THE ROLE OF OXYGEN RADICALS IN CARDIOVASCULAR DISEASES

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CONVERSION OF RAT XANTHINE DEHYDROGENASE TO XANTHINE OXIDASE DURING OXIDATIVE STRESS.

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ABSTRACT

The enzyme "xanthine oxidase" is one of the best characterized sources of superoxide anion and hydrogen peroxide. "In vivo" it mainly acts as a dehydrogenase which, by reducing NAD^+, appears to be the physiologic form. Nevertheless under a variety of conditions the enzyme can undergo a conversion to an oxidase which delivers the electrons to oxygen to form superoxide and hydrogen peroxide. The transformation xanthine dehydrogenase ⟶ xanthine oxidase is irreversible when it is induced by a proteolytic attack or reversible when the SH groups of the enzyme have been oxidized.

In the present study the oxidative stress induced by ischemia and ischemia followed by reperfusion was compared to that induced by hydrogen peroxide or diamide in normoxic conditions. In particular we intended to characterize the type (reversible or irreversible) of the xanthine dehydrogenase ⟶ xanthine oxidase conversion and the importance of glutathione and protein SH groups in this conversion. In the fresh heart and in the control after normoxic perfusion we detected a 80% of xanthine dehydrogenase and a 20% of xanthine oxidase (5% reversible xanthine oxidase and 15% irreversible xanthine oxidase). There appears to be a considerable increase in reversible xanthine oxidase (from 5% to 15%) after reperfusion following 1 hour of global ischemia; in the same conditions there is a decrease of total glutathione and of total SH groups of about 70% and 25% respectively. After perfusion with hydrogen peroxide or with diamide, a well known thiol oxidizing agent, a very marked increase in reversible xanthine oxidase of about 40% and 60% respectively is apparent. When the infusion of hydrogen peroxide or diamide ends, the reversible xanthine oxidase partially reverts to xanthine dehydrogenase. The changes in the redox state of glutathione and of total thiol groups are then in agreement with the changes of the enzyme. From the reported data it can be concluded that: 1) in the rat heart there is a basal level of reversible + irreversible xanthine oxidase which, in the presence of hypoxanthine and xanthine, contributes to the background production of oxygen free radicals. 2) There is no significant increase of irreversible xanthine oxidase during ischemia nor during reperfusion. 3) Reversible xanthine oxidase increases during the reperfusion that follows an ischemic period bringing the total amount of xanthine oxidase to about 30%. 4) Oxidizing agents like diamide and hydrogen peroxide markedly convert xanthine dehydrogenase to reversible xanthine oxidase. The latter can be reconverted to xanthine dehydrogenase after withdrawing diamide or hydrogen peroxide.

OVERVIEW AND INTRODUCTION

The catabolism of purine nucleotides is accomplished by several enzymes which, ultimately, form uric acid, the final product of purine catabolism in man and primates. The last two steps of the catabolic process, namely the transformation hypoxanthine ⟶ xanthine and xanthine ⟶ uric acid are catalyzed by the enzyme generally called xanthine oxidase [xanthine-oxygen oxidoreductase, E C (1.2.3.2)]. The enzyme from bovine milk has been the most extensively studied, although it appears to be largely distributed in many different species and tissues (Krenitsky et al., 1974; Parks and Granger, 1986).

The native xanthine oxidase protein from liver and other sources is a dimer having a molecular weight of about 300 000 daltons and each subunit contains an internal electron transport chain formed by FAD, molybdenum, iron and acid labile sulfide (Fig. 1). In the enzyme from animal sources (mammalian and avian) these groups are present in a molar ratio of 1:1:4:4. Within xanthine oxidase the oxidation-reduction potential of the various prosthetic groups, i.e. Mo ⩽ Fe.S ⩽ FAD (Olson et al., 1974), determines the direction of the electron flux from Mo to FAD. Mo is the site of oxidation of hypoxanthine and xanthine while FAD is the site which donates the electrons to the acceptor. The electron acceptor of the xanthine-converting enzyme isolated from mammalian sources is mainly, if not exclusively, NAD^+, which is reduced to NADH and, consequently, the enzyme behaves like a dehydrogenase (EC 1.2.1.37) considered to be its physiologic form (Stirpe and Della Corte, 1969). Under a variety of heterogenous conditions, first studied by Stirpe and coworkers (Stirpe and Della Corte, 1969; Della Corte and Stirpe, 1972), the enzyme is converted to an oxidase form in which the acceptor is the dioxygen in turn transformed to both $O_2^{\cdot-}$ and H_2O_2. The relative amounts of $O_2^{\cdot-}$ and H_2O_2 produced depend on factors like pH, concentration of oxygen and substrates (Fridovich, 1970). In the milk enzyme, in fact, it has been shown that, at pH 7, only the 20% of oxygen is transformed into the superoxide anion while the remaining is transformed to H_2O_2 (Fridovich, 1970). It has also been shown that the amount of univalently reduced oxygen increased by decreasing the concentration of xanthine.

