Electrical Heterogeneity and Conduction Block in Reoxygenated Guinea Pig Papillary Muscles

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SUMMARY

To investigate the changes in electrical activity after reoxygenation, guinea pig papillary muscles were reoxygenated with 5 mM glucose solution after various durations (30–120 min) of substrate-free hypoxia. Action potential duration recovered to the control level on reoxygenation following 30–120 min hypoxia. The recovery of developed tension or resting tension was incomplete after reoxygenation following longer periods of hypoxia. There was a high incidence of arrhythmias on reoxygenation after 60 min hypoxia, which have been shown to be triggered activities due to delayed afterdepolarizations. In some muscles reoxygenated after prolonged hypoxia (90–120 min), there was electrical heterogeneity which was shown by variable action potential durations and configurations. There was also conduction block around the microelectrode site in muscle. It was suggested that the electrical heterogeneity and conduction block could predispose to reentry, and that triggered activities and reentry could be involved in the genesis of arrhythmias on reperfusion. (Jpn Heart J 1996; 37: 383–391)

Key words: Delayed afterdepolarization Triggered activity Action potential duration Calcium

THE electrophysiological mechanisms of arrhythmias during reperfusion may involve reentry, automaticity, and triggered activity.[1-3] It has been suggested that the electrical heterogeneity (especially the dispersion of action potential duration) is the electrophysiological basis of reentry occurring on reperfusion.[1,2] Reperfusion has been shown to induce marked electrical inhomogeneity; that is, the desynchronization of electrical depolarizations as indicated by widening or fractionation of the electrogram.[4,5]

Although strong analogy has been drawn between reperfusion damage and reoxygenation damage in the heart,[6] there have been few reports on electrical heterogeneity during reoxygenation. It is likely that reoxygenation leads to the

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electrical heterogeneity in isolated cardiac preparations. One reason is that reoxygenation provokes a large increase in Ca^{2+} influx, and in the rabbit septum the added influx was 'patchy'.[7] Arrhythmias occur in a high percentage of reoxygenated guinea pig papillary muscles, and this has been attributed to triggered activity arising from Ca^{2+} overload of the cells.[8] In that study, a mapping of the muscle surface after reoxygenation indicated electrical heterogeneity in regard to the amplitudes of delayed afterdepolarizations.

The purpose of this study was to investigate the electrophysiological changes on reoxygenation following variable periods of substrate-free hypoxia. Heterogeneity in the electrical activity was also studied in guinea pig papillary muscles subjected to reoxygenation.

Methods

Papillary muscles were obtained from the right ventricles of guinea pig hearts. Animals weighing 250–300 g were sacrificed by cervical dislocation and the heart was removed and placed in the control Krebs solution (in mM): NaCl 113.1, KCl 4.6, $CaCl_2$ 2.45, $MgCl_2$ 1.2, NaH_2PO_4 3.5, $NaHCO_3$ 21.9 and glucose 5, equilibrated with 95% O_2-5% CO_2 (pH 7.4). Papillary muscles (about 3 mm long, 0.5 mm wide) were excised and mounted in a Perspex bath (1 m*l* volume) superfused with the solution at 37 ± 0.2°C at a flow rate of 1 m*l*/min. Mural ends of muscles were secured in a clamp and tendinous ends were tied by a silk thread to a stainless steel rod extending from the head of a force transducer (Statham, UC2). Stimulating pulses (1 Hz, 2 msec duration, twice threshold intensity) were applied to muscles through a bipolar Ag-AgCl electrode. Muscle length was adjusted until resting tension was 75–125 mg. At similar resting tensions, muscles in control resting tension-developed tension experiments developed about 75% maximum response. The action potentials were recorded with a 3 M KCl-filled microelectrode (8–10 MΩ) connected to a high input impedance amplifier (M-707, WPI, New Haven) via an Ag-AgCl pellet. Action potentials and tension were displayed on a storage oscilloscope (model 5113, Tektronix, Beaverton) and a pen recorder (Gould, Cleveland).

All muscles were equilibrated for 60 min in the control Krebs solution prior to an experimental procedure. Substrate-free hypoxic solution was gassed with 95% N_2-5% CO_2, which was the same composition as the control Krebs solution except glucose was excluded. The pO_2 was 20–30 mmHg in the middle of the bath where muscles were positioned. Stimuli were applied at a basic cycle length (BCL) of 1,000 msec, while some preparations were stimulated at BCLs shorter than 1,000 msec after reoxygenation. In some preparations, muscle surfaces were mapped with an electrode during reoxygenation.

