused with modified Krebs solution gassed with 95% O₂-5% CO₂ at a rate of 2 ml/min. The temperature and pH of the solution were maintained at 36 ± 0.2°C and 7.4, respectively. A TV system was employed for visual observation of the cells.

Rod-shaped cells were quiescent, had a normal appearance, and comprised 20–40% of the cell population. They were impaled with 3 M KCl-filled microelectrodes (20–50 MΩ) connected to a high-input impedance amplifier (model 8100, DAGAN, Minneapolis, Minn.) via an Ag-AgCl pellet; the reference electrode was a 3 M KCl/Ag-AgCl unit. Action potentials elicited by passing short-current pulses (5–20 nA, <1 msec, BCL 1,000 msec) through the microelectrode were displayed on a storage oscilloscope.

**ATP Content**

Papillary muscles were horizontally mounted in a perfused bath (2-ml volume) that accommodated up to 3 muscles. After individual adjustment of resting tension, muscles were stimulated and superfused under conditions that were similar to those used in the electrophysiological experiments ATP was determined using a firefly luminescence technique (for complete details, see McDonald and MacLeod⁸). Two or three muscles were pooled for each determination after procedures that depressed ATP to very low concentrations.

**Drugs**

Ryanodine was generously supplied by Merck Sharp & Dohme Research Laboratory, Rahway, N.J. Sodium cyanide (CN) was purchased from Sigma and was added in small volumes to the bathing solution. The pH of CN-Krebs solution was adjusted with HCl, and solutions were freshly prepared every 10 minutes during CN experiments.

**Results**

There were marked changes in the electrical activity, contractile activity, and ATP content of guinea pig papillary muscles that were superfused with substrate-free hypoxic solution for 60 minutes and then reoxygenated with 5 mM glucose solution. These changes are summarized in Figure 1. Hypoxia shortened the action potential duration to about 18% of control, and there was prompt recovery with reoxygenation (Figure 1A). There were large concomitant changes in action potential overshoot (maximum depression 15–25 mV) and smaller changes in resting potential and Vmax (maximum depressions of 5 mV and 10%, respectively). Developed tension declined to about 25% of control during hypoxia and only recovered to 65% of con-
FIGURE 1

Time course of electrical, contractile, and metabolic changes during 60 minutes of substrate-free hypoxia and subsequent 30 minutes of reoxygenation with 5 mM glucose solution. Muscles were stimulated at BCL 1,000 msec. A. Action potential duration (APD) was measured at 75% repolarization and expressed as percent of prehypoxia (control) value. B: Developed tension as percent of prehypoxia control. C: Increase in resting tension above control value (75–125 mg). D: ATP content (mmol/kg tissue wet wt). ○, control values on normoxic muscle. Values are mean ± SEM, n = 12 muscles for A, B, C, and n = 6–12 determinations for D.

Factors Affecting Incidence of Reoxygenation-Induced Arrhythmia

STIMULATION The emergence of arrhythmic activity was dependent on muscle stimulation during the reoxygenation phase. This conclusion was reached from trials on 35 muscles that were conditioned for 60 minutes in substrate-free hypoxic solution and then reoxygenated for 30 minutes in 5 mM glucose solution. These muscles were divided into 4 groups according to control on reoxygenation (Figure 1B). Resting tension rose slowly during the first 30 minutes of hypoxia and then quickly for the next 30 minutes. Approximately 80% of the increase in resting tension was dissipated during the reoxygenation phase (Figure 1C). Muscle ATP content declined rapidly during the first 30 minutes of hypoxia and then more slowly to about 0.8 mM/kg wet wt (<20% of control ATP) at 60 minutes of hypoxia (Figure 1D). Recovery was incomplete (~60% of control) after 30 minutes of reoxygenation.

