Effect of ischemia-reperfusion on the post-rest inotropy of isolated perfused rat heart

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Abstract

This paper aims to study the effect of ischemia-reperfusion on the post-rest inotropy and to characterize post-rest B1:B2 ratio as an index of intracellular Ca^{2+} overload. When the rest interval between the cardiac beats is increased, the magnitude of the post-rest beats is increased. First beat (B1) is maximally potentiated with exponential decline of the second (B2) and subsequent beats, thereby establishing a normal B1:B2 ratio of post-rest inotropy of the cardiac muscle. The rest potentiation of B1 and subsequent decay in the magnitude B2 is thought to develop from the time-dependent changes in the Ca^{2+} -uptake and release from the sarcoplasmic reticulum (SR). Ca^{2+} -kinetics of SR can be modulated by a variety of interventions which produce Ca^{2+} loading of the SR.

Methods: Isolated perfused (K-H buffer, 34°C) rat hearts were paced at 1 Hz (steady state frequency). Interbeat intervals between 1s and 10s were introduced and the recovery in the left ventricular contractile force (Pmax) of post-rest B1 and B2 for each interval was recorded. Their relative relationship was computed and compared under control and experimental conditions.

Results: High extracellular Ca^{2+} (2.50 to 7.0 mM) or low extracellular Na^+ (50% of control), and ischemia (60 min, 34°C) – reperfusion (30 min, 34°C) caused the reversal of the control relationship of the B1 to B2, with B2 being more potentiated than B1, accompanied by the appearance of after-contractions during the rest intervals of 4s or more. The mean ($\pm$ SE) control B1:B2 ratio (at 4s interval) of 1.12 ± 0.05 was significantly ($P < 0.001$) reduced to 0.93 ± 0.07 ; 0.89 ± 0.01 ; and 0.96 ± 0.02 after high Ca^{2+} (6 mM) perfusion, low Na^+ (50%) perfusion and ischemia-reperfusion respectively. Simultaneous perfusion with ryanodine (1 μM) abolished the after-contractions and significantly increased the reduced ratios. The time course of changes in B1:B2 ratio after graded ischemia-reperfusion showed a significant fall in the ratio between 30 and 60 min of ischemia. A parallel change in Pmax and a significant rise in the left ventricular end-diastolic pressure, indicating an irreversible phase of the injury was recorded. No significant changes in B1:B2 ratio were detected during the reversible phase (< 30 min) of the ischemia-reperfusion injury.

Conclusions: Ischemia-reperfusion induces significant alterations in the relative ratio of the post-rest contractions of the left ventricle in isolated perfused rat heart. The altered ratios were characterized to predict the irreversibility of the reperfusion injury and to index the extent of Ca^{2+} -loading of the sarcoplasmic reticulum.

Keywords: after-contractions • Ca^{2+} -overload • interval-force relationship • irreversible ischemia • post-rest contractions • ryanodine

Introduction

Post-rest inotropy in cardiac muscle has previously been studied in isolated cardiac tissue and in isolated

perfused rat and rabbit hearts [1-10]. This inotropy manifests itself when the rest interval between the beats is increased to more than the steady state interval. Under control experimental conditions, there is an increase in the magnitude of the developed force of the post-rest beats with the first post-rest beat being maximally potentiated. Thereafter the post-rest poten-

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Table 1. Post-rest inotropy of left ventricle of perfused rat heart under control and experimental conditions

PROTOCOL	B1:B2 RATIO	
	CONTROL	EXPERIMENTAL
HIGH Ca^{2+} PERFUSION		
$[\text{Ca}^{2+}]_o$, 4 mM (n = 6)	1.12 ± 0.05	1.02 ± 0.05
$[\text{Ca}^{2+}]_o$, 4 mM (n = 6)	1.12 ± 0.05	$0.93 \pm 0.07^{***}$
LOW Na^+ PERFUSION		
$[\text{Na}^+]_o$ = 50% (n = 10)	1.09 ± 0.01	$0.89 \pm 0.01^*$
ISCHEMIA-REPERFUSION		
45 min (n = 6)	1.14 ± 0.03	$1.06 \pm 0.02^*$
60 min (n = 6)	1.10 ± 0.02	$0.96 \pm 0.02^{**}$

