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Renal antioxidant enzyme mRNA levels are increased in rats with experimental diabetes mellitus

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Summary Exposure to high glucose concentrations increases the mRNA levels of oxygen radical scavenging enzymes in cultured endothelial cells, suggesting a compensatory response to increased free radical production. To test the hypothesis that this response also occurs in vivo, Cu,Zn-superoxide dismutase (Cu,Zn-SOD) and catalase mRNA levels, were measured in the kidneys of Sprague-Dawley rats 17 days after intravenous injection of streptozotocin (60 mg/kg body weight) and compared with those of control rats. Diabetic rats were either left untreated or given differing insulin regimens (2, 3–8, 6–10 IU/day) in two different experiments that were designed to achieve varying degrees of metabolic control. Cu,Zn-SOD and catalase mRNA levels were measured by Northern blot hybridization and standardized by 28S ribosomal RNA determination. Renal Cu,Zn-SOD and catalase mRNA levels were significantly greater in untreated diabetic and in low-dose (2 IU/day) insulin-treated rats than in controls. Treatment with a moderate dose (3–8 IU/day) of insulin normalized catalase but not Cu,Zn-SOD mRNA levels. The highest insulin regimen (6–10 IU/day), in addition to achieving complete metabolic control as evidenced by normal growth and plasma glucose levels, normalized both catalase and Cu,Zn-SOD mRNA levels. Thus, in rats with streptozotocin-induced diabetes Cu,Zn-SOD and catalase renal mRNA levels are greater than in normal rats. This difference is prevented by sufficient insulin dosage to normalize plasma glucose and might be due to an increased production of free radicals. [Diabetologia (1997) 40: 23–29]

Keywords Catalase, messenger RNA, kidney, insulin, superoxide dismutase.

Although the pathophysiology of diabetic complications is probably multifactorial, the results of in vitro [1], animal [2], and human [3] studies suggest a role for oxidative stress via an increased formation of free radicals. Increased generation of reactive oxygen metabolites such as superoxide anion and hydrogen peroxide has been shown to occur in diabetes mellitus in association with hyperglycaemia [4]. An increased glucose concentration can induce formation of free radicals and activation of oxidative stress through nonenzymatic glycation of protein substrates [5], auto-oxidative glycation [6], activation of protein kinase C [7] and increased polyol pathway [8].

Excessive generation of reactive oxygen metabolites also plays a role in the pathophysiology of a variety of clinical and experimental renal diseases [9]. These diseases include acute and chronic, glomerular and tubular disorders through both immunological and non-immunological mechanisms. In the kidney, as in other organs, endogenous antioxidant enzymes (AOEs) protect cells against the toxic effect of free radicals and are an essential defence system against oxidant injury. Superoxide dismutases (SODs) exist in two forms containing copper/zinc (Cu,Zn-SOD) and manganese (Mn-SOD), respectively, and

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Abbreviations: SOD, Superoxide dismutase; STZ, streptozotocin; AOE, endogenous antioxidant enzymes; SDS, sodium dodecyl sulphate; SSC, standard sodium citrate

transform the superoxide anion into hydrogen peroxide. Catalase catalyses the transformation of hydrogen peroxide to yield oxygen and water. Previous studies have indicated that the activity of SODs and catalase is modulated by many stimuli and is regulated to meet the biological need imposed by oxidant stress [10]. In some of these studies, transcriptional regulation of steady-state mRNA levels appeared to be a major determinant of local antioxidant enzyme levels [11, 12]. The present study was designed to evaluate the expression of the genes for Cu,Zn-SOD and catalase in kidney tissue of rats with experimentally induced diabetes which were either left untreated or given different insulin regimens designed to achieve varying degrees of metabolic control.

Materials and methods

Animals. Male Sprague-Dawley rats (Bantin and Kingman, Fremont, Calif., USA), ranging in weight from 150 to 200 g, were maintained on standard rat chow and tap water ad libitum with 12-h light/dark cycles in a quiet environment. Diabetes was induced by intravenous administration of streptozotocin (STZ) (Sigma, St. Louis, Mo., USA) 60 mg/kg body weight, dissolved in sodium citrate buffer (0.1 mol/l, pH 4.5) at a concentration of 20 mg/ml immediately before use. Control rats, which were matched for age and weight at the time of STZ administration, received an equal volume of the vehicle. Rats were considered diabetic if blood glucose levels were greater than 6.7 mmol/l 72 h after the injection.