Aftercontractions, delayed afterdepolarizations, and extrasystoles were recorded from reoxygenated papillary muscles. Examples from 3 muscles that were reoxygenated for 2–8 minutes after 60 minutes of substrate-free hypoxia are presented in Figure 2. The more important observations are as follows: 1) Extrasystolic action potentials arose from near-normal resting potentials (approximately ~85 mV) and had V_{max} values similar to those of action potentials initiated by external stimuli (approximately 220 V/sec). 2) The amplitude of delayed afterdepolarizations varied from about 20 to 2 mV (Figures 2A and 2C, respectively). When a given reoxygenated muscle was constantly stimulated at BCL 1,000 msec, the amplitude of the delayed depolarization increased during the first 3–5 minutes of reoxygenation and then gradually declined over the next 10–20 minutes. However, the amplitude was also dependent on BCL (see Figure 4B) and on the positioning of the microelectrode (see Figure 6). 3) The peak of the delayed afterdepolarization preceded the peak of the aftercontraction by 40–100 msec (Figure 2; also see Figure 4C); the converse was never observed. 4) The intervals between stimulated upstrokes and spontaneous electrical and contractile events (coupling indexes) were about 300–450 msec (Figure 2). In a given muscle, these intervals were affected by BCL (see Figure 4C). 5) The amplitude of the delayed afterdepolarization was relatively small after a short rest on the first stimulus and then increased with the next few stimuli (Figure 2B). Conversely, on cessation of stimulation and spontaneous activity, amplitudes of delayed events damped rapidly (Figure 2A).

FIGURE 2. Arrhythmic activity, delayed afterdepolarizations, and aftercontractions in three papillary muscles after 60 minutes of substrate-free hypoxia and reoxygenation in 5 mM glucose solution. Stimulated events are marked by arrowheads. A. After 3 minutes of reoxygenation during stimulation at BCL 1,000 msec. B. After 5 minutes of reoxygenation. A 10-second rest (not shown) preceded the stimulation at BCL 300 msec. C. After 8 minutes of reoxygenation during stimulation at BCL 1,000 msec.
the stimulation pattern during reoxygenation: 1) stimulation at BCL 1,000 msec \((n = 11)\), 2) stimulation at variable BCL \((n = 16)\), 3) no stimulation \((n = 5)\), and 4) stimulation after 3–4 minutes of rest imposed at the onset of reoxygenation \((n = 3)\). Under the variable BCL pattern, stimulation at BCL 1,000 msec was interrupted for 30 seconds each minute by trains of 10 stimuli (BCL 200–800 msec) separated by 5-second rests. The first interruption began at 45 seconds after reoxygenation.

Extrasystolic beats were detected in 9 of the 11 muscles stimulated at BCL 1,000 msec during reoxygenation, and in 13 of 16 muscles stimulated at variable BCL, but not in any of the 5 muscles that were not stimulated.

The time course of automaticity was estimated from observations on the muscles that displayed automaticity at least once during the 30-minute period after reoxygenation \((n = 9\) at BCL 1,000 msec, \(n = 13\) at variable BCL). The occurrence of an extrasystolic action potential during the period 45–75 seconds after reoxygenation, and during subsequent 30-second periods spaced 1 minute apart, was scored for each muscle. Scores were expressed by an index, incidence of automaticity, that ranged from 0 to 100% (100%: all muscles in the group registered an extrasystole during the given 30-second interval). Figure 3 indicates that the incidence-time relation climbed from 0 to 100% within 5 minutes, remained above 75% for 3–5 minutes, and then decayed to 0% over the next 5–10 minutes. The variable BCL stimulation regimen (open circles) was slightly more provocative than the BCL 1,000-msec regimen (solid circles).

Arrhythmic activity did not arise in any of the 5 muscles allowed to rest during the 30-minute period after reoxygenation (Group 3). However, in all 3 of the muscles (Group 4) rested for the first 3–4 minutes of reoxygenation, a single postrest stimulus provoked a burst of activity that lasted 10–20 seconds. Single postrest pulses applied before hypoxia \((n = 15)\), during hypoxia \((n = 8)\), or later than 20 minutes after reoxygenation \((n = 8)\) were not followed by spontaneous activity.

During the series of experiments connected with Figure 3, it was apparent that stimulation at some BCLs provoked automaticity more frequently than stimulation at other BCLs. In addition, when automaticity was not provoked at any BCL during a variable BCL trial, the amplitudes of delayed afterdepolarizations and aftercontractions following the last stimulated event varied with BCL.