The xanthine dehydrogenase (XD) ⟶ xanthine oxidase (XO) transformation can be operated by many different conditions like proteolysis, heating at 37°C, storage at -20°C, anaerobiosis, treatment with organic solvents and SH groups reagents (Stirpe and Della Corte, 1969; Della Corte and Stirpe, 1972); all these treatments may fall into two categories which are (I) proteolysis and (II) oxidation or binding of thiol groups (Battelli et al., 1973). All these conditions might probably induce a conformational change at the flavin site which, becoming unable to interact with its natural substrate NAD^+, interacts with molecular oxygen. According to the type of the experimental condition used for the transformation of XD to XO, the latter can be of the reversible (XOrev) or irreversible (XOirrev) type. The irreversible type is obtained from the dehydrogenase or from the reversible type by

proteolytic treatment (trypsin, chymotrypsin, papain, subtilisin) (Battelli et al., 1973) which determines the cleavage of a polypeptidic fragment not essential for the oxidase activity but necessary to warrant a proper conformation at the flavin site for the interaction with NAD^+; there appears to be a direct relationship between the formation of a stable flavin semiquinone radical (FADH•) and the ability for NAD^+ reduction (Waud and Rajagopalan, 1976). The reversible oxidase (XOrev) can be obtained from the dehydrogenase by oxidation of some sulfhydryl groups to disulphides or by treatment with thiol modifying reagents (Della Corte and Stirpe, 1972; Waud and Rajagopalan, 1976) by sulfhydryl oxidase (Clare et al., 1981) or thiol-transferase (Battelli and Lorenzoni, 1982). The conversion from XD to XO is progressive so that a XD/XO form, intermediate between the dehydrogenase and the oxidase form, and characterized by a partial modification of the SH groups was reported (Kaminski and Jezewska, 1979). The XD/XO form interacts both with NAD^+ and oxygen. With the exception of the intestine (Battelli et al., 1972), the back conversion XOrev $\longrightarrow$ XD can be catalyzed in other tissues and is obtained with thiol compounds, the most effective being dithioerythritol (DTE), dithiothreitol (DTT) and dihydrolipoic acid while GSH, cysteine, cysteamine and other low molecular weight thiols are scarcely effective (Battelli et al., 1973).

Of the various organs of the rat, the liver and intestine exhibit the highest activity of the xanthine-converting enzyme (Battelli et al., 1972) while the heart appears to have only 13% of the activity found in the intestine. Moreover, comparing the levels reported for other mammals, liver and intestine of man have relatively low values of xanthine oxidase (Parks and Granger, 1986). As far as xanthine oxidase of human heart is concerned, there is a report of just one experiment showing its presence (Krenitsky et al., 1974) while another author does not find any activity (Ramboer, 1969). In our laboratory, in three different measurements on hearts removed after heart transplantation from patients suffering from dilatative cardiomyopathy we did not find any significant activity (not shown).

By the use of immunohystochemical techniques (Jarasch et al., 1981), xanthine oxidase was localized preferentially in the cytoplasm of endothelial cells of the mammary gland, intestine, liver, heart and lung.

Recently there has been a surge of interest on this enzyme as generator of oxygen free radicals since the latter appear to be involved in the pathogenesis of injury provoked by reperfusion following a period of ischemia. Oxygen free radicals appear in fact to play a major role in inducing microvascular and parenchimal damage. The most important sources of free radicals in the infarcting myocardium, recently reviewed by Hearse et al. (1986), are: a) catecholamines which are catabolized by monoamino oxidases producing hydrogen peroxide b) mitochondria that, particularly after an ischemic period, could leak superoxide and hydrogen peroxide c) leucocytes which, after having invaded the myocardium are able to convert oxygen to superoxide by their NADPH oxidase

activity and, finally d) xanthine oxidase. The production of superoxide by xanthine oxidase is considered responsible for ischemia-reperfusion disorders appearing in different tissues such as the intestine (Granger et al., 1981), the kidney (Hansson et al., 1983), the heart (Hearse et al., 1986), and the liver (Adkison et al., 1986). The conversion XD⟶XO was reported to occur in several organs during ischemia concomitantly with a marked catabolism of adenine nucleotides which brings to an accumulation of hypoxanthine. The latter acts as a substrate for the enzyme during the reperfusion following the ischemic period, leading to a rapid formation of superoxide and hydrogen peroxide. Since superoxide can dismutate, spontaneously or through the superoxide dismutase (SOD) activity, to hydrogen peroxide, the latter can accumulate. Hydrogen peroxide can either oxidize glutathione through glutathione peroxidase or initiate lipid peroxidation by interaction with myoglobin or with iron released from its stores during ischemia. Glutathione oxidation can secondarily induce the oxidation of protein SH groups.

The accumulation of activated forms of oxygen together with the impairement of the endogenous scavenging systems (GSH, protein SH groups, glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase (Ursini et al., 1985) can be the beginning of the peroxidative damage of the ischemic/reperfused organs.

In the present work the oxidative stress induced by ischemia and ischemia followed by reperfusion was compared to that induced by hydrogen peroxide and diamide in normoxic conditions. The parameters considered are the conversion XD⟶XO and particularly the type of conversion (reversible or irreversible), the redox state of glutathione and of the protein SH groups. Other parameters which are directly or indirectly indicative of an oxidative stress like formation of malondialdehyde (MDA), and left ventricular pressure developed were also considered.

