

Low Molecular Weight Iron and the Oxygen Paradox in Isolated Rat Hearts

Arthur Voogd, Wim Sluiter, Henk G. van Eijk,* and Johan F. Koster

Departments of Biochemistry and *Chemical Pathology, Medical Faculty, Erasmus University Rotterdam, 3000 DR Rotterdam, The Netherlands

Abstract

Little is known about changes in the amount of iron in the intracellular low molecular weight pool, which catalyzes the Fenton reactions during reperfusion after ischemia. In this study a new approach is presented to measure low molecular weight iron and it is applied to normal hearts during ischemia and to iron-loaded hearts during anoxia and reoxygenation. The results of this study show that (a) during ischemia in normal hearts a progressive 30-fold increase occurs in low molecular weight iron after 45 min of ischemia, whereas (b) during 45 min of anoxic perfusion the low molecular weight iron does not increase. This means that the reductive release from the storage protein ferritin is greatly enhanced by the acidification that occurs during ischemia. (c) Anoxic perfusion of iron-loaded hearts does increase low molecular weight iron and there is a further increase upon reoxygenation, which is prevented by (+)-cyanidanol-3. Based on these findings it is concluded that oxygen deprivation enhances the susceptibility of rat hearts to oxygen radicals by increasing the amount of catalytic, ferrous iron in the low molecular weight pool. (*J. Clin. Invest.* 1992;90:2050–2055.) Key words: oxygen free radicals • deferoxamine • ischemia • anoxia • reperfusion

Introduction

The generation of oxygen-centered free radicals is thought to contribute to the exacerbation of tissue injury upon restoration of the oxygen supply to ischemic organs (1, 2). This hypothesis is based on protective effects of the addition of scavengers of superoxide, hydrogen peroxide, and the hydroxyl radical (3–7). Direct measurements of the radical adducts of spintrap agents have since confirmed that these radicals are generated (8, 9). However, neither superoxide nor hydrogen peroxide are very toxic in themselves, but in the presence of a transition metal, such as iron or copper, the very toxic hydroxyl radical or ferryl ion is formed (10). The significance of iron to ischemia and reperfusion is supported by studies in which iron chelation protects postischemic tissue (11–13). In addition to the circumstantial evidence on the basis of iron chelation, we have shown that the hearts from iron-loaded rats are very sensitive to reoxygenation after a mild anoxic insult (14).

Address correspondence and reprint requests to Dr. A. Voogd, Department of Biochemistry I, Medical Faculty, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

Received for publication 27 January 1992 and in revised form 1 June 1992.

J. Clin. Invest.

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0021-9738/92/11/2050/06 \$2.00

Volume 90, November 1992, 2050–2055

Under normal conditions only a minute quantity of the intracellular iron is present in a low molecular weight pool. This iron is bound to weak chelators such as AMP and ATP (15) and in this form iron is able to catalyze hydroxyl radical formation (16, 17). The bulk of the intracellular iron exists as $\text{Fe}(\text{OH})_3$ stored in ferritin and as heme iron in a variety of proteins. This iron is not available to catalyze hydroxyl radical formation. It has been shown that superoxide can mobilize iron from ferritin and that this iron can catalyze hydroxyl radical formation (18, 19). In addition, other reducing agents such as reduced flavins can cause the reductive release of iron from ferritin (20, 21). This makes ferritin a potentially hazardous biomolecule under pathological conditions when sufficient reducing equivalents are available. It is therefore important to investigate whether there is a rise in low molecular weight iron during ischemia or anoxia and after reoxygenation to appreciate the contribution to tissue injury under these conditions.

There are reports in which an increase in low molecular weight iron has been found during ischemia. However, both Holt et al. (22) and Komara et al. (23) neglected the possibility that iron was released from ferritin during their procedures to isolate the low molecular weight pool. Healing et al. (24) realized the possibility but argue that since the iron they measure is chelatable, it must have been loosely bound and therefore potentially catalytic.