The dependence of these aspects on BCL (200–1,000 msec) was examined in 8 muscles reoxygenated for 7–15 minutes after 60 minutes of substrate-free hypoxia. Figure 4A shows that the incidence of automaticity (percent of 42 trials) was W-shaped, with the maxima near 60% (BCL 200, 500, and 1,000 msec) and the minima near 35% (BCL 300 and 800 msec). Similar, though less distinct, shapes characterized the BCL relations for delayed afterdepolarization and aftercontraction amplitudes \((n = 12\) trials) (Figure 4B).

These experiments were also used to measure coupling indexes, or time intervals, between the upstrokes of stimulated action potentials and 1) the upstrokes of spontaneous action potentials, 2) the peaks of the delayed afterdepolarizations, and 3) the peaks of the aftercontractions. The results from 12 variable BCL trials are shown in Figure 4C. The average coupling intervals for extrasystolic action potentials and delayed afterdepolarizations were usually within 20 msec of each other and ranged from 345 to 430 msec. However, the intervals for aftercontractions (415–485 msec) were distinctly higher than those of the electrical parameters. The relations between coupling intervals and BCL were roughly M-shaped for all three events, i.e., the inverse of the relations found for the other 3 aspects (Figures 4A and 4B).

**Severity of hypoxic conditioning.** We examined the incidence of arrhythmias in muscles that were reoxygenated after hypoxia of varying duration in solutions containing 0, 5, and 50 mM glucose (Figure 5). The incidence is presented on a plot of duration of hypoxia vs increase in resting tension at the termination of hypoxia. Muscles that exhibited reoxygenation-induced arrhythmias are scored by the solid symbols, and those that did not are indicated by the open sym-
Automalicity

200 400 600 800 1000
Basic Cycle Length (msec)

200 400 600 800 1000
Basic Cycle Length (msec)

400 600 800 1000
Basic Cycle Length (msec)

FIGURE 4
Modulation of electrical and contractile activity in reoxygenated muscle by stimulation BCL. Results are from 8 muscles reoxygenated for 7–15 minutes after 60 minutes of substrate-free hypoxia. Up to 8 variable BCL trials were conducted on each muscle, n values below refer to number of completed trials. A: Incidence of automaticity (% of trials) vs BCL, n = 42 trials, each of which elicited at least 1 extrasystole. B: Amplitudes of delayed afterdepolarizations (DAD, left-hand axis) and aftercontractions (AC, right-hand axis) vs BCL. Amplitudes were measured after last (tenth) stimulus at each BCL in trials (n = 12) that did not elicit extrasystoles. C: Coupling indexes vs. BCL. Coupling indexes are intervals between the upstrokes of stimulated action potentials and the following, upstrokes of spontaneous action potentials (Auto), peaks of delayed afterdepolarizations (DAD), and peaks of aftercontractions (AC); n = 12. All symbols indicate mean values; vertical bars in B and C indicate SEM.

FIGURE 5. Emergence of automaticity during reoxygenation depends on severity of hypoxic conditioning. Muscles (n = 42) stimulated at BCL 1,000 msec were conditioned with one of following hypoxic solutions: 0 mM glucose (substrate-free) for 30 and 60 minutes (circles), 5 mM glucose for 60 minutes (squares), and 50 mM glucose for 60 minutes (triangles). During reoxygenation in 5 mM glucose solution, muscles were stimulated at BCL 1,000 msec or variable BCL 200–1,000 msec. A muscle was scored as automatic if a spontaneous event was detected during the 30 minutes after reoxygenation (but note that spontaneity in these muscles was always more extensive than this criterion (Figure 3)). Data are presented on plots of increase in resting tension at end of hypoxia vs. duration of hypoxia. Muscles that displayed automaticity (Auto +) are denoted by solid symbols, and those that did not (Auto -) by open symbols.

other way, the incidence of arrhythmias after 60 minutes of substrate-free hypoxia was 100% (18 of 18 muscles) when the increase in resting tension during hypoxia exceeded 150 mg. The latter result establishes a useful reference by which the outcome of interventions (e.g., drug treatments) can be judged (see ryanodine section below).

Heterogeneity of Electrical Activity in Reoxygenated Muscle

Threshold potential is about -65 mV in guinea pig ventricular preparations. The upstrokes of extrasystolic action potentials often arose from potentials that were distinctly negative to this level. Therefore, threshold was reached elsewhere in the preparation, and resultant action potentials were propagated to the microelectrode site.

