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Antioxidative State of the Myocardium and Kidneys in Acute Diabetic Rats

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Summary

The influence of acute diabetes (8 days), induced by streptozotocin (45 mg.kg^{-1} body weight) on myocardial and renal antioxidative conditions was investigated. The animals were given subtherapeutical doses of insulin (Interdep 6 U. kg^{-1} body weight, s.c.). Considerably increased levels of malondialdehyde (MDA), as well as of superoxide dismutase (SOD) and catalase (CAT) activity were found in the myocardium of diabetic animals. The oxidized glutathione (GSSG) level and glutathione peroxidase (GSH-PX) activity remained unchanged. The reduced glutathione (GSH) level as well as the activity of glutathione S-transferase (GST) were significantly lower. The activity of GSH-PX in the kidneys of diabetic rats increased by 60 % and that of GST by 105 %, respectively. CAT and SOD activity values were unchanged.

Key words

Rat – Streptozotocin – Diabetes – Antioxidative enzymes – Malondialdehyd – Glutathione

Introduction

Highly reactive species of oxygen are produced in every cell and are critical for the normal operation of a wide spectrum of biological processes, e.g. immunity (Allen 1979, Badwey and Karnovsky 1980), cell growth and division (Cerutti *et al.* 1990, Saran and Bors 1989, 1990). Mitochondria are the main source of the superoxide anion ($.O_2^-$) where it is formed by autooxidation of ubisemichinon and of electron-transferring proteins. H_2O_2 is generated by spontaneous or enzymatic $.O_2^-$dismutation, whereas the highly reactive hydroxyl radical (.OH) is a product of the Haber-Weiss and Fenton reactions. The levels of toxic intermediate products of O_2 metabolism are controlled by cellular defense mechanisms (antioxidative enzymes – SOD, CAT, GSH-PX, GST – as well as non-enzymatic free oxygen radical scavengers). If the balance between the formation of free oxygen radicals and their elimination by detoxication is impaired, oxidative damage of the membrane and changes in the structural and functional integrity of subcellular organelles can occur (Mimnaugh *et al.* 1981, Doroshow 1983). Lipid peroxidation has been implicated in the pathogenesis of many degenerative disorders (Simmons 1984, Halliwell and Gutteridge 1984) including naturally occurring and chemically induced diabetes (Gandy *et al.* 1982, Hagglof *et al.* 1983). Sato *et al.* (1979) were the first to report increased levels of plasma thiobarbituric acid-reactive substances in IDDM and NIDDM patients. Subsequently, increased lipid peroxidation was found in various tissues from rats with streptozotocin- or alloxan-induced diabetes (Higuichi 1982, Karpen *et al.* 1982, Matkovics *et al.* 1982). Increased or uncontrolled oxidative activity is probably associated with the development of secondary diabetic complications – cardiomyopathy, nephropathy and retinopathy (Crouch *et al.* 1978, Paller *et al.* 1984).

The majority of reviews has been concerned with the influence of chronic diabetes on the antioxidation status of tissues. The objective of our present work was to find out if the changes in antioxidative conditions of tissues known to be involved in diabetic complications (myocardium and kidneys) were already detectable in the case of acute diabetes (8 days).

Materials and Methods

Male Wistar rats (Dobrá Voda) were used in the experiment in which diabetes was induced by i.v. administration of streptozotocin (45 $mg.kg^{-1}$ body weight in a 0.1 $mol.l^{-1}$ citrate buffer, pH 4.5). The animals were given insulin (Interdep 6 $U.kg^{-1}$ body weight s.c.) for 8 days. Healthy male Wistar rats were used as the control group. Both groups were fed a standard laboratory diet and decapitated in satiated conditions (without food limitation).