The purpose of this study is to present an alternative procedure to measure low molecular weight iron that circumvents the drawbacks mentioned above. Using this method, we have investigated the changes in the low molecular weight iron pool during no-flow ischemia, which causes a profound acidosis ($\text{pH} < 6$), and during anoxic perfusion where the cellular pH remains constant. In addition we have extended this study to hearts from iron-loaded rats. These hearts, which contain twice as much total iron as control hearts, were shown to be very sensitive to anoxia and reoxygenation (14). The iron in these hearts is predominantly located in the endothelium but the dramatic loss of contractility that occurs could not be correlated with morphological indications of injury in endothelial cells (Voogd, A., A. M. M. van der Kraaij, H. G. van Eijk, C. E. Essed, and J. F. Koster, manuscript submitted for publication). To evaluate the role of low molecular weight iron in these hearts we have measured the size of the low molecular weight pool during anoxia and reoxygenation.

Methods

Animals and perfusion protocol

12–14-wk-old male Wistar rats were used. After a brief anesthesia with diethyl ether the hearts were excised and placed in ice-cold tyrode buffer. The excised hearts were cannulated through the aorta and perfused retrogradely according to Langendorff (25). The perfusions were carried out at 37°C and pH 7.4 using a modified Tyrode's buffer containing the following (mM): 128 NaCl, 4.7 KCl, 1.3 CaCl_2 , 20.2 NaHCO_3 , 0.4 NaH_2PO_4 , 1.0 MgCl_2 , and 11 glucose saturated with

95% O₂ and 5% CO₂. Perfusion pressure was held constant at 80 cm H₂O.

Iron overloading

Iron overload in male Wistar rats, 12 wk of age, was achieved by injection of 0.5 ml Imferon (iron-dextran, 50 mg Fe/ml; Fisons Corporation, Leusden, The Netherlands) in the gluteus muscles, after a brief anesthesia with diethyl ether, once a week for a period of 6 wk and by adding a supplement of iron (FeSO₄·7H₂O; 7.5 mg Fe/g standard food) to their food during this period (14).

Ischemia and anoxia

Hearts were always perfused with normoxic Tyrode for ≥ 15 min before either ischemia or anoxia was induced. Warm, no-flow ischemia was induced by stopping coronary flow completely while the heart was submerged in Tyrode buffer saturated with 95% N₂ and 5% CO₂ and kept at 37°C. Reperfusion is achieved by restoring coronary flow. Anoxia was caused by perfusing the hearts with tyrode containing glucose (11 mM) saturated with 95% N₂ and 5% CO₂. Reoxygenation is achieved by changing to the normoxic tyrode.

Low molecular weight iron measurements

The hexadentate (26) iron chelator deferoxamine forms a 1:1 complex (HDFX-Fe, $K_{as} = 10^{31}$ or H₂DFX-Fe, $K_{as} = 10^{21}$, [27]) with iron, which is known as ferrioxamine. Therefore there is a linear relation between the ratio ferrioxamine/deferoxamine and original iron concentration after addition of excess deferoxamine to iron-containing solutions or tissue homogenates. The very high stability of the complex ensures that all iron is extracted from the weaker intracellular chelators. In this study iron was measured as the ratio of ferrioxamine to deferoxamine formed in incubations at 37°C of 10,000 g supernatant of heart homogenates with 2 mM deferoxamine as described by Gower et al. (28). In this procedure all the iron in the low molecular weight pool is chelated within 1 h. However, it also leads to the removal of iron from ferritin, which is linear in time over ≥ 2 h (29). To obtain the actual amount of low molecular weight iron we have followed the increase in ferrioxamine in these incubations in time by measuring ferrioxamine/deferoxamine after 5, 30, 60, 90, and 120 min. Next, we have subtracted the linear increase between 60 and 120 min from the 60-min value.