Experiment 1. Diabetic rats were further divided into two subgroups: untreated rats ($n = 6$), and rats to which human recombinant insulin (Humulin N, Lilly, Indianapolis, Ind., USA) was administered once daily (at 20.00 hours) in moderate doses (3–8 IU/day), that were adjusted to prevent severe hyperglycaemia ($n = 6$). Insulin treatment was begun 3 days after STZ injection. In control rats ($n = 6$) and untreated diabetic rats, blood glucose levels were measured every day by tail vein sampling (Accu-chek bG, Bio-Dynamics, Boehringer-Mannheim, Indianapolis, Ind., USA), whereas in the treated animals, insulin dosage was adjusted on the basis of daily determinations at 08.00 and 20.00 hours. Rats were killed by decapitation 17 days after administration of STZ. Trunk blood was collected in fasted animals to measure plasma glucose and insulin levels. The kidneys were removed quickly, rinsed in phosphate buffered saline, weighed, and frozen in liquid nitrogen for subsequent RNA isolation.

Experiment 2. This experiment was performed with the same protocol except that one of the diabetic subgroups received a fixed low dose of insulin ($n = 6$) (2 IU/day) to prevent ketosis, and the other subgroup ($n = 6$) a higher dose (6–10 IU/day), which was adjusted to achieve euglycaemia. Seventeen days after STZ administration rats were killed and kidneys handled as indicated above.

RNA analysis. Total RNA was isolated from frozen tissue by a modification of the guanidine thiocyanate method of Chirgwin et al. [13], as described previously [14]. Briefly, after storage at – 80 °C, the tissue was homogenized in a solution containing 4 mol/l guanidine thiocyanate, 25 mmol/l sodium citrate (pH 7.0), 0.5 % N-lauroylsarcosine, and 0.1 mol/l β-mercaptoethanol in a ratio of 16 ml/g tissue mass. Total nucleic acid was precipitated by the addition of 0.025 % volume of 1 mol/l acetic acid, 50 % volume of ethanol, and incubation at – 20 °C for a minimum of 4 h, followed by centrifugation at 12 000 *g* at – 10 °C for 20 min. The pellet was drained briefly and resuspended in a solution containing 7.5 mol/l guanidine hydrochloride, 25 mmol/l sodium citrate, and 4.5 mmol/l dithiothreitol, in a ratio of 8 ml/g tissue. Total RNA was then precipitated as indicated above. The resultant pellet was dissolved in sterile water and the total RNA concentration was quantitated by ultraviolet absorbance at 260/280 nm. RNA integrity was verified by agarose gel electrophoresis.

A 0.45-kilobase (kb) ^{32}P-labelled antisense Cu,Zn-SOD cRNA was synthesized using a *Xho* I linearized SP65 vector containing the 450-bp *Alu* I to *Taq* I human Cu,Zn-SOD cDNA insert [15], as previously described [16]. A 0.468-kb ^{32}P-labelled antisense catalase cRNA was synthesized using a *Xho* I linearized SP65 vector containing the 1250-bp *Hind* III to *Pvu* II human catalase cDNA insert [17], as previously described [16]. Probe purification was performed by spin column centrifugation using a Sephadex G-50 RNA purification Quick Spin column (Boehringer Mannheim). Cu,Zn-SOD and catalase mRNA levels were quantitated by Northern blot hybridization analysis [14]. Nylon membranes were prehybridized in a solution containing 50 mmol/l Tris (pH 7.5), 0.1 % sodium pyrophosphate, 1 % sodium dodecyl sulphate (SDS), 0.2 % poly(vinylpyrrolidone), 0.2 % Ficoll, 5 mmol/l EDTA, 50 % formamide, 0.2 % bovine serum albumin, 1 × standard sodium citrate (SSC), and 150 μg/ml denatured salmon sperm DNA at 65 ° C for 6 h. Hybridization was performed using fresh prehybridization solution with the addition of radiolabelled Cu,Zn-SOD or catalase antisense cRNA at a concentration of 1×10^6 counts · min^{-1} · ml^{-1}. Hybridization was performed at 65 °C for 16–18 h. After hybridization membranes were washed sequentially at 65 °C, twice for 15 min with 2 × SSC – 0.1 %. SDS and twice for 15 min with 0.1 × SSC – 0.1 % SDS. Autoradiographs were obtained by exposing the membranes to Cronex X-ray film (DuPont, Boston, Mass., USA) with an intensifying screen at – 80 °C for 15 h for Cu,Zn-SOD and for 5 days for catalase. Films were scanned with a laser densitometer (LKB Ultroscan, Piscataway, N. J., USA). Duplicate membranes were prepared and hybridized with a ^{32}P-labelled oligonucleotide probe complementary to bases 4011–4036 of human 28S ribosomal RNA [14].

Plasma glucose and insulin concentrations. Plasma glucose and insulin samples were obtained at time of killing. Plasma glucose was determined using a YSI Model 23A glucose autoanalyser (Yellow Springs Instrument, Yellow Springs, Ohio, USA). Plasma insulin levels were determined by radioimmunoassay, using a double antibody technique [18].

Statistical analysis. Data are presented as means ± SEM. Comparisons between groups were done by analysis of variance (Statview, Abacus, Berkeley, Calif., USA), and the relationships between blood glucose, plasma insulin, and mRNA levels were examined by linear regression analysis. Differences were considered to be statistically significant when p was less than 0.05.