Glutathione peroxidase (GSH-PX) activity was determined by the method of Paglia and Valentine (1967), using cumene hydroperoxide as substrate. The activity of superoxide dismutase (SOD) was determined by the Randox Lab. Ltd. commercial test and catalase (CAT) activity was assessed by the modified method of Cavarocchi *et al.* (1986). 1-chloro-2,4-dinitrobenzene was used as substrate in glutathione S-transferase (GST) activity determinations (Habig *et al.* 1974). The levels of substances reacting with thiobarbituric acid, expressed in malondialdehyde (MDA) concentration were determined (Buege and Aust 1978), as were the reduced glutathione (GSH) and oxidized glutathione (GSSG) (Bergmeyer 1974), proteins in the homogenate using bovine albumin standard (Lowry *et al.* 1951), and blood sugar levels (Bio La Test, Lachema).

Data are given as means ± S.E.M. Statistical significance was determined by Student's t-test.

Results

Diabetic animals had three times higher blood sugar values compared to the controls. At the beginning of the experiment, the animals had a mean body weight of 258±9 g; prior to decapitation, the body weight of the diabetic group was lower by 9.7 % than the controls. Heart protein levels were approximately the same in both groups, whereas the renal protein contents in the diabetic group were higher than in the control group (Table 1).

Table 1
General characteristics of control and diabetic animals

	Control n=10	Diabetic n=10
Body weight (g)	329±6	300±5^{c}
Blood sugar ($mmol.l^{-1}$)	5.75±0.10	18.08±1.52^{e}
Heart proteins ($mg.g^{-1}$)	30.31±0.52	31.87±0.58
Renal proteins ($mg.g^{-1}$)	74.01±2.41	82.72±2.56^{a}

Results are expressed as means ± S.E.M. Statistical significance: $^{a}p < 0.05$, $^{c}p < 0.01$, $^{e}p < 0.001$.

Acute diabetic animals showed, in addition to increased MDA levels, also higher heart SOD and CAT activity values compared with the controls. GSSG levels and GSH-PX activity were not significantly different from those of the control animals. A statistically significant reduction of GSH level and GST activity was found (Table 2).

Table 2
Malondialdehyde levels and heart antioxidative status in control and diabetic animals

	Control	Diabetic
MDA ($nmol.gP^{-1}$)	295.40±29.21	425.50±33.20^{a}
GSH ($\mu mol.g^{-1}$)	3.22±0.10	2.50±0.18^{c}
GSSG ($\mu mol.g^{-1}$)	0.79±0.12	0.65±0.06
SOD ($U.mgP^{-1}$)	6.35±0.32	7.50±0.24^{a}
GSH-PX ($U.mgP^{-1}$)	0.40±0.02	0.40±0.02
CAT ($kU.mgP^{-1}$)	0.68±0.04	0.90±0.02^{e}
GST ($mU.mgP^{-1}$)	144.90±6.50	103.30±2.50^{e}

Results are expressed as means ± S.E.M. Statistical significance: $^{a}p < 0.05$, $^{c}p < 0.01$, $^{e}p < 0.001$.

The kidneys of diabetic animals had considerably increased GSH-PX and GST activity values compared with the controls. GSH-PX activity was increased by 60 % and GST activity by 105 %, respectively. No significant differences were observed in either CAT or SOD activities in this tissue (Table 3).

Table 3
Activity of antioxidative enzymes in the kidneys of control and diabetic animals

	Control	Diabetic
GSH-PX ($U.mgP^{-1}$)	0.10±0.01	0.16±0.01^{e}
CAT ($kU.mgP^{-1}$)	5.18±0.10	4.89±0.17
SOD ($U.mgP^{-1}$)	3.05±0.16	3.07±0.13
GST ($mU.mgP^{-1}$)	43.31±3.01	89.06±6.21^{e}

Results are expressed as means ± S.E.M. Statistical significance: $^{e}p < 0.001$.

Discussion

The results have shown that already in acute diabetes lasting 8 days, peroxidative damage occurred in the heart tissue resulting in activity changes of various components of the antioxidative system. The excess levels of intermediate reduction products of

oxygen metabolism are primarily detoxicated by CAT and SOD.