Tissue preparation and ferrioxamine/deferoxamine determination

Immediately after the experiment rat hearts were blotted dry, weighed, and homogenized to a 20% wt/vol homogenate in 100 mM Tris/HCl, pH 7.4. Homogenate was centrifuged at 10,000 g for 15 min and 1-ml samples were taken from the supernatant, was brought to 2 mM deferoxamine by adding 100 μl of 22 mM deferoxamine, and was incubated at 37°C. Incubations were stopped by passing the sample through a cartridge (Sep Pak C18; Millipore Corp., Milford, MA) preeluted with 5 ml methanol followed by 5 ml distilled water, on which ferrioxamine and deferoxamine are retained. After application of the sample the cartridge was washed with 5 ml distilled water and deferoxamine and ferrioxamine were eluted with 2 ml methanol and the eluate was applied to the HPLC column.

HPLC analysis

HPLC was performed with dual LKB 2150 pumps (LKB Instruments, Inc., Gaithersburg, MD), controlled through the LKB 2152 LC controller equipped with automatic sample injector. Effluent from the column was passed through two LKB 2151 variable wavelength detectors to allow simultaneous detection of ferrioxamine at 430 nm and deferoxamine at 229 nm. Pump heads and high pressure tubing are all made of titanium. The stationary phase is a 250 × 4 mm i.d. stainless steel cartridge (model RP18; E. Merck, Darmstadt, Germany). The mobile phase consisted of 88% 20 mM Na₂HPO₄/NaH₂PO₄, 2 mM Na EDTA, 1 M ammonium acetate (pH 6.6), and 12% acetonitrile. All

reagents were of HPLC grade and buffers were filtered and degassed before use. Recovery of deferoxamine and ferrioxamine peaks was measured using standard dilutions in methanol and always exceeded 75%. The relation between the ferrioxamine/deferoxamine ratio and the amount of iron was calculated from standards incubated and extracted as the samples.

Experimental design

Ischemia and reperfusion. The effect of ischemia on low molecular weight iron was determined in hearts that were subjected to warm ischemia for 15, 30, and 45 min. Control hearts were perfused with normoxic buffer for 60 min. To investigate the effect of reperfusion on the low molecular weight iron pool, hearts were subjected to 30 min of ischemia and low molecular weight iron was determined after 20 min of reperfusion. The influence of superoxide or iron chelation on low molecular weight iron during reperfusion was determined in two groups of hearts subjected to 30 min of ischemia and 20 min of reperfusion in the presence of 30 mg/liter SOD or 50 μM deferoxamine.

Anoxia and reoxygenation in iron-loaded rat hearts. To evaluate the effect of anoxia and reoxygenation on the size of the low molecular weight iron pool in iron-loaded hearts, low molecular weight iron was analyzed in normoxic hearts ($n = 3$) and hearts that were perfused with anoxic buffer for 45 min ($n = 3$) and were not reoxygenated or were reoxygenated for 10 min ($n = 3$). To evaluate the effect of iron chelation and antioxidant intervention under those conditions, these experiments were performed with tyrode only or with tyrode containing 50 μM deferoxamine or 20 μM (+)-cyanidanol-3, which has been shown to scavenge both superoxide and the hydroxyl radical (30).

Statistical evaluation

All low molecular weight measurements are expressed as nmol/g wet weight and presented as mean ± SEM. Intergroup differences were evaluated by one-way analysis of variance with the Bonferroni option using STATA release 2 (Computing Resource Center, Los Angeles, CA).

Chemicals

Deferoxamine methanesulfonate (Desferrioxamine mesylate, Desferal) was purchased from Ciba-Geigy (Basel, Switzerland) and (+)-cyanidanol-3 was purchased from Zyma (Nyon, Switzerland). (+)-cyanidanol-3 buffer was protected from light by wrapping the perfusion apparatus in aluminium foil. Bovine erythrocyte Cu-Zn SOD (3,000 U/mg) was purchased from Sigma Chemical Co. (St. Louis, MO).