We looked for an additional indication of electrical heterogeneity by quickly "mapping" reoxygenated preparations with a roving microelectrode. The results from an example muscle are shown in Figure 6. At the original impalement site, extrasystoles were evident.
Reoxygcnation Arrhythmia

BCL 500 msec  BCL 200 msec

FIGURE 6. Heterogeneity in amplitude of delayed afterdepolarizations along surface of papillary muscle reoxygenated after 60 minutes of substrate-free hypoxia. Impalement sites (left) numbered 1 to 5 were 0.3–0.4 mm apart and were sampled at 14, 18, 20, 22, and 25 minutes after reoxygenation, respectively. Automaticity was recorded in site 1 at 14 minutes (top record) but not at 16 minutes (not shown). There was no automaticity at any site after this, but there was considerable intersite variation in amplitudes of delayed afterdepolarizations during stimulation at BCL 500 or 200 msec. Note that the intersite variation of activity was not simply related to time after reoxygenation. Stimulated events are marked by arrowheads. Central vertical bars indicate potential levels (see top bar).

after 14 minutes of reoxygenation (1, left-hand panel) but not after 16 minutes (not shown). Four additional sites separated from each other by distances of 0.3–0.4 mm were then explored in order (numbers, left-hand side). After securing a stable impalement (1–2 minutes) in each new site, records were collected during stimulation at BCL 500 msec (left-hand traces) and 200 msec (right-hand traces). Automaticity was not detected at any of these new sites. At site 1, delayed afterdepolarization amplitude declined from 4 mV (5 minutes of reoxygenation) to about 1 mV at 14–16 minutes. At site 2, the delayed events were comparable to those at site 1, but at site 3, they were considerably larger (approximately 8 mV) despite the elapsed time after site-1 sampling. Finally, small- and intermediate-sized events were recorded at sites 4 and 5, respectively.

Suppression of Reoxygcnation Arrhythmias by Ryanodine

A factor that may link delayed afterdepolarizations, aftercontractions, and extrasystoles is oscillatory release of calcium from the SR. Since ryanodine has been reported to inhibit calcium release from the SR of cardiac tissue, we examined whether it could moderate dysfunction in reoxygenated muscle.

In control muscles (n = 3) stimulated at BCL 1,000 msec, 1 μM ryanodine for 30 minutes had little effect on the action potential, but it depressed developed tension by about 30% within 5–10 minutes. This is in agreement with the findings of Sutko and Kenyon on guinea pig papillary muscle and of Mitchell et al on guinea pig myocytes. For the reoxygenation experiments, 1 μM ryanodine was introduced during the last 10 minutes of the 60-minute substrate-free hypoxia and was present during the reoxygenation phase. Each muscle in this series (n = 5) had an increase in resting tension that exceeded 180 mg. Therefore, the expected outcome without drug interference was spontaneity in all 5 muscles (Figure 5). In fact, none of the ryanodinetreated muscles displayed dysfunction on reoxygenation (e.g., Figure 7).

FIGURE 7. Absence of automaticity, aftercontractions, and delayed afterdepolarizations in reoxygenated muscle treated with 1 μM ryanodine. The drug was added after 50 minutes of 60 minutes of substrate-free hypoxia and was present during reoxygenation in 5 mM glucose solution. Example records were collected at 5–6 minutes after reoxygenation. Stimulated events are marked by arrowheads.
Hypoxia-Reoxygenation in Isolated Ventricular Myocytes

Control action-potential configuration in guinea pig ventricular myocytes was similar to that in papillary muscles (Figure 8) (Watanabe et al). However, 60-minute superfusion with substrate-free hypoxic solution only induced a variable moderate reduction (10–40%) in the action-potential duration. Others have had similar experiences with guinea pig myocytes superfused with hypoxic solution (e.g., G. Isenberg, personal communication), and the likely explanation is contaminant oxygen from the air (also see Stern et al16). The presumed oxygen contamination was negated by adding 1 mM sodium cyanide to the hypoxic solution. This concentration of cyanide moderately accelerated hypoxia-induced shortening of the action potential in papillary muscle.