CAT activity showed the highest increases in the myocardium. This may be an important adaptive response to conditions of increased peroxidative stress in this tissue (Matkovics 1977). Since H_2O_2 is used for substrate by CAT, increased activity of this enzyme indicates increased formation of endogenous H_2O_2 in the heart of diabetic rats: *1) H_2O_2 is formed in the β-oxidation process of fatty acids,* which, along with ketone bodies, are the main source of energy for the diabetic heart (Bowman 1966, Cahill 1971). Hypoinsulinaemia is linked to the stimulation of acyl-CoA-oxidase activity which initiates β-oxidation of fatty acids (Osumi and Hashimoto 1978); *2)., increased SOD activity indicates increased H_2O_2 formation by enzymatic dismutation of the $.O_2^-$ radical.* Increased quantity of $.O_2^-$ can be formed by AMP degradation due to effects of the xanthin oxidase enzyme. The diabetic heart has increased AMP levels due to defects in generation of ATP (Warner *et al.* 1986); *3) when the formation of $.O_2^-$ is increased, the probability of H_2O_2 formation is enhanced by spontaneous $.O_2^-$ dismutation* (Boveris and Chance 1973).

The reaction of CAT, one of the two H_2O_2-detoxicating enzymes (i.e. CAT and GSH-PX), indicates that excessive amounts of peroxide are present since CAT protects cells against high H_2O_2 levels, whereas a GSH-dependent mechanism is sensitive to its low concentrations (Doroshow *et al.* 1980). GSH-PX has a high substrate affinity but its rate is limited by sluggish glutathione recycling. In accordance with the results obtained by Wohaieb and Godin (1987), who have studied the influence of chronic diabetes (STZ) on the antioxidative state of rat tissues no changes of GSH-PX activity were found in our experiment. Reduced GST activity and unchanged GSH-PX activity indicated that neither of these enzymes consumed GSH at an increased rate. Nevertheless, the GSH concentration in the heart of diabetic animals decreased. The GSH levels are maintained by GSSG reduction catalyzed by glutathione reductase (GSH-RD), an NADPH-dependent enzyme. The glucose metabolism relates to GSH regeneration through NADPH formation in the pentose cycle, due to activity of the glucose-6-phosphate-dehydrogenase enzyme. Metabolic defects of the myocardium in diabetes can be manifested by NADPH deficiency due to the reduced ability of GSH-RD to react to the increased formation of reactive oxygen species. GSH-RD activity was not determined in the present report but Wohaieb and Godin (1987) found enhanced activity of this enzyme in the myocardium of diabetic rats (STZ) in the course of 12 weeks. However, the duration of the disease can affect the antioxidative state of this tissue, as was confirmed by the results of Armstrong and Al-Awadi (1991) who studied the levels of MDA in the plasma of diabetic rats (STZ) depending on the duration of the disease.

Acute diabetes also influenced the activity of antioxidative enzymes in the kidneys. The highest increase concerned the activity of GST and GSH-PX. Activity of these enzymes is approximately three times lower in the kidney that in the heart; that of CAT and SOD (whose activity is fairly high in the kidney, particularly if expressed per gram of tissue) showed no statistically significant changes. Wohaieb and Godin (1987) found significantly increased GSH-PX activity levels in 12-week-old diabetes (STZ), and even decreased renal CAT and SOD activity. In connection with this finding these authors postulated that, under oxidative stress conditions, the activity of those enzymatic components of the antioxidative defense mechanisms whose activity in a given tissue is relatively low in comparison with other tissues is increased (Wohaieb and Godin 1987); this is in accordance with our experimental results.

In conclusion, our data have shown that peroxidative damage of the myocardium is manifested in acute diabetes, with corresponding changes in the antioxidative state of this tissue. Increased SOD activity points to increased $.O_2^-$ formation, and the considerably increased CAT activity probably indicates increased endogenous H_2O_2 production. Acute diabetes causes changes in the activity of antioxidative enzymes in the kidneys as well, with excess toxic radicals and H_2O_2 which are primarily decomposed by a GSH-dependent mechanism.

A further study is necessary to explain the role of reactive oxygen-derived radicals in the development of diabetes and its complications.

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