MATERIALS AND METHODS

Rat heart were perfused at 37°C by the non-recirculating Langendorff procedure with a medium composed by 118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.5 mM $CaCl_2$ and 11 mM glucose at pH 7.4; the solution was kept at 37°C and saturated with 95% O_2 and 5% CO_2. Before any experiment the hearts were perfused for 20 minutes to allow hemodynamic and metabolic stabilization of the preparation. Left ventricular pressure (LVP) was measured by introducing into the left ventricular cavity a liquid-filled balloon connected to a pressure transducer. During normoxic perfusion the flow rate was 12 ml/min; in the experiments where total ischemia was induced the flow was reduced to nought. Fractions of the effluent were collected for the determination of glutathione released. At the end of the perfusion experiment the hearts were rapidly removed and homogenized with Ultra Turrax in 10 ml of 0.1 M Tris buffer (pH 8.1), containing 1.5 mM EDTA, 1.5 mM EGTA and 1 mM phenylmethylsulfonylfluoride (PMSF). One ml of the homogenate was saved for the estimation of total sulphydryl groups and malondialde-

hyde (MDA), while the remaining volume was centrifuged at 105 000 x g for 40 minutes; pellet was discarded and supernatant was eluted through a 10 x 1.5 cm column of Sephadex G-50. This fraction was utilized for the estimation of the various forms of the xanthine-oxidizing enzyme. The assay was performed at 37°C by following the formation of uric acid at 292 nm (εM = 9 900). About 0.2 mg/ml of supernatant protein was incubated in 0.1 M phosphate buffer (pH 7.8) containing 80 μM xanthine and, when requested, 0.3 mM NAD^+, 2 mM DTE, 2 mM pyruvate and 0.1 U/ml of lactate dehydrogenase. The total enzyme activity (XD + XOrev + XOirrev) was measured in the aerobic medium in the presence of NAD^+; pyruvate and lactate dehydrogenase were also added in order to reoxidize the NADH formed that otherwise could inhibit the enzyme activity. The total oxidase activity (XOrev + XOirrev) was measured in the absence of NAD^+. The percentage of the total oxidase activity versus the total enzymatic activity could then be calculated and, by difference, the amount of XD obtained. The activity of XOirrev was estimated in the absence of NAD^+ after preincubating the supernatant at 37°C for 10 minutes in the presence of 10 mM DTE; an aliquot, about 0.4 mg/ml, was withdrawn for the assay to achieve a final concentration of 2 mM DTE. The percentage of XOirrev was obtained from the total enzymatic activity measured as reported above but in the presence of DTE in order to avoid any aspecific inhibitory effect by this reducing compound. Finally the percentage of XOrev was calculated by substracting the % of XOirrev from the % of total oxidase.

Total sulfhydryl groups of the homogenate were titrated by using the Ellman's reagent, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). Homogenate (0.5 mg protein) was incubated in 0.2 M Tris-HCl buffer (pH 8.1) containing 5 mM EDTA and 0.8% SDS to a final volume of 3 ml. The reaction was started by adding DTNB to a final concentration of 2 mM and the increase of absorbance was followed at 412 nm until a constant value was obtained. The concentration of sulfhydryl groups was calculated using εM = 13 600.

Glutathione was essentially measured with the procedure of Tietze (1970) modified for the determination of oxidized glutathione (GSSG) as described by Xia et al. (1985) where reduced glutathione (GSH) was enzymatically trapped by methylglyoxal in the presence of glyoxalase I.

The malondialdehyde formed was determined on homogenate aliquots of 0.3-0.6 mg protein with the method described by Yagi (1978) originally used for the determination of serum MDA. The solution for the final assay was formed by 12% acetic acid, 0.17% thiobarbituric acid (TBA), 0.01% butylated hydroxytoluene (BHT) and 0.25% Nonidet P-40. The suspension was left to react for 1 hour at 95°C, cooled and centrifuged. The absorption of the adduct formed between MDA and TBA was read at 532 nm and the concentration of MDA calculated after substraction of the 580 nm absorption utilizing an εM = 156 000.

Protein of the homogenate were determined by the biuret method (Gornall et al., 1949), while those of the xanthine oxidase preparation were determined with the method of Lowry et al. (1951).

RESULTS

When perfused hearts are maintained in ischemic conditions (perfusion flow = 0 ml/min) at 37°C for 1 hour and then reperfused with a normoxic medium a massive coronary efflux of glutathione (80% reduced) ensues, as shown in Fig. 2. Fig. 3 shows the effect of total ischemia and subsequent reperfusion on contractile performance of isolated and perfused rat hearts. The reduction of the perfusion flow from 12 to 0 ml/min resulted in a rapid decline of the developed pressure so that contractile activity completely ceased five minutes after the onset of the ischemia. The resting pressure began to rise as early as twenty minutes after ischemia and, during the post-ischemic reperfusion, there was a marked increase of the resting pressure and a poor recovery of the developed pressure (less than 20% of the control value). The distribution of the various forms of the xanthine-converting enzyme in the above reported conditions is shown in Tab. 1. After 1 hour of ischemia the dehydrogenase activity is poorly affected and decreases from 80% to 75%; a simultaneous small increase of the reversible form (4%) is observed while the irreversible form does not significantly change. When ischemia is followed by 1 hour of reperfusion there is a significant increase (9%) of only the reversible form; two hours of reperfusion do not determine any substantial increase with respect to the values found after 1 hour (data not reported). The lack of conversion XD $\longrightarrow$ XOirrev during ischemia indicates that probably during ischemia there is no involvement of cytosolic proteases. Only if extreme experimental conditions, like autolysis for 18 hours at room temperature, are used it becomes possible to find a significant conversion of xanthine dehydrogenase which is converted 42% in XOirrev and 45% in XOrev. Glutathione was almost completely lost from the autolyzing heart and recovered in the medium partially in the oxidized form (31%).