Mean $\pm$ SE of the data was computed at the rest interval of 4 s for each protocol of study. Control values were obtained with $[\text{Ca}^{2+}]_o = 2.50$ mM, and $[\text{Na}^+]_o = 100\%$. *p < 0.05, ** p < 0.01, ***p < 0.001 with respective controls.

tiation declines exponentially to the steady state level [11-14]. The exponential decay in the magnitude of the post-rest potentiated beats reflects the time-dependent efflux of Ca^{2+} from the sarcoplasmic reticulum (SR) [4, 6]. Sutko *et al.* [6] have studied the effect of interventions retarding the efflux of Ca^{2+} on the post-rest inotropy in the rabbit papillary muscle. Alterations in the transmembrane distribution of Na^+ or Ca^{2+} enhanced the contractile force of post-rest beats after variable rest periods from 0.25-10 min. By halving the extracellular Na^+ , but by maintaining a constant $[\text{Ca}^{2+}]$ to $[\text{Na}^+]$ ratio, or by increasing extracellular Ca^{2+} , Sutko *et al.* [6] reported additional potentiation of post-rest contractions with no observed changes in the ratio of the first two post-rest beats of the rabbit papillary muscle. The present study describes the altered potentiation of the post-rest contractions and the changes observed in the relative ratio of the first two post-rest beats by interventions causing Ca^{2+} loading of the left ventricle in isolated perfused rat heart exposed to shorter rest periods of 1 s to 10 s. The effects of ischemia-reperfusion on post-rest inotrophy were also investigated.

Animals and methods

Normal Wistar rats of both sexes and weighting 350-400 g were lightly anesthetized with ether-oxygen. Heparin (400 u) was injected intravenously and hearts were rapidly excised and mounted on the perfusion apparatus for retrograde aortic perfusion with oxygenated (95% O_2 + 5% CO_2) Krebs-Henseleit buffer (K-H, 34°C) with the following composition (mM): Na^+ 137, K^+ 5.5, Ca^{2+} 2.5, Mg^{2+} , HCO_3^- 20, glucose 12, pH 7.35. Hearts were made quiescent by cutting the atria and destruction of the atrioventricular node. A pair of stainless steel electrodes were attached to the free wall of the right ventricle and hearts were stimulated at a desired frequency by a programmable stimulator. Under basal conditions hearts were paced at 1 Hz. An isovolumic latex balloon was inserted and secured into the cavity of the left ventricle. A short polyethylene tube connected the balloon to a pressure transducer wired to a direct writing ink-jet mingograph (Siemens Elema, AB, Sweden). The balloon volume was adjusted to give an initial control left ventricular end-diastolic pressure of 5 mm Hg.

Solutions

High Ca^{2+} concentration solutions were prepared by the addition of appropriate amounts of calcium chloride to the K-H buffer. Low Na^+ K-H buffer was prepared by 50% reduction in the Na^+ concentration and replacing it with equiosmolar sucrose. Ryanodine was added to these solutions, when indicated, in 1 μM concentration.

Global ischemia was produced by complete stoppage of the aortic root perfusion, for periods ranging from 15 min to 60 min. During the ischemic arrest heart temperature (monitored by septal needle thermister probe), was kept constant at 34°C. All ischemic hearts were reperfused with oxygenated K-H solution at 34°C for 15 min and the recovery in contractile force was recorded.