Results

Low molecular weight iron determinations. The standard curve (Fig. 1) relating the quantity of iron to the ferrioxamine/deferoxamine ratio follows a linear relation with $R^2 = 0.970$ ($P < 0.001$). Contaminating iron from the chemicals used, determined by extrapolation of this curve to ferrioxamine/deferoxamine = 0, was consistently low at 2.1 ± 0.2 nmol/ml ($n = 3$ separate curves). Both slope and background were similar to those presented by Gower et al. (28). To measure the low molecular weight iron pool the 10,000 g supernatant was incubated with deferoxamine, aliquots are drawn at different time points, and the ferrioxamine/deferoxamine ratio was determined. Initially a rapid rise was found that turns into a linear increase after 1 h (Fig. 2). The initial rate of iron chelation was lower in the normoxic hearts than in hearts that had been ischemic for 45 min. After 1 h both curves leveled off to a constant rate of ferrioxamine formation. To evaluate the slope of both curves, regression analysis was performed for the time points 60, 90, and 120 min using time and ischemia (0 or 1) as independent variables. This showed that the data can be described by the linear increases as indicated in Fig. 2. Inclusion of the interaction term (time × ischemia) in the regression analysis

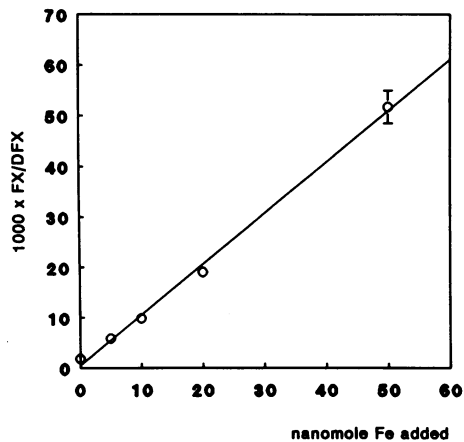


Figure 1. Standard curve. Relationship between the added amount of iron and ferrioxamine/deferioxamine ratio of standard samples extracted and incubated as the experimental samples. Mean \pm SEM of triplicate determinations of one set of samples. Linear regression of these data shows $R^2 = 0.970$, $P < 0.001$. Background iron is calculated by extrapolation to ferrioxamine/deferioxamine = 0.

showed a nonsignificant contribution, indicating that there is no significant difference in the slope of these curves. No attempt was made to perform nonlinear regression on the earlier data points. This result fits the observations that all the iron in the low molecular weight pool is chelated within 1 h (28) and that the release of iron from ferritin is linear over ≥ 2 h (29). Therefore, to obtain the actual low molecular weight iron in individual hearts, we correct for this ferritin-derived iron by subtracting the amount of iron released between 1 and 2 h from the amount measured at 1 h.

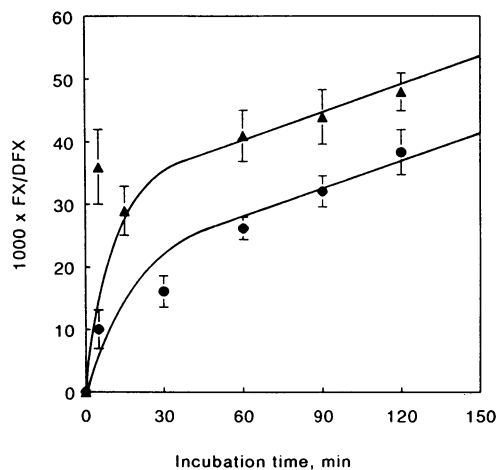


Figure 2. Ferrioxamine formation during incubation of supernatants. Effect of the duration of incubation on the ferrioxamine/deferioxamine ratio of 1 ml supernatant of normoxic rat hearts (circles) or rat hearts subjected to 45 min ischemia (triangles) with 2 mM deferioxamine. Aliquots of 1 ml from supernatants of individual hearts ($n = 3$) were incubated during the times indicated and processed as described under Methods. Regression analysis using time and ischemia (0 or 1) as independent variables results in the equation: ratio = $0.147 \times \text{time} + 12.0 \times \text{ischemia} + 19.4$ ($R^2 = 0.624$). Inclusion of the interaction term time \times ischemia showed a nonsignificant contribution, indicating that there is no significant difference in the slope of both curves. Each point represents the mean \pm SEM.