The action potential duration (APD) was measured at 75% repolarization. Data are expressed as mean ± SE. Statistical analysis was performed using ANOVA and a post-hoc test. P values < 0.01 were considered significant.

Results

Twenty-three papillary muscles were reoxygenated with 5 mM glucose solution after 30 ($n = 5$), 60 ($n = 10$), 90 ($n = 4$) or 120 ($n = 4$) min superfusion with substrate-free hypoxic solution (Figure 1). During hypoxic perfusion (open circles), there were depressions of APD and developed tension, and an increase in resting tension. Reoxygenation (filled circles) produced full recovery of the APD (Figure 1A), but only partial restoration of developed tension (Figure 1B) and partial dissipation of the increase in resting tension (Figure 1C). Statistically significant differences between the values at 10 min reoxygenation after variable periods of hypoxia are marked in Figure 1. The improvement of mechanical performance on reoxygenation was best after 30 min hypoxia and became progressively worse as the period of hypoxia increased. Although reoxygenation after 30 min hypoxia did not cause arrhythmia in any of the muscles ($n = 5$), there was a high incidence of arrhythmias during the first 15 min of reoxygenation after 60 min hypoxia (8 of 10 muscles). There were arrhythmias in 2 of 4 muscles reoxygenated after 90 and 120 min of substrate-free hypoxia.

We investigated 16 additional reoxygenated preparations displaying arrhythmias after 90–120 min hypoxia by (a) recording the electrical activity continuously by an electrode at a site chosen before the hypoxia-reoxygenation regimen ($n = 4$), (b) mapping the muscle surface to search for "uncommon" electrical activity during reoxygenation by an electrode ($n = 6$; Figure 2), and (c) imposing short trains of stimuli at BCLs shorter than the standard 1,000 msec ($n = 6$; Figure 3).

Ten of these 16 reoxygenated muscles displayed spontaneous activities between 2 and 16 min post-reoxygenation. The extrasystolic action potentials often arose on the humps of delayed afterdepolarizations, and all action potentials were accompanied by contractile twitches. This pattern was the same as that observed in muscles reoxygenated after 60 min of hypoxia.[8]

In the other 6 muscles, we observed various degrees of electrical heterogeneity. When the muscle surface was mapped with an electrode during reoxygenation after 120 min of substrate-free hypoxia, there were disparate action potential configurations, as shown in Figure 2. Shortly after reoxygenation, the action potential at the initial microelectrode site was shortened but still had a near normal configuration (Figure 2A). Four min later, at a site less than 1 mm away, the resting potential was depolarized by about 10 mV, and the action

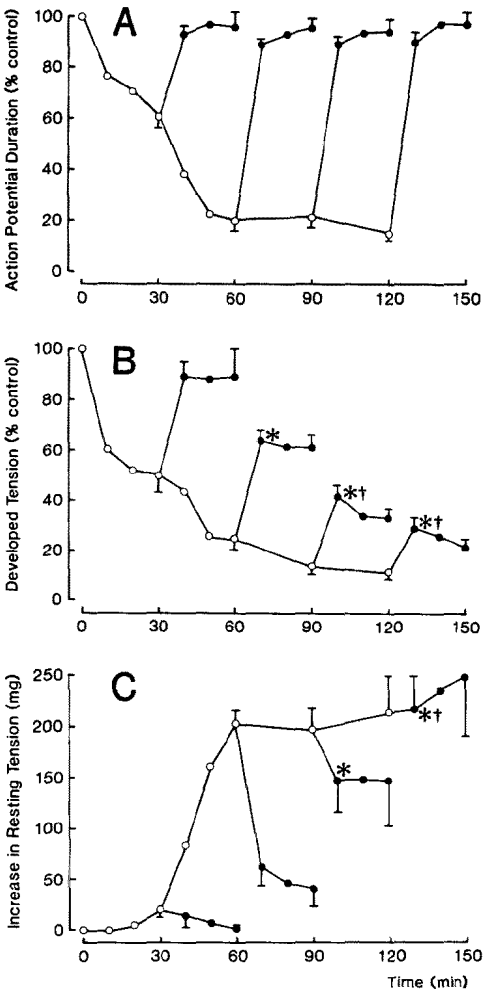

Figure 1. The recovery of electrical and contractile activity in 23 guinea pig papillary muscles reoxygenated after 30 ($n = 5$), 60 ($n = 10$), 90 ($n = 4$) and 120 ($n = 4$) min of substrate-free hypoxia. (A) Action potential duration (% pre-hypoxia control). (B) Developed tension (% control). (C) Increase in resting tension (mg above the pre-hypoxia value of 75–125 mg). Mean ± SE; stimulation at BCL 1,000 msec. *$p < 0.01$ vs the values at 10 min after 30 min hypoxia, and †$p < 0.01$ vs the values at 10 min after 60 min hypoxia, using ANOVA and a post-hoc test.