Protocol

The quiescent hearts were stimulated by an unidirectional square wave pulse of 1 ms duration and with an amplitude of 150% of the threshold value for excitation. The pulses were delivered from a programmable stimulator at constant frequency. The interval between pulses could be varied, in one ms steps from 1 s to 10 s, with a variable current intensity of 0-20 mA and a constant current output. The steady state frequency for all the protocols was kept constant at 1 Hz. At selected time, the rest interval was increased in 500 ms steps, from 1 s to 10 s, and one rest interval was introduced for each step. Steady state (SS) values were ensured before entering a new interval. Left ventricular maximum developed pressure (Pmax, mm Hg) was used as the index of contractility. Pmax was recorded for each steady state control beat (SSC), first post-interval beat (B1) and second post-interval beat (B2). (Fig. 1, Control panel). The relative relationship of post-rest B1 to B2 was computed for each rest interval.

The data are expressed as mean $\pm$ SE and were analysed statistically by using SPSS XTM package (V6.0.1, Windows, SPSS Inc., Chicago, Ill.). Paired data were using Student's t test.

Results

Control B1:B2 ratio

A representative control record of the relationship of the first two post-rest beats, B1 and B2, for the left ventricle of the isolated perfused heart

Fig. 1. Representative records of left ventricular pressure (LVP) obtained from left ventricle of the isolated perfused rat heart, illustrating the post-rest inotropy under control and experimental conditions. Records were obtained at the rest interval of 4 s and at the steady state priming frequency of 1 Hz. Note the reversal of the control B1:B2 relationship after high Ca^{2+} (6mM) perfusion, low Na^+ (50%) perfusion and after ischemia-reperfusion. Simultaneous perfusion with ryanodine (1 μM) restored the altered relationship to the control level.

SSC, Steady state control

SS, Steady state after experimental intervention.

B1, First post-rest beat.

B2, Second post-rest beat.

Isch. Rep, Ischemia (60 min, 34°C) – reperfusion (15 min, 34°C).

Ryan., ryanodine.

(obtained at a steady state priming frequency of 1 Hz) is shown in Fig. 1. The B1:B2 ratio increased with the increase in the rest interval from 1 s to 3 s, when it reached the mean ($\pm$ SE, $n = 28$) value of 1.13 ± 0.05 (Fig. 2). Thereafter it established a plateau, up to the rest interval of

Fig. 2 Post-rest B1:B2 ratios for the left ventricle of the isolated perfused rat heart obtained at variable rest intervals of 1 s to 10 s. Note significant depression of the ratios at all the rest intervals after high Ca^{2+} (4 mM) perfusion, low Na^+ (50%) perfusion after ischemia (60 min) – reperfusion. Each point represents mean $\pm$ SE for at least 6 measurements. Control and experimental hearts were perfused with K-H buffer containing $[\text{Ca}^{2+}]_o = 2.5$ mM.

Fig. 3 Effect of increasing steady state priming frequency from 1 Hz to 3.3 Hz on the left ventricular Pmax and post-rest (2 s rest interval) B1:B2 ratios of isolated rat heart perfused with K-H buffer containing $[\text{Ca}^{2+}]_o 2.5$ mM. Each data point represents mean $\pm$ SE for at least 6 measurements.

** $P < 0.01$, *** $P < 0.001$ compared with 1 Hz frequency.

6 s, when it showed a slight decline and stabilized at a value of 1.09 ± 0.04 for the rest intervals of 7 s and 10 s. The mean ($\pm$ SE) control value at the rest intervals of 4 s and 6 s (used for comparison of the results of different protocols of this study) was 1.12 ± 0.05 and 1.12 ± 0.04 , respectively. These results indicate that under control conditions, the rest interval induced optimal potentiation of the B1 in the rat heart occurs within a narrow range of increase in the rest interval from 1 s to 3 s.

Change in the steady state priming frequency from 1 Hz to 3.3 Hz, significantly increased the B1:B2 ratio. The mean ($\pm$ SE) ratio of 1.12 ± 0.04 at 1 Hz significantly ($P < 0.01$) increased to 2.48 ± 0.35 at the steady state priming frequency of 3.3 Hz (Fig. 3). Steady state priming frequency of 3.3 Hz corresponds to the average spontaneous frequency of the rat heart at 34°C (under the experimental conditions of this study). This considerable rest-induced potentiation of B1 was accompanied by a negative inotropic effect of increase in the stimulation frequency on the rat heart (Fig. 3).