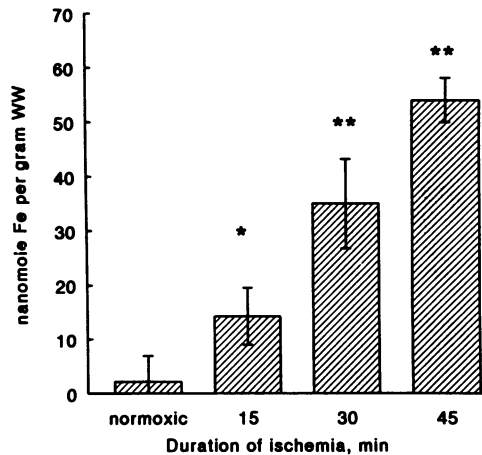


Figure 3. Low molecular weight iron and ischemia. Effect of the duration of warm no-flow ischemia on low molecular weight iron content of rat hearts. Normoxic hearts were perfused with normoxic tyrode for 60 min. Ferrioxamine/deferioxamine ratio was determined in supernatants after 60, 90, and 120 min and low molecular weight iron calculated as described under Methods. Each bar represents mean \pm SEM, $n = 6$. * $P = 0.09$. ** $P < 0.01$ vs. normoxic controls. Linear regression shows a significant increase in time $P < 0.001$, $R^2 = 0.660$.

The effect of ischemia and reperfusion. To evaluate the effect of ischemia on the low molecular weight iron pool, rat hearts were subjected to warm ischemia for 15, 30, and 45 min. The results (Fig. 3) show a progressive increase in low molecular weight iron due to ischemia from 2.1 ± 4.8 nmol/g wet weight in normoxic hearts to 54.2 ± 4.1 nmol/gram wet weight after 45 min of ischemia ($R^2 = 0.660$, $P < 0.001$).

To investigate the effect of reperfusion in the presence or absence of SOD or deferioxamine on the size of the low molecular weight iron pool, hearts were subjected to 30 min of ischemia followed by 20 min of reperfusion. Addition of SOD does not affect low molecular weight iron during reperfusion, whereas iron chelation results in a tremendous decrease in low molecular weight iron (Fig. 4). Both the SOD group and the

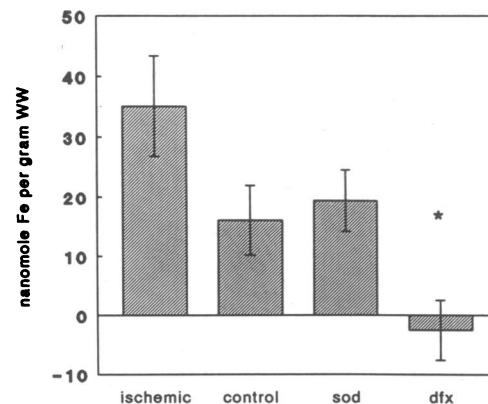


Figure 4. Low molecular weight iron and reperfusion. Effect of 30 min of ischemia (ischemic) and 20 min of reperfusion after 30 min of ischemia on low molecular weight iron in hearts perfused in the absence (control) or in the presence of SOD (sod) or deferioxamine (dfx). Bars represent mean \pm SEM, $n = 6$ for ischemic; $n = 3$ for control, sod, and dfx. * $P < 0.05$ compared with ischemic hearts.

control group were lower than the ischemic group but the difference does not reach significance levels. The chelation of the iron into ferrioxamine probably facilitates the washout from the cells.

The effect of anoxia. Anoxia differs substantially from no-flow ischemia in that, although there is oxygen depletion, the coronary bed is still perfused. This means that there still is a supply of glucose and removal of metabolites and acidosis is prevented. This makes the latter a mild insult (14). To evaluate the effect of anoxia, the size of the low molecular weight iron pool was compared in hearts that were perfused for 45 min under normoxic or anoxic conditions. Anoxic perfusion for 45 min does not lead to an increase in the low molecular weight iron content. (Fig. 5, left).