potentials had a reduced amplitude and triangular configuration. Damped oscillatory activity followed each stimulated event and extrasystoles generated elsewhere were conducted to the site.

The electrical activity at the next 3 sites was near normal for this stage of reoxygenation (6–14 min), and by 15 min both electrical and mechanical records suggested that arrhythmic activity had ceased (not shown). However, a new impalement shortly afterwards indicated otherwise (Figure 2C). At this site, slow

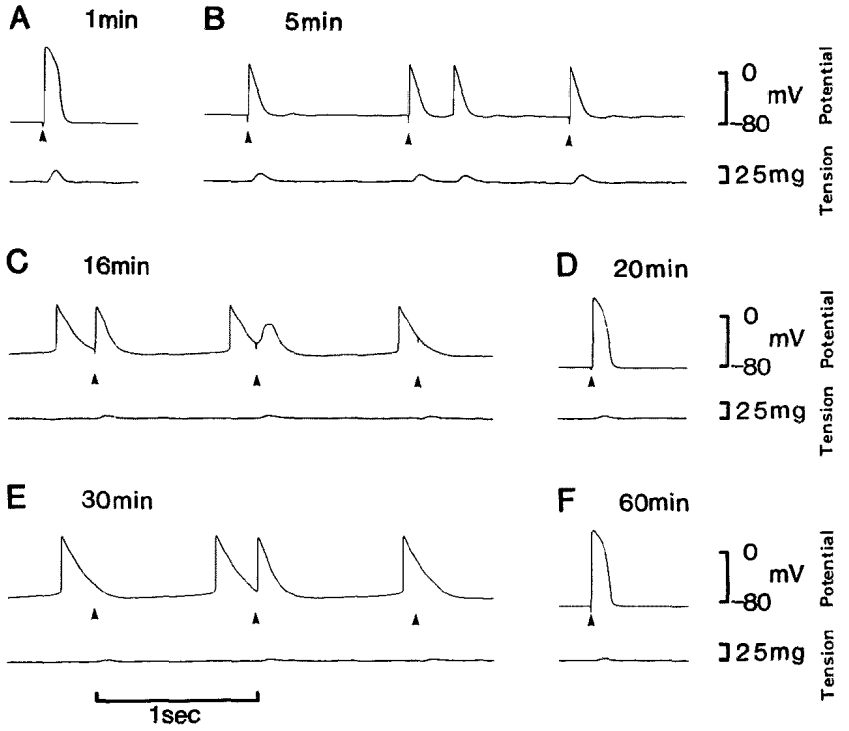

Figure 2. Heterogeneous action potential configurations in a guinea pig papillary muscle reoxygenated after 120 min of substrate-free hypoxia. Arrowheads mark the timing of the stimuli, and the muscle surface was mapped with an electrode. (A) Near normal configuration during early recovery from hypoxia. (B) Depolarization, afterdepolarizations and arrhythmias recorded from a different site after 5 min reoxygenation. (C) Spontaneous action potentials with phase 4 depolarization at a different site after 16 min reoxygenation. Note slow terminal repolarization of the spontaneous action potentials, electrical refractoriness of the microelectrode site when stimulus arrived during the spontaneous action potential and detectable twitches in response to each applied stimulus but not in response to spontaneous action potentials. (D) Action potential of near normal configuration from another impalement after 20 min reoxygenation. (E) Responses similar to those in C at a different site after 30 min reoxygenation. (F) An action potential of near normal configuration at a different site after 60 min reoxygenation. All records were obtained during the constant stimulation at BCL 1,000 msec.