Effect of positive inotropic interventions

Effect of various positive inotropic interventions on the post-rest inotropy of left ventricle of the perfused rat heart was evaluated by: (i) a graded increase in $[Ca^{2+}]_o$ from 1 mM to 8 mM; (ii) reducing $[Na^+]_o$ by 10% to 50%. With control perfusion ($[Ca^{2+}]_o = 2.5$ mM), when the rest interval between the beats was increased and was more (4 s) than the steady state interval (1 s), the contractile force (P_{max}) of the post-rest beats B1 and B2 increased; B1 was more potentiated than B2, thereby establishing the normal B1:B2 ratio (Fig. 1). However, this normal post-rest B1:B2 relationship was reversed when hearts were perfused with higher Ca^{2+} concentration of 4 mM and above, and B2 showed more potentiation than B1 (Fig. 1). The B1:B2 ratio declined and was negatively correlated ($r^2 = 0.97$) with graded increase in $[Ca^{2+}]_o$ from 2.5 mM to 8.0 mM (Fig. 4). The mean ($\pm$ SE) control ratio of 1.12 ± 0.05 at the rest interval of 4s, was significantly reduced to 1.02 ± 0.05 and 0.93 ± 0.07 with $[Ca^{2+}]_o$ of 4 mM and 6 mM respectively (Table 1).

Fig. 4 Effect of $[Ca^{2+}]_o$ on the post-rest (4 s rest interval) B1:B2 ratios of the left ventricle of isolated perfused rat heart. A highly significant inverse relationship ($r^2 = 0.97$) is evident after graded increase in $[Ca^{2+}]_o$ from 1 mM to 7 mM. Each data point represents mean $\pm$ SE for at least 6 measurements.

Graded reduction in $[Na^+]_o$ also caused a significant depression in the B1:B2 ratios for all rest intervals and this effect was most prominent with $[Na^+]_o$ of 50% of the control (Fig. 2). Identical to high $[Ca^{2+}]_o$ perfusion, control post-rest B1:B2 relationship was reversed by low $[Na^+]_o$ perfusion (Fig. 1). In addition, during the prolonged interval between the beats, after-contractions were observed (Fig. 5). These after-contractions are thought to result from the oscillatory release of Ca^{2+} from the Ca^{2+} -loaded SR, during the prolonged rest interval [15]. Ryanodine (1 μ M) perfusion selectively abolished these after-contraction (Fig. 5). This effect was evident after 2 min of perfusion with ryanodine. Whereas left ventricular developed pressure (P_{max}) showed a progressive rise with graded reduction in $[Na^+]_o$, B1:B2 ratio declined with 20% to 50% reduction in $[Na^+]_o$ (Fig. 6). Significant fall in the B1:B2 ratio indicates the inotropic status, and hence the

Fig. 5 Representative original records of left ventricular pressure (LVP) showing the diastolic oscillations in the left ventricular pressure occurring during prolonged rest intervals (6s) after low Na^+ perfusion (Panel A), and after ischemia (60 min, 34°C) – reperfusion (Panel B). Note the diastolic oscillations in the LVP representing left ventricular after-contractions occurring almost immediately at the onset of the rest interval. These after-contractions were selectively abolished after simultaneous perfusion with ryanodine ($1\ \mu\text{M}$) for 2 min.

Fig. 6 Dose-response curves relating left ventricular post-rest B1:B2 ratios at 4 s and 6 s rest intervals, end-diastolic pressure (LVEDP) and P_{max} to $[\text{Na}^+]_o$. Each data point represents mean $\pm$ SE for at least 6 measurements.

extent of Ca^{2+} loading of the left ventricle. No significant changes in left ventricular end-diastolic pressure were observed during experiments. The mean ($\pm$ SE) control B1:B2 ratio of 1.09 ± 0.01 at 4s interval was significantly ($P < 0.001$) reduced to 0.89 ± 0.01 , after 50% $[\text{Na}^+]_o$ perfusion. Ryanodine ($1\ \mu\text{M}$) perfusion significantly ($P < 0.001$) improved this ratio, to 1.03 ± 0.01 (Table 1, Fig. 7).