Stable impalements were achieved in 10 cells exposed to substrate-free cyanide hypoxia for 60 minutes and then reoxygenated with 5 mM glucose solution. The response of the action-potential duration to cyanide hypoxia was variable. In 3 cells (Figure 8A), the duration declined at a rate comparable to that in hypoxic papillary muscles (Figure 1A), cell shortening was visually discernible at 30–40 minutes, and there was full recovery of the action potential along with partial relaxation within 10 minutes of reoxygenation. In 4 cells (Figure 8B), there was a more rapid depression of the action potential, full contracture (rounding up) within the 60-minute hypoxia, and very little recovery after 10 minutes of reoxygenation. In the remaining 3 cells (Figure 8C), there was a slow decline in the action potential duration, slight cell shortening, and prompt recovery with reoxygenation.

Delayed afterdepolarizations were recorded in the cells that responded to reoxygenation with a recovery of the action potential. Figure 9A illustrates that the postreoxygenation development of the afterdepolarizations was similar to that in the tissues. The delayed afterdepolarizations led to episodes of automaticity in 3 cells, and records from one of these is shown in Figure 9B. The small delayed afterdepolarization at 2 minutes after reoxygenation increased in amplitude and resulted in triggered activity within the next 3 minutes. The automaticity was sporadic for nearly 15 minutes but eventually gave way to control-like stable activity. The coupling intervals in the records of Figures 9A and 9B are 300–400 msec.

Discussion

The reoxygenation of hypoxic guinea pig ventricular muscle often led to dysfunction characterized by aftercontractions, delayed afterdepolarizations, and arrhythmic activity that persisted for as long as 15 minutes. Prerequisite conditions for the emergence of arrhythmic activity included sufficiently severe hypoxic conditioning and electrical stimulation during the reoxygenation.

Ferrier et al17 have described an electrical dysfunction precipitated by the reoxygenation of canine Purkinje fibers. In the Purkinje fibers, partial depolarization and oscillatory afterpotentials facilitated the generation of single poststimulus extrasystoles during the first few minutes of reoxygenation. Thereafter, rapid depolarization produced inexcitability for about 20 minutes, and subsequent gradual repolarization was marked by episodes of depolarization-induced automaticity. This pattern contrasts with that observed in reoxygenated guinea pig muscle (no depolarization or inexcitability, a high degree of automaticity shortly after reoxygenation, normal rhythm after 10–20 minutes). In the Purkinje fibers, the intervals between oscillatory afterpotentials and the last driven upstrokes in the fibers were approximately equal to stimulus BCL (250, 500, and 700 msec). By contrast, coupling indexes for delayed afterdepolarizations, extrasystoles, and aftercontractions in reoxygenated muscles had M-shaped dependencies on stimulus BCL (200–1,000 msec). The latter difference may be tissue-related since calcium-overloaded dog Purkinje fibers (digitalis treatment) had a near-linear dependence of coupling index on BCL, whereas calcium-overloaded dog ventricular muscles (low-K treatment)18 had an M-shaped dependence similar to that reported here.

With reference to concepts already developed by others (see below), the present results are consistent with the following chain of events: 1) high calcium influx upon reoxygenation raises Ca, and leads to oscillatory spontaneous release of calcium from overloaded SR in the same cells, 2) following an action potential and associated twitch, there is a temporary synchronization of the spontaneous release, 3) a large synchronized spontaneous release elicits an aftercontraction and a transient inward current that is observed as a delayed afterdepolarization, and 4) a delayed afterde-
**Calcium and ATP During Hypoxia and Reoxygenation**

The reoxygenation of hypoxic muscle triggers a rapid influx of calcium. If this influx is large enough, it may set the stage for the emergence of arrhythmia. The degree of calcium gain on reoxygenation has been correlated with the degree of ATP depletion and increase in resting tension achieved during the hypoxic conditioning. The latter findings may explain the lack of reoxygenation-induced disturbances when substrate-free hypoxia was of short duration (30 minutes) or when glucose was present during lengthier (60 minutes) hypoxic episodes. Since increases in resting tension under these protocols than under our standard (60 minutes, substrate-free) hypoxic conditions, it is likely that the influx of calcium on reoxygenation was also less severe. In this context, the small fraction of muscles that did not exhibit automaticity after standard conditioning (Figure 5) may have experienced a smaller than average depletion of ATP and influx of calcium.