Earlier observations from our laboratory have shown that normal rat hearts, perfused with anoxic buffer for 45 min, regain 80% of preanoxic contractility upon reoxygenation (14) whereas normal hearts subjected to 15 min of ischemia regain only 40% of preischemic contractility (12) upon reperfusion. From the results described above it is clear that ischemia leads to a tremendous increase in low molecular weight iron and to severe damage, whereas anoxia leads to little or no increase in low molecular weight iron and the consequences are mild. Iron-loaded hearts, however, regain only 20% of pre-anoxic contractility after 45 min of anoxic perfusion (14). Therefore we measured the amount of low molecular weight iron in iron-loaded hearts. Normoxic iron-loaded hearts have a higher low molecular weight iron pool than normal hearts (Fig. 5). In contrast to normal hearts, the size of this low molecular weight pool increases dramatically during anoxia.

Effect of iron chelation and antioxidant protection in iron-loaded hearts. The dramatic loss of function of iron-loaded hearts during anoxia and reoxygenation could be prevented by perfusion with either (+)-cyanidanol or deferoxamine (14). Therefore, the size of the low molecular weight iron pool during anoxia and reoxygenation of iron-loaded rat hearts was evaluated under these conditions in a separate experiment.

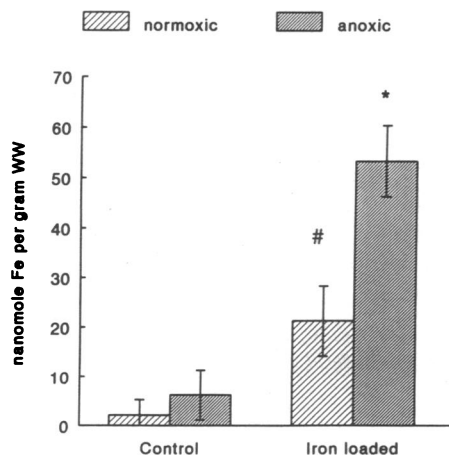


Figure 5. Low molecular weight iron and anoxia. Effect of 45 min of anoxic perfusion on low molecular weight iron of normal (*Control*) and iron-loaded rat hearts compared with hearts that were perfused with normoxic tyrode for 60 min. Each bar represents the mean±SEM. ($n = 6$ in noniron-loaded hearts and $n = 3$ in the iron-loaded hearts. * $P < 0.05$ vs. normoxic value. # $P < 0.05$ vs. noniron loaded).

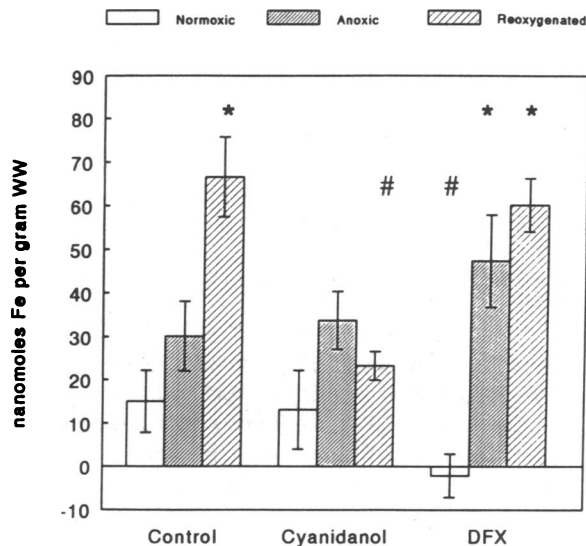


Figure 6. Low molecular weight iron during anoxia and reoxygenation in iron-loaded rat hearts. Effect of 60 min normoxic perfusion (*Normoxic*), 45 min anoxic perfusion (*Anoxic*), and reoxygenation after 45 min anoxia for 10 min (*Reoxygenation*) in the absence (*Control*) or presence of 20 μM (+)-cyanidanol (*Cyanidanol*) or 50 μM deferoxamine (*DFX*). Each bar represents the mean±SEM ($n = 3$). * $P < 0.05$ vs. normoxic hearts in that group. # $P < 0.05$ vs. untreated control.