In Tab. 2 MDA formation, total SH groups and glutathione content after ischemia and ischemia followed by reperfusion are reported. MDA content, even though very low, appears to increase after ischemia. There are no significant alterations in the concentration of total SH groups and glutathione after the period of ischemia while they are strongly decreased when reperfusion follows the ischemic period. After 1 hour of reperfusion total glutathione is decreased to about a third of that present in the control and about 25% of this residue is in the oxidized form.

Normoxic perfusion in the presence of hydrogen peroxide or diamide were carried out in order to understand if some of the damaging effect observed during ischemia and ischemia followed by reperfusion could be partly due to the accumulation of H_2O_2 or to the oxidation of glutathione. Treatment of the heart with thiol oxidizing agents can also give information about the extent of reversible conversion of the xanthine-converting enzyme.

Under aerobic conditions, the addition of H_2O_2 or diamide (Fig. 4) to the perfusion medium induced alterations of the mechanical functions. As soon as hydrogen peroxide was administered, an initial

increase in the heart rate was observed, and the developed pressure was maintained at a constant level even though quite erratic and lasted also when the oxidant was withdrawn. When diamide was added, there was a slow decline of contractile activity with no major changes in the resting pressure. Under the latter conditions, even after 1 hour, low values of developed pressure were still maintained; nevertheless the contractile activity was resumed almost completely when the infusion of diamide was suspended.

The effects of hydrogen peroxide and diamide on the xanthine-converting enzyme activity are reported in Tab. 3. About 40% of the xanthine dehydrogenase is transformed into reversible oxidase after perfusion in the presence of hydrogen peroxide; when hydrogen peroxide is removed from the perfusion medium, the reversible oxidase is extensively reconverted to the dehydrogenase. The transformation XD$\longrightarrow$XO is more dramatic when diamide is used instead of hydrogen peroxide, since in this case, almost 60% of the dehydrogenase is transformed into the oxidase. Also with diamide the enzyme reconverts to the native form upon withdrawal of the reagent from the perfusion medium. Both with diamide and hydrogen peroxide, the transformation affects only the reversible form of the oxidase while the irreversible form remains constant at the basal level of about 15%.

Tab. 4 shows that hydrogen peroxide but not diamide can slightly increase MDA content at a level close to that found after ischemia. Total SH groups are extensively oxidized during the perfusion with hydrogen peroxide and diamide, and a decrease of 25% and 35% respectively is apparent (Tab. 4); the decrease in glutathione is proportionally greater since about 50% of it is lost from the heart after 30 minutes of perfusion in the presence of hydrogen peroxide or diamide; the amount of glutathione lost was recovered in the pooled perfusate (Fig. 5 and 6). The fraction remaining in the heart is about 50% in the oxidized form after perfusion with either hydrogen peroxide or diamide (Tab. 4). The reperfusion with the medium without the oxidant determines an increase of both the total sulfhydryl groups and of reduced glutathione, the reduced/oxidized ratio of which reaches a value close to that of the control.

DISCUSSION

Current literature dealing with xanthine oxidase reports that in most tissues this enzyme is present "mainly, if not exclusively" as xanthine dehydrogenase; the ratio XD/XO existing "in vivo" has not been clearly defined. From our data it appears that an average amount of 15% XOirrev is always present in the rat heart tissue and that this amount does not significantly increase after ischemia, ischemia followed by reperfusion or after oxidative stress induced by oxidizing agents like H_2O_2 or diamide. This amount of XOirrev corresponds, in terms of mU, to about 10 mU/g wet weight; consequently when hypoxanthine concentration increases as during ischemia (Granger et al., 1986; Schosten et al., 1983) a flux of reduced forms of oxygen is produced and could contribu-

te to the well established reperfusion injury.

The basal level of XOirrev can be found even when the homogenization is carried out in the presence of DTT (not reported), EDTA, EGTA and PMSF in order to prevent any reversible or irreversible conversion of the enzyme. The XOirrev, which is always present, could represent a xanthine oxidase with different catalytic properties (Schousten et al., 1983) or a particular catabolic form of this enzymatic protein.