Effect of ischemia-reperfusion

Similarly to high Ca^{2+} and low Na^+ perfusions, ischemia-reperfusion (34°C , 60 min.) significantly reduced the post-rest B1:B2 ratios, at the rest intervals between 2 s and 10 s (Fig. 2). Control relationship of the first two post-rest beats was reversed (Fig. 1) and diastolic oscillations in the left ventricular pressure were observed after 60 min of ischemia-reperfusion (Fig. 5). These after-contractions were abolished by ryanodine ($1\ \mu\text{M}$)

Fig. 7 Effect of simultaneous perfusion with ryanodine (1 μ M) on post-rest (4s) B1:B2 ratios after low Na⁺ (50%) perfusion and after ischemia (60min, 34°C)-reperfusion. Note significant increase in the B1:B2 ratios after ryanodine perfusion. Each bar represents mean $\pm$ SE for at least 10 measurements.

*P<0.05 compared with control.

**P<0.05 compared with 50% Na

***P<0.05 compared with ischemia-reperfusion.

perfusion, indicating that they resulted from the oscillatory diastolic release of Ca²⁺ from the Ca²⁺-loaded SR as a result of the ischemia-reperfusion insult. Ryanodine also prevented the decline in the B1:B2 ratio after ischemia-reperfusion and restored this relative relationship to near normal (Fig. 7). The mean ($\pm$ SE) ratio of 0.96 ± 0.02 after ischemia-reperfusion significantly ($P<0.01$) increased to 1.03 ± 0.02 after ryanodine perfusion (Fig. 7). Time-course of changes in B1:B2 ratios, LV Pmax and LVEDP after graded global ischemia-reperfusion are shown in Fig. 8. During the first 30 min of ischemia-reperfusion, the Pmax declined with no significant change in the B1:B2 ratios. However, in the next 30 minutes of ischemia, there was a dramatic fall in the Pmax and the B1:B2 ratios

Fig. 8 Time course of changes in left ventricular post-rest (4 s) B1:B2 ratio, Pmax and end-diastolic pressure (LVEDP) following graded ischemia-reperfusion (34°C). Note the precipitous fall in the B1:B2 ratio and Pmax and a considerable rise in LVEDP at and after 30 min ischemic time. Each data point represents the mean $\pm$ SE for at least 6 measurements.

* P<0.05, ** P<0.01, ***P<0.001 compared with 0 time.

and a significant rise in the LVEDP. The mean ($\pm$ SE) B1:B2 ratios were significantly reduced to 1.06 ± 0.02 and, respectively 0.96 ± 0.02 for the protocols of ischemia-reperfusion time of 45 min and 60 min (Table 1). These results indicate that post-ischemic negative inotropy occurred in the presence of a significant intracellular Ca^{2+} loading as indicated by the peak reduction in the B1:B2 ratios.