The size of the low molecular weight iron pool in normoxic iron-loaded rat hearts amounted to 15.4 ± 6.1 nmol/g wet weight (Fig. 6, *Control*). Perfusion with anoxic buffer led to the expected increase of low molecular weight iron. Upon reoxygenation a further increase was found. The low molecular weight pool in iron-loaded hearts is unaffected by normoxic perfusion with 20 μM (+)-cyanidanol-3 for 1 h and during anoxic perfusion the same increase was found as in control hearts (Fig. 6, *Cyanidanol*). However, upon reoxygenation no further increase was found. Normoxic perfusion with 50 μM deferoxamine for 1 h led to a very small low molecular weight iron pool (Fig. 6, *DFX*). This could be due to washout from ferrioxamine from the heart. Anoxic perfusion in the presence of deferoxamine again increased low molecular weight iron, and upon reoxygenation there is a further increase of low molecular weight iron.

Discussion

In the present paper we have presented a method to determine the size of the intracellular low molecular weight iron pool and applied this method to ischemic or anoxic rat hearts. The chelated iron in normoxic rat hearts as measured in 60-min incubations by Gower et al. (28) was 15.44 ± 6.37 nmol/g wet weight. In our hands the 60-min incubations yielded 21.6 ± 3.6 ($n = 6$) nmol chelated iron/g wet weight. After our correction for ferritin-derived iron, the low molecular weight pool contains 2.1 ± 4.8 nmol/g wet weight. This figure means that the low molecular weight pool in normoxic rat hearts is so small that it escapes more exact measurement. However, in hearts that had been ischemic, the biphasic time curve of iron chelation from the supernatant shows a faster initial phase whereas there is no significant difference between normoxic and isch-

emic hearts in the slope after 1 h. Thus correction for this ferritin-derived iron leads to a higher value for the low molecular weight pool.

Our results show that the amount of low molecular weight iron increases dramatically during ischemia from 2.1 ± 4.8 to 54.2 ± 4.1 nmol/g wet weight. Healing et al. (24) have studied the amount of iron in rabbit kidney homogenates after cold ischemia by measuring ferrioxamine/deferioxamine ratios in homogenates in 60-min incubations. It was shown that the amount of iron increases from ~ 15 nmol in nonischemic control to 60 nmol/g tissue after 72 h of cold ischemia. However, these authors did not correct for the iron released from ferritin that occurred during this incubation. Others (22) showed that the low molecular weight iron in ischemic and nonischemic myocardium after a 2-h in vivo coronary artery occlusion in dogs increased from 130.6 to 183.2 nmol/g tissue. In dog brain an increase in low molecular weight iron from 90.5 to 370.4 nmol/g wet wt was found after cerebral ischemia induced by cardiac arrest (23). No measurements of total iron were presented in those studies, but mammalian tissue, except liver and spleen, contains $\sim 1,000$ nmol Fe/g wet weight (20, 31). Recalculation of the above results would imply that in mongrel dog brain and heart 10% of the total iron is in the low molecular weight iron pool under normoxic conditions. This is very high with regard to other estimates in which the low molecular weight iron pools amounts to only 0.2% (28) or 3% (32) of the total iron. Possibly these high values can be explained by the fact that during ultrafiltration the homogenate was not buffered and contained only EDTA, which may have led to acidification. Since the iron release from ferritin depends on reducing equivalents and is enhanced by acidification (20, 21), additional iron release may have occurred during those incubations.