The activity of XOrev in fresh rat heart is about 5% and after reperfusion following an ischemic period there appears to be a net conversion of XD ⟶ XOrev. This conversion, which is determined by the oxidation of some SH groups of the enzyme, appears to depend on the production of oxidizing species during reperfusion, a likely candidate being H_2O_2. Concomitantly with the reversible conversion of the enzyme there is a very dramatic decrease of cellular glutathione so that the cell is left without one of the most relevant defence-systems against the oxidative stress. The decrease in glutathione concentration is paralleled by a decrease in the total SH groups, which represent the sum of glutathione and protein thiol groups; this indicates that a consistent number of protein SH groups were transformed to disulfides because of lack of the reducing buffer capability of glutathione. The amount of glutathione which remains in the heart even after severe ischemia followed by reperfusion probably is the fraction contained within the mitochondria, the membrane of which appears then to be damaged secondarily; the bulk of glutathione lost is mainly represented by the cytosolic fraction. The extention of the reperfusion damage is also shown by the decrease in the left ventricular pressure which, after reperfusion, is about 10-20% of the control value.

From the above reported results obtained in an ischemic/reperfused model it appears that the conversion XD ⟶ XOrev occurs only during the reperfusion period. Perfusion experiments in the presence of H_2O_2 and diamide were then devised in order to: a) establish a relationship between the oxidative stress and the reversible conversion of the enzyme. b) clarify the role of glutathione and protein SH groups in the reversible conversion of xanthine oxidase.

Perfusion with H_2O_2 or with diamide determines a large conversion XD ⟶ XOrev which is of about 35% and 60% respectively. If the basal level of XOirrev is added to the concentration of XOrev, the amount of total oxidase increases to about 50% and 75% respectively. The different effects of diamide and H_2O_2 on the conversion XD ⟶ XOrev could be explained by the direct action of diamide upon GSH and protein SH groups, while the action of H_2O_2 is mediated through the oxidation of glutathione. If, after a period of perfusion, these oxidizing reagents are withdrawn from the perfusion medium, the XOrev partly reconverts to XD. The reconversion increases when perfusion time is longer (not shown). A behaviour similar to that of the enzyme is shown by cellular glutathione and protein SH groups; they are oxidized upon the action of diamide and restored to the reduced state upon withdrawal of the reagents. Consequently, it appears that the redox state of glutathione

could influence the status of protein thiols like those of xanthine dehydrogenase.

After H_2O_2 or diamide perfusion, the sum of the total pooled glutathione of the perfusates with the amount of the glutathione found in the heart is lower than the amount of the glutathione of the controls; in addition there is an apparent "increase" of the cellular glutathione after the perfusion in the absence of the oxidizing agents. This discrepancy can be explained considering that the oxidizing agents can induce the formation of mixed disulfides between glutathione and protein thiol groups; the reduction of these disulfides determines an intracellular release of GSH.

The conversion XD⟶XOrev appears to depend upon the presence of oxidized glutathione and on an enzymatic system. A GSSG-dependent thiol: disulfide oxidoreductase has been extensively characterized in rat liver (Battelli and Lorenzoni, 1982) and found to have a molecular weight of 40 000; this enzyme exhibits an activity also in rat heart even though to a lower extent, being of about a quarter of that present in the liver. This enzyme acts on XD in the presence of GSSG and converts XD into a XOrev.

The sulfhydryl oxidase that could act directly on the SH groups of XD without the intermediation of GSSG is present in the milk but does not appear to be involved in the heart tissue.

It has been reported that XOrev from rat liver cannot be reconverted to XD by GSH (Battelli and Lorenzoni, 1982), nevertheless we were able to get the XOrev partially reconverted to the dehydrogenase after perfusion without the oxidizing agents. The reducing equivalents are supplied by NADPH which, after oxidation, could be reduced both by the pentose phosphate shunt or by the malic enzyme. The latter could have an important role since a special feature of the myocardium is its poor activity of the pentose phosphate pathway (Zimmer et al., 1984). NADPH can reduce GSSG through glutathione reductase which, in turn, by a thioltransferase can reduce the disulfides of the XOrev to the native dithiols so that the XD activity is recovered. NADPH, instead of acting on GSSG, could reduce thioredoxin, through thioredoxin reductase, which, in turn, can operate the transformation XOrev XD. As pointed out by Mannervik et al., (1983) the two systems could act complementary and, according to the nature of a particular substrate, the one or the other of the two reducing systems could be operative.

Many papers indicate a protective effect of antioxidants, superoxide dismutase, catalase and allopurinol on ischemic and reperfused rat heart (Guarnieri et al., 1978; Manning et al., 1984; Jolly et al., 1984). In the present research we observed both a marked decrease of sulfhydryl groups during ischemia/reperfusion and an oxidative stress-induced conversion of XD to XOrev which nevertheless can be restored to its original function by the reducing pool mainly represented by glutathione. The recovery of the contractile performance after removal of diamide confirms the capability of the tissue to repair the damage induced by oxidative stress as shown by the heart functional parame-

ter like left ventricular pressure (L.V.P.). Consequently, the infusion of thiols or thiols-delivering agents could limit the injury caused by ischemia and by the action of oxidizing species thereby protecting the myocardium from a potential damage.

The xanthine-converting enzyme appears to be sensitive to the redox state of the cellular glutathione. A decrease in the ratio GSH/GSSG could shift the xanthine dehydrogenase towards the XO form with the consequent production of reduced forms of oxygen, provided that a sufficient concentration of hypoxanthine is present. Heart tissues, compared to other tissues, exhibit a lower activity of xanthine oxidase and consequently the role of this enzyme in the production of free radicals in the heart could be less relevant than in other tissues like the liver and the intestine.