phase 4 depolarization gave rise to action potentials that generally occurred a few hundred milliseconds prior to the applied stimulus (BCL 1,000 msec). The spontaneous action potentials were triangular in shape, had an extremely slow rate of terminal repolarization, and were not accompanied by detectable twitches. The applied stimuli always triggered twitches but not necessarily action potentials at the recording site. The reason for the electrical refractoriness lay in the timing of the stimuli relative to the spontaneous action potentials. As shown in Figure 2C, the stimulus response ranged from a full action potential (left), to an aborted

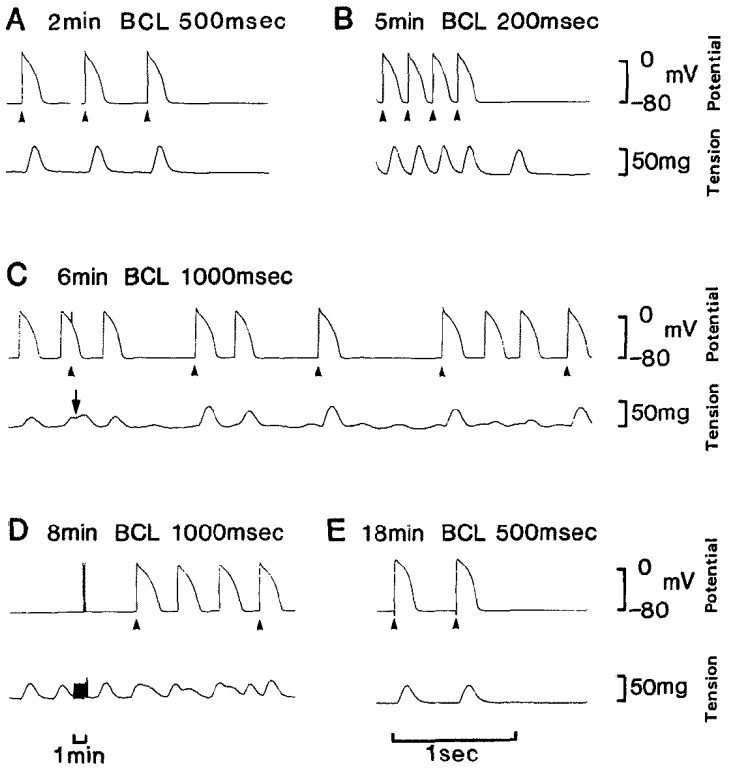

Figure 3. Conduction block in a guinea pig papillary muscle reoxygenated after 90 min substrate-free hypoxia. The muscle was impaled continuously with an electrode. The arrowheads indicate the timing of the stimuli. (A) Partial recovery of the action potential and developed tension after 2 min reoxygenation (BCL 500 msec). (B) Contractile twitch during electrical quiescence at the microelectrode site upon termination of a train of 10 stimuli at BCL 200 msec after 5 min reoxygenation. (C) The record after 6 min reoxygenation (BCL 1,000 msec). Note the extra twitch (downward arrow) that created a fused contractile event during the second action potential of the record. The stimulus (arrowhead) that arrived during the plateau phase of the spontaneous action potential at the microelectrode site must have triggered the extra twitch by exciting another (non-refractory) region of the muscle. (D) Contractile activity during electrical quiescence at the microelectrode site after 8 min reoxygenation (BCL 1,000 msec). (E) Near normal electrical and mechanical activity after apparent cessation of arrhythmic activity at 18 min reoxygenation (BCL 500 msec).

event (middle), to no response at all (right), depending on the timing of the stimulus relative to the upstroke of the spontaneous event.

Examples of records from 2 other sites at later stages in the same experiment are shown in Figure 2D and Figure 2E. At the first site (20 min post-reoxygenation), the electrical activity was near normal. At the next site (30 min post-reoxygenation), we again recorded long, triangular-shaped spontaneous action potentials and phase 4 depolarization. This activity was recorded for the next 25 min. The microelectrode was then moved to a new site about 0.5 mm

away and the experiment terminated after the observation of action potentials having an almost normal appearance (Figure 2F). There was, however, no evidence of disparate action potential configurations when the muscle surface was mapped during reoxygenation after 60 min of substrate-free hypoxia ($n = 6$).