**Calcium Overload, Aftercontractions, and Delayed Afterdepolarizations**

Aftercontractions and delayed afterdepolarizations are synonymous with calcium overload in heart tissue. These contractile events are almost certainly the consequences of spontaneous releases of calcium from overloaded SR. In the absence of stimulation, there is cell-to-cell variation in the timing and amount of spontaneous calcium release in calcium-overloaded tissue. Stimulation may synchronize post-twitch spontaneous release with consequent enhancement of delayed-event amplitude. The likely membrane conduits for the transient inward currents underlying delayed afterdepolarizations are nonspecific cation channels opened by high Ca, and the electrogenic sodium–calcium transporter. These concepts can be applied to explain the responses observed in reoxygenated muscle. Asynchrony of the spontaneous release in quiescent muscle hinders the generation of detectable (at our gains) aftercontractions and delayed afterdepolarizations. Action potentials and associated calcium releases promote, after short lags, synchronous spontaneous releases that are large enough to elicit resolvable aftercontractions and delayed afterdepolarizations. With the termination of action potential generation, there is a rapid desynchronization of release that translates to a rapid attenuation of the delayed events (Figure 2A).

In general, the magnitude and timing of spontaneous releases are likely to be determined by the interplay of factors such as diastolic Ca, extent of SR filling, and availability of the release mechanism. Since these determinants are affected by membrane electrical activity, the pattern of electrical stimulation will exert a complex influence on the amplitude and timing of delayed events. The complexity is illustrated by the W- and M-shaped relations between delayed-event descriptors and stimulus BCL (Figure 4), and it is likely to be more pronounced in muscular (abundant SR) than in nonmuscular cardiac tissue. For example, in dog sinus tissue treated with norepinephrine, Henning and Wu observed an inverse relation between delayed-afterdepolarization amplitude and coupling interval and a positive linear relation between action potential duration and delayed-afterdepolarization amplitude. Although the inverse relation appeared to be expressed in reoxygenated muscle (Figures 4B and 4C), a positive linear effect of action potential lengthening (occasioned by increasing BCL) was not observed (Figure 4B).

Ryanodine abolished aftercontractions and delayed afterdepolarizations in calcium-overloaded papillary muscle (K-free treatment). These drug actions were duplicated in reoxygenated muscle; correspondingly, arrhythmic activity was not detected at any stimulation BCL. Since ryanodine inhibits the release of calcium from the SR but does not affect transsarcolemmal calcium movements, it is likely that the amelioration of sufficient amplitude promotes a triggered action potential.
tion of dysfunction in reoxygenated muscle stemmed from a drug block of spontaneous calcium release from the SR.

**Electrical Heterogeneity and Arrhythmia in Reoxygenated Muscle**

In reoxygenated myocytes, delayed afterdepolarizations arose from potentials (approximately −85 mV) that were close to normal resting potentials. During bouts of spontaneous activity, the ascending phase of large-amplitude, delayed afterdepolarizations led smoothly to regenerating upstrokes. This phenomenon was rarely recorded in arrhythmic reoxygenated tissue; i.e., the delayed afterdepolarizations at microelectrode sites were usually less than 10 mV in amplitude, and the upstrokes of spontaneous action potentials were sharply rising events. Since the recorded resting potentials in the tissues were about −85 mV, delayed afterdepolarizations of 15–25 mV amplitude would have been required to reach threshold and trigger smoothly rising upstrokes. Threshold must have been attained elsewhere in the tissue, perhaps due to large-amplitude delayed afterdepolarizations or to smaller ones superimposed on depressed resting potentials. The likely cause of this electrical heterogeneity is that some cells in the tissue are more adversely affected than others in regard to metabolic function, calcium permeability, and capacity to deal with calcium overload. These disparities may arise during the hypoxia and become further aggravated during the reoxygenation.38

Triggered activity may reflect moderate injury from which cells in the tissue, and in isolation,29 can recover as the calcium overload is dissipated. In preliminary experiments, we have found that the reoxygenation of tissue after more severe deprivation than detailed here provokes increased patchiness of electrical activity, partial uncoupling, and conduction disturbances. This sets the scene for a range of activity varying from near normal to triggered activity to parasystolic foci. If a similar range of activity develops in ventricular tissue after reperfusion and if electrical dysfunction in Purkinje fibers27 is also considered, it is not surprising that more than a single mechanism is required to explain reperfusion arrhythmias.5,6

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