Xanthine oxidase might be both a cause of oxidative stress and a consequence of it. It is a cause since about 20% of the total enzyme is always present in the oxidase form in the rat cardiac tissue. The transformation XD $\longrightarrow$ XO is also a consequence of the oxidative stress since H_2O_2 and other oxidizing agents, directly or through the oxidation of glutathione, can induce the XD $\longrightarrow$ XOrev conversion and, thereby, amplify the damage.

Tab. 1. Conversion of xanthine dehydrogenase to xanthine oxidase during ischemia and ischemia followed by reperfusion.

	Xanthine dehydrogenase	Xanthine oxidase (reversible)	Xanthine oxidase (irreversible)
Control	80.00 ± 1.9 (8)	5.00 ± 0.2 (8)	15.25 ± 0.59 (8)
Ischemia (60 min)	75.25 ± 5.0 (10)	9.00 ± 0.5 (10)	16.00 ± 1.0 (10)
Ischemia (60 min) + Reperfusion (60 min)	67.00 ± 3.5 (10)	18.00 ± 1.2 (10)	15.00 ± 0.6 (10)

Values, expressed as percentage of the total activity, are mean ± S.D. and figures in brackets show the number of experiments.

Tab. 2. Malondialdehyde, sulfhydryl groups and glutathione content of rat heart after ischemia and ischemia followed by reperfusion.

	Malondialdehyde	Total SH groups	GSH + GSSG	GSSG
Control	0.316 ± 0.06(3)	90 ± 9(7)	11.8 ± 1.0(5)	1.1 ± 0.3(3)
Ischemia (60 min)	0.413 ± 0.04(3)	88 ± 3(8)	12.0 ± 0.6(3)	1.9 ± 0.2(3)
Ischemia (60 min) + Reperfusion (60 min)	0.400 ± 0.07(3)	70 ± 6(7)	3.6 ± 1.4(3)	0.9 ± 0.2(3)

Values, expressed as nmol/mg protein, are mean ± S.D. and figures in brackets show the number of estimations, each obtained with a different heart.

Tab. 3. Conversion of xanthine dehydrogenase to xanthine oxidase after perfusion with hydrogen peroxide and diamide

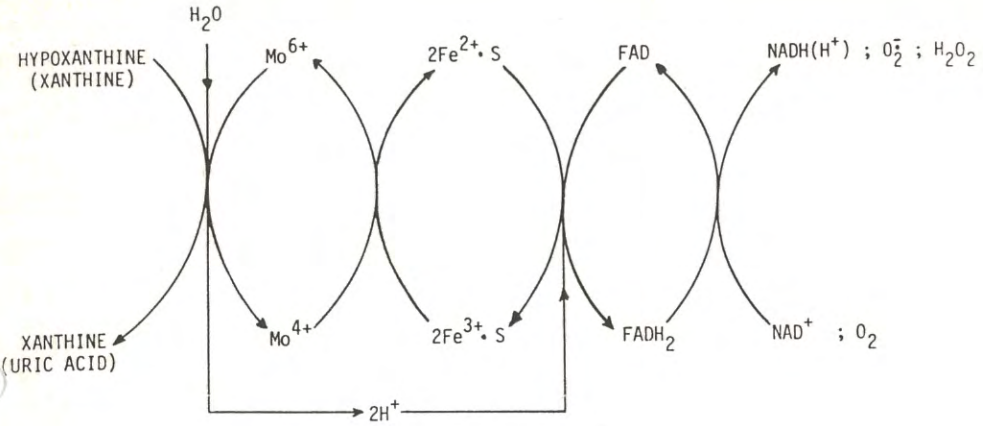
	Xanthine dehydrogenase	Xanthine oxidase (reversible)	Xanthine oxidase (irreversible)
Control	80.00 ± 1.9 (8)	5.00 ± 0.2 (8)	15.25 ± 0.6 (8)
H_2O_2 (30 min)	48.00 ± 4.0 (10)	38.00 ± 4.0 (10)	14.00 ± 0.5 (10)
H_2O_2 (30 min) + H_2O_2 omitted (30 min)	71.50 ± 1.5 (10)	18.00 ± 1.0 (10)	14.00 ± 0.5 (10)
Diamide (30 min)	28.00 ± 4.0 (3)	59.00 ± 3.0 (3)	13.00 ± 1.0 (3)
Diamide (30 min) + Diamide omitted (30 min)	47.00 ± 1.0 (3)	39.00 ± 2.0 (3)	14.00 ± 1.0 (3)

Hydrogen peroxide and diamide are 50 µM and 100 µM respectively. Values, expressed as percentage of the total activity, are mean ± S.D. and figures in brackets show the number of experiments.

Tab. 4. Malondialdehyde, sulfhydryl groups and glutathione content of rat heart after perfusion in the presence of hydrogen peroxide or diamide.