Figure 3 shows a series of records with a continuous electrode at variable BCLs in a muscle that had been superfused with substrate-free hypoxic solution for 90 min. After 2 min reoxygenation, there was partial recovery of the APD and developed tension, and no sign of errant electrical or contractile activity during or after stimulation at a BCL of 500 msec (Figure 3A). This was not the case after 5 min of reoxygenation (Figure 3B) when the stimulation was changed to a BCL of 200 msec. During the BCL 200 msec train, each stimulus elicited an action potential and a contraction. Although cessation of stimulation led to electrical quiescence at the microelectrode site, there was a large spontaneous twitch that peaked about 500 msec after the last stimulated one. This indicates the presence of a conduction block at the microelectrode site. The stimulation rate was then reduced to a BCL of 1,000 msec, and arrhythmic activity was recorded from the microelectrode site over the next 10 min. Clear evidence of a conduction block at the microelectrode site during the early part of this dysfunction is shown in Figure 3C. The first 3 action potentials of the record segment were spontaneous events conducted to the site; each of these was accompanied by a twitch. Since the regular stimulus arrived during the plateau phase of the second action potential, the electrical response was limited to a stimulus artifact spike. Despite the electrical refractoriness at the microelectrode site, the stimulus caused a contraction (downward arrow) which fused with the spontaneous beat. During the next 4 sec, action potential was elicited by each stimulus (arrowhead). However, there was erratic mechanical activity during electrical diastole at the microelectrode site. After 8 min of reoxygenation, conduction block at the microelectrode site during periods of rest and stimulation at a BCL of 1,000 msec was even more striking (Figure 3D). By 18 min post-reoxygenation, there was no further evidence of conduction block or spontaneous activity at any BCL (Figure 3E).

Discussion

There has been a high incidence of arrhythmias upon reoxygenation after 60 min of substrate-free hypoxia. The arrhythmias and the related delayed afterdepolarizations and aftercontractions have been shown to be caused by increased $[Ca^{2+}]i$ and oscillatory Ca^{2+} release from the sarcoplasmic reticulum.[8-10] We have already shown that there were variable amplitudes of delayed afterdepolarizations, showing heterogenous changes upon reoxygenation.[8] A more clear and probably more severe indication of the heterogenous changes

during reoxygenation was shown in this study following longer periods of substrate-free hypoxia. There was a striking difference in the action potential configurations in a muscle shown in Figure 2.

It is possible that the electrical heterogeneity could be due to different levels of oxygenation[11] at different parts of the papillary muscles (interior vs surface). Electrical heterogeneity could be also explained by heterogenous cellular response on reoxygenation. Li et al[12] have reported the heterogenous response of rat myocytes to oxidative repletion, that is, cells with low $[Ca^{2+}]i$ remained in the square configuration with cell aligned striations for 30 min. Other cells, which experienced high $[Ca^{2+}]i$, hypercontracted into a round form soon after restoration of oxidative phosphorylation. They concluded that cell survival during reperfusion depends on previous levels of $[Ca^{2+}]i$. We have also reported that there was a mixed population of myocytes with some showing normal $[Ca^{2+}]i$ and shape, and others showing high $[Ca^{2+}]i$ and contracture, during and after the washout of sodium cyanide.[13]

The dissociation between electrical activity and mechanical activity at the recording site in a muscle which was shown in Figure 2 and Figure 3, indicates that there was conduction block around the microelectrode site. The lower degree of recovery of developed tension after reoxygenation following longer periods of hypoxia could be due to the lower degree of recovery of mitochondrial function and ATP production.[14] However, the lower degree of recovery of developed tension and resting tension has been also related to the Ca^{2+} uptake upon reoxygenation.[15] Smith and Allen[16] reported that $[Ca^{2+}]i$ continued to rise and the muscle failed to recover if $[Ca^{2+}]i$ exceeds 2.5 μM when oxidative metabolism is restarted. Since the lesser degree of recovery and even the increase in resting tension following longer hypoxia in this study (Figure 1) suggest Ca^{2+} accumulation, it may be possible that conduction block could be related to cell-to-cell uncoupling due to the increased $[Ca^{2+}]i$.[17-19]

In summary, there was a high incidence of triggered activity due to delayed afterdepolarizations shortly after reoxygenation, which are likely to be caused by increased $[Ca^{2+}]i$. We have shown that there was heterogeneity in the electrical activity during reoxygenation after prolonged hypoxia, shown by variable action potential durations and configurations. There was also conduction block around the microelectrode site in the muscle. It is suggested that electrical heterogeneity (especially dispersion of action potential duration) and conduction block may play a role in reentrant arrhythmia shown in larger preparations of reperfusion.[20] It is, therefore, likely that reentry and triggered activity due to delayed afterdepolarizations could be involved in the genesis of arrhythmias on reperfusion.

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