Discussion

Rest inotropy in the cardiac muscle is thought to develop as a result of: time dependent increase in Ca^{2+} uptake and release from the SR [5, 6]; reduced Ca^{2+} -efflux during the rest intervals [6]; increased Ca^{2+} -entry during depolarization [4, 9, 16]; and, recently, from more significant end-diastolic binding of Ca^{2+} to sites on troponin C [10]. The decay in the magnitude of first post-rest contraction is reported to reflect the time-dependent loss of Ca^{2+} from the intracellular stores. Because of the relative efficacy of Ca^{2+} -efflux mechanisms during post-rest inotropy, the second post-rest beat (B2) is less potentiated as compared to the first post-rest beat (B1), thereby establishing a normal B1:B2 ratio. Steady state contractile force in the rat myocardium is determined mainly by the SR Ca^{2+} release. The contractile force of the post-rest B1 is relatively high, due to enhanced restitution during the prolonged rest interval, in the rat myocardium [5]. Furthermore, the post-rest decay in contractile force in rat myocardium is slower due to less efficient Ca^{2+} -efflux. Slower rest decay, therefore, accounts for the relatively lower B1:B2 ratio in the rat as compared to other species, notably the rabbit [5, 6]. However, this difference was abolished when the steady state priming frequency of rat ventricle was increased from the base-line 1 Hz to 3.3 Hz (Fig. 3). The mean ($\pm$ SE) B1:B2 ratio was considerably increased indicating enhancement of post-rest inotropy of B1 and a faster decay in the contractile force of B2, possibly due to more efficient Ca^{2+} -efflux. Although the post-rest inotropy of B1 was enhanced, the steady state contractile force (P_{max}) declined considerably in the rat left ventricle at high rates of stimulation (Fig. 3). Reduced

time for restitution of steady state force of contraction at higher frequencies [9, 17, 18] may be responsible.

The presented results revealed three inter-related Ca^{2+} -dependent events occurring after high $[\text{Ca}^{2+}]_o$, low $[\text{Na}^+]_o$ perfusions or ischemia-reperfusion protocols: (i) alterations in the size of the first two post-rest beats; (ii) reversal of the control B1:B2 ratio; and (iii) occurrence of after-contractions. These changes can be attributed to the increase of intracellular Ca^{2+} as a result of: high $[\text{Ca}^{2+}]_o$ [19], low $[\text{Na}^+]_o$ [6, 9, 20] perfusions or ischemia-reperfusion [20-23]. As the muscle becomes Ca^{2+} overloaded, oscillatory release of Ca^{2+} from SR occurs during the prolonged rest intervals. It has been implicated in the genesis of after-contractions and enhanced diastolic myofilament motion [15, 24, 25]. Diastolic oscillatory loss of Ca^{2+} from SR also limited the potentiation of the first post-rest beat, B1 [24, 25], and hence produced the observed alterations in the B1:B2 ratio. Ryanodine abolished the occurrence of after-contractions and increased the B1:B2 ratio confirming SR as the source of these perturbations in the Ca^{2+} -loaded heart. Ryanodine is known to inhibit both Ca^{2+} -induced- Ca^{2+} release, as well as spontaneous release of Ca^{2+} from the SR [26].

Time course of changes in the B1:B2 ratio after ischemia-reperfusion indicate that major changes in the intracellular Ca^{2+} homeostasis, occurred after 30 min of ischemia (or more) (Fig. 8). Longer periods of ischemia are known to increase free cytosolic Ca^{2+} by stimulating spontaneous release of Ca^{2+} from the Ca^{2+} -loaded SR, which is maintained during the reperfusion period [23]. Left ventricular contracture development, as indicated by a considerable rise in the LVEDP, (Fig. 8) is another manifestation of the same phenomenon. During the first 30 min of ischemia, no significant changes in the B1:B2 ratio were observed. However, a significant decrease in P_{max} occurred during this period, which was possibly due to loss of energy [27] rather than disturbances in the intracellular Ca^{2+} homeostasis. During "no-flow" ischemia of less than 30 min, no spontaneous release of Ca^{2+} from SR has been demonstrated, indicating that SR is not loaded with Ca^{2+} during this period [22, 23]. This phase of ischemia-reperfusion has been characterized as

the reversible phase [27]. The irreversible phase of ischemia-reperfusion occurs after a “no-flow” period of 30 min or more, when Ca^{2+} -loading of the SR and spontaneous release of Ca^{2+} from the SR have been demonstrated [22, 23]. A significant decrease in the B1:B2 ratios, occurring at (or after) 30 min of “no flow” ischemia, may indicate the transition from the reversible to the irreversible phase of the injury. B1:B2 ratio recorded after the rest intervals of 4s or more may, therefore, serve as an index of the Ca^{2+} loading of the SR during ischemia-reperfusion.

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