	Malondialdehyde	Total SH groups	GSH + GSSG	GSSG
Control	0.316 ± 0.06(3)	90 ± 9(7)	11.8 ± 1.0(5)	1.1 ± 0.3(3)
H_2O_2 (30 min)	0.380 ± 0.03(3)	63 ± 17(8)	6.2 ± 0.5(3)	3.3 ± 0.2(3)
H_2O_2 (30 min) + H_2O_2 omitted (30 min)	0.395 ± 0.05(4)	78 ± 17(9)	6.8 ± 0.8(4)	1.0 ± 0.1(3)
Diamide (30 min)	0.368 ± 0.04(3)	68 ± 4(3)	5.7 ± 1.6(3)	3.0 ± 1.1(3)
Diamide (30 min) + Diamide omitted (30 min)	0.300 ± 0.05(3)	82 ± 3(3)	7.1 ± 1.2(3)	1.1 ± 0.9(3)

Values, expressed as nmol/mg protein, are mean ± S.D. and figures in brackets show the number of experiments, each obtained with a different heart.



$$\text{XANTHINE} + H_2O + NAD^+ \longrightarrow \text{URIC ACID} + NADH + H^+$$

$$\text{XANTHINE} + H_2O + 2O_2 \longrightarrow \text{URIC ACID} + 2O_2^- + 2H^+$$

$$\text{XANTHINE} + H_2O + O_2 \longrightarrow \text{URIC ACID} + H_2O_2$$

Fig. 1. Mechanism of action of xanthine oxidoreductase.

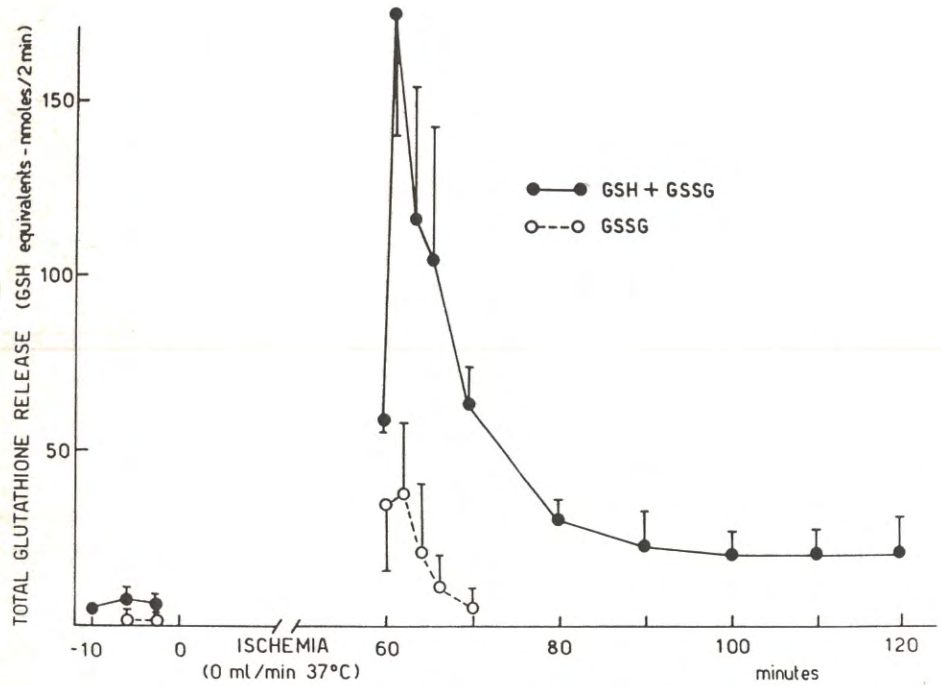


Fig. 2. Time course for release of glutathione from rat heart during reperfusion following global ischemia.

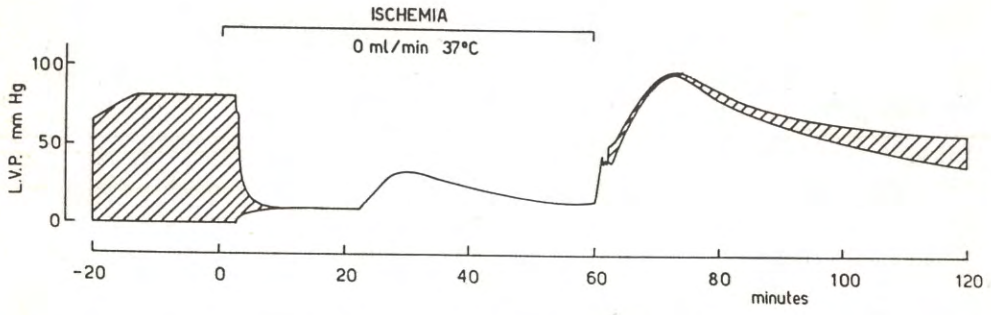


Fig. 3. Time course of left ventricular pressure (L.V.P.) during global ischemia and reperfusion of rat heart.