We have shown that ischemia causes a progressive increase in low molecular weight iron whereas during anoxia low molecular weight iron does not increase in noniron-loaded hearts. To release iron from ferritin a reduction to Fe(II) must take place and the ferrous iron must be chelated (20, 21). One possible mechanism is a superoxide-dependent reduction. However, the iron released through this mechanism has been shown not to exceed 1.5 mol Fe/mol ferritin (33). Furthermore, it is unlikely that superoxide is produced during ischemia and we found no significant difference in the amount of low molecular weight iron in hearts subjected to ischemia and reperfusion with or without SOD. Funk et al. (20) studied the reductive mobilization of iron from isolated horse spleen ferritin. Here it was shown that reduced flavin mononucleotide induces the release of 1,200 Fe atoms/ferritin molecule within 3 min at pH 7. At pH 5.1 this was increased to 1,900 atoms Fe. Ischemia causes a drop in intracellular pH to 5.9 (34) and even to 5.7 after 25 min (35). Thus, the reducing equivalents present in ischemic tissue and the acidification will facilitate the reductive release of iron from ferritin. During anoxic perfusion coronary flow is maintained, allowing the supply of glucose and the efflux of metabolites. The metabolic consequence is that acidosis is prevented. Therefore, reducing equivalents will be present during anoxia but no acidification occurs. It has to be concluded that the acidification contributes tremendously to the mobilization of iron from ferritin.

The increment of low molecular weight iron during reoxygenation of iron-loaded hearts must have a mechanism other than the increase due to reducing agents during anoxia. This is

substantiated by our finding that low molecular weight iron in iron-loaded hearts increased during anoxic perfusion in the presence of (+)-cyanidanol-3, whereas the additional increase during reoxygenation was prevented by (+)-cyanidanol (Fig. 6, *Cyanidanol*). This implies that the increase of low molecular weight iron in iron-loaded hearts during reoxygenation is due to superoxide.

Normoxic iron-loaded hearts perfused with deferioxamine for 1 h show a very small low molecular weight pool. This is probably due to a loss of ferrioxamine to the perfusate, which will deplete the low molecular weight pool first. An increase occurs during anoxia because apparently the reductive release of iron exceeds the washout effect. 10 min after reoxygenation low molecular weight iron in these hearts was higher than in the (+)-cyanidanol-3 hearts. Again, this could be caused by superoxide production since this is not prevented by deferioxamine.

Normoxic iron-loaded rat hearts have a higher low molecular weight iron content compared with the noniron-loaded hearts. This is without apparent effect on the physiological parameters such as coronary flow or contractility (14). Due to the presence of oxygen, the iron in the low molecular weight iron pool in normoxic iron-loaded hearts is in the ferric state, in which it will not catalyze the formation of hydroxyl radicals directly. In contrast, the low molecular weight iron in anoxic or ischemic hearts must be ferrous iron. Moreover, we have shown here that the amount of low molecular weight ferrous iron is increased by ischemia or anoxia. This will directly form hydroxyl radicals together with the hydrogen peroxide that arises from the dismutation of superoxide that is generated upon reoxygenation. Indeed, the effect of reoxygenation after the anoxic insult is quite dramatic in the iron-loaded hearts and this could be prevented by perfusion with (+)-cyanidanol-3 (14). Despite the increase in low molecular weight iron, the iron-loaded hearts perfused with deferioxamine recover up to 80% of preanoxic contractility (14), which shows that the low molecular weight iron in these hearts is not in a form that catalyzes the Fenton reaction because it is chelated as ferrioxamine (17).

Taken together our results show that the low molecular weight iron pool increases during oxygen deprivation and that this release is due to the reducing equivalents generated in the absence of oxygen. The drop in pH that occurs in ischemic hearts dramatically increases this reductive iron release. Furthermore, free radicals generated during reoxygenation release additional iron. From these results it can be concluded that ischemia increases the susceptibility to the oxygen radicals generated during reoxygenation by increasing the amount of catalytic, ferrous iron in the low molecular weight pool.

Acknowledgments

This research was supported solely by the Erasmus University Rotterdam, The Netherlands.

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