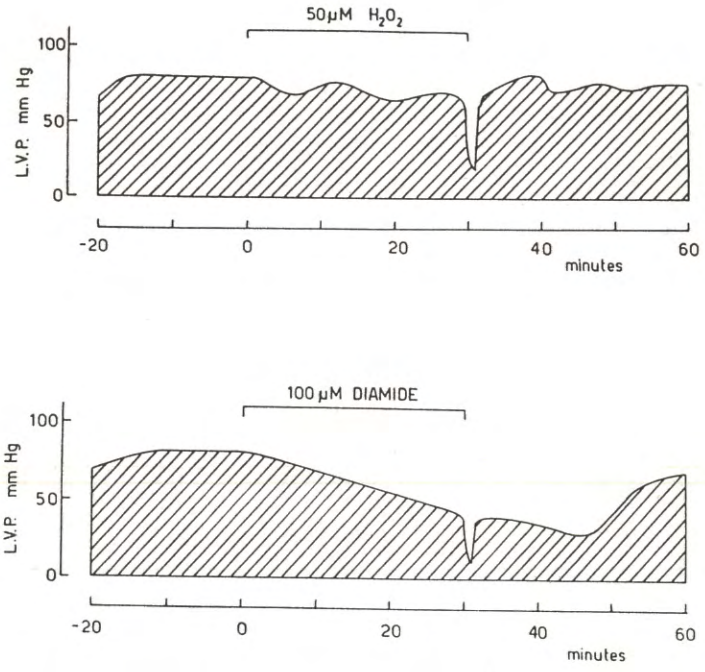


Fig. 4. Time courses of left ventricular pressure (L.V.P.) during perfusion in the presence of hydrogen peroxide and diamide in rat heart.

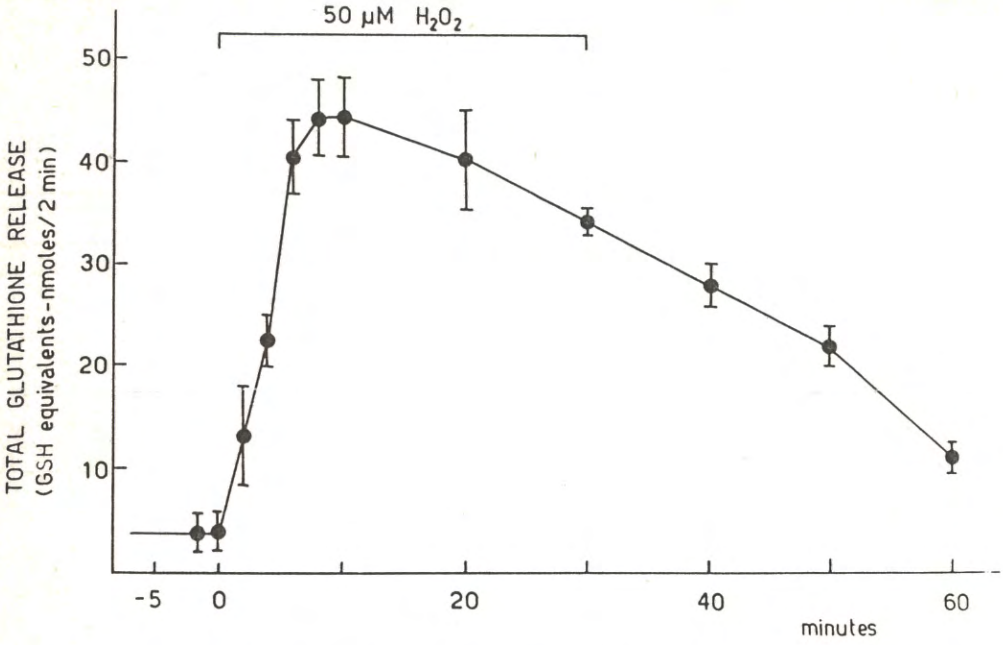


Fig. 5. Time course for release of glutathione from rat heart perfused for 30 minutes with medium containing H_2O_2.

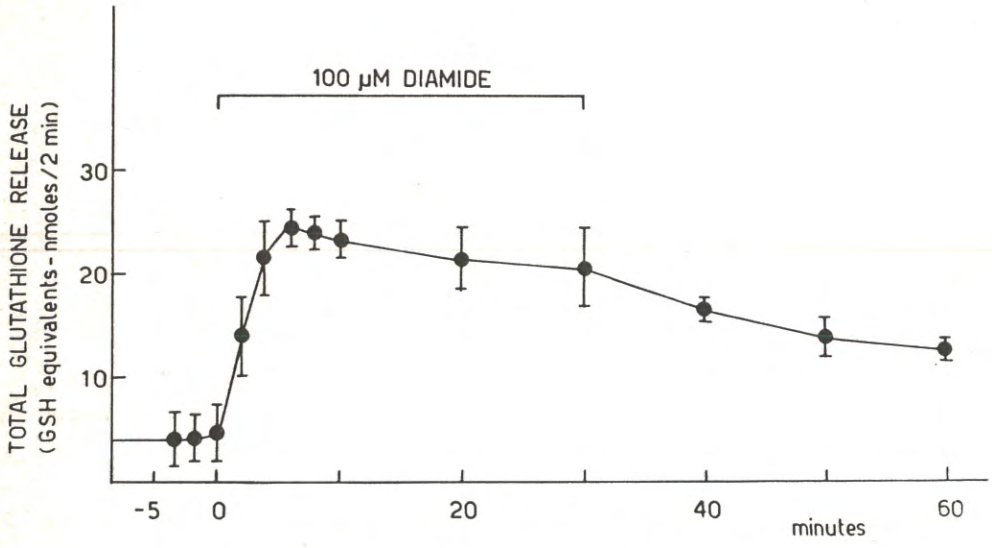


Fig. 6. Time course for release of glutathione from rat heart perfused for 30 minutes with medium containing diamide.

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