Results

Hyperglycaemia occurred within 3 days of STZ administration in both experiments. Because of slight differences in age of rats and activity of STZ, blood

Table 1. Body weight and blood glucose in control and diabetic rats with and without insulin treatment

Group	(Rats) (n)		Day 0	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16
Experiment 1										
Control	6	BW	153 ± 2	183 ± 2	198 ± 3	213 ± 2	230 ± 1	243 ± 3	255 ± 3	270 ± 3
		BG	6.7 ± 0.2	7.7 ± 0.2	5.6 ± 0.3	6.3 ± 0.4	6.7 ± 0.4	5.6 ± 0.5	5.9 ± 0.5	6.3 ± 0.3
Diabetes	6	BW	152 ± 2	162 ± 2	173 ± 3	180 ± 3	185 ± 4	193 ± 4	201 ± 7	211 ± 8
		BG	6.6 ± 0.2	18.5 ± 1.5	19.8 ± 1.8	20.1 ± 0.7	20.6 ± 0.8	21.5 ± 0.6	22.1 ± 0.1	> 22.2
Diabetes + insulin (3–8 IU/day)	6	BW	149 ± 1	163 ± 6	183 ± 5	204 ± 4	216 ± 5	240 ± 6	257 ± 8	268 ± 7
		BG	6.8 ± 0.3	18.2 ± 1.8	18.8 ± 1.5	15.9 ± 3.3	11.6 ± 2.8	9.8 ± 0.9	10.9 ± 0.6	13.6 ± 2.3
Experiment 2										
Control	6	BW	198 ± 2	237 ± 2	249 ± 3	261 ± 3	276 ± 4	288 ± 3	297 ± 4	304 ± 6
		BG	–	6.7 ± 0.3	6.9 ± 0.2	6.7 ± 0.3	6.8 ± 0.2	8.4 ± 0.6	6.7 ± 0.3	6.6 ± 0.2
Diabetes + insulin (2 IU/day)	6	BW	198 ± 2	201 ± 2	207 ± 2	210 ± 3	221 ± 4	230 ± 6	231 ± 4	235 ± 8
		BG	–	> 22.2	> 22.2	> 22.2	> 22.2	> 22.2	> 22.2	> 22.2
Diabetes + insulin (6–10 IU/day)	6	BW	198 ± 2	199 ± 2	222 ± 3	236 ± 2	264 ± 4	276 ± 4	283 ± 4	300 ± 4
		BG	–	> 22.2	19.2 ± 1.7	21.3 ± 0.6	7.4 ± 1.3	9.8 ± 1.7	10.1 ± 2.1	4.9 ± 0.4

Values are means ± SEM. BW, Body weight in g; BG, blood glucose in mmol/l. Blood glucose was measured by tail vein sampling and Accu-Check.

Table 2. Effect of STZ-induced diabetes with and without insulin treatment on body weight, kidney weight/body weight ratio, plasma glucose, insulin concentration, renal Cu,Zn-SOD mRNA, renal catalase mRNA, and renal 28S rRNA in the rat

Group	Rats (n)	Body weight (g)	Plasma glucose (mmol/l)	Kidney weight/ body weight ratio (× 1000)	Plasma insulin (pmol/l)	Cu,Zn-SOD mRNA (scanner units)	Catalase mRNA (scanner units)	28S rRNA (scanner units)
Experiment 1								
Control	6	270 ± 3	8.1 ± 0.2	6.8 ± 0.3	52 ± 5	14.5 ± 0.8	4.3 ± 0.5	14.0 ± 0.6
Diabetes	6	211 ± 8[a]	29.6 ± 2.6[a]	9.9 ± 0.2[a]	29 ± 4[b]	39.4 ± 5.2[c]	7.2 ± 0.6[c]	13.2 ± 0.8
Diabetes + insulin (3–8 IU/day)	6	268 ± 7	14.4 ± 2.8	7.7 ± 0.3	62 ± 4	33.7 ± 3.6[c]	5.0 ± 0.4	13.2 ± 0.5
Experiment 2								
Control	6	304 ± 6	6.9 ± 0.2	7.6 ± 0.2	35 ± 3	10.7 ± 1.2	4.4 ± 0.3	15.4 ± 1.0
Diabetes + insulin (2 IU/day)	6	235 ± 8[a]	26.1 ± 1.0[a]	11.4 ± 0.3[a]	10 ± 1[a]	18.3 ± 2.6[d]	5.6 ± 0.3[e]	14.9 ± 0.7
Diabetes + insulin (6–10 IU/day)	6	300 ± 4	6.2 ± 0.6	8.3 ± 0.1[c]	80 ± 10[a]	12.9 ± 1.3	3.4 ± 0.4	15.7 ± 0.8

Values are means ± SEM. Measurements of body weight, kidney weight, plasma glucose, and plasma insulin were done at time of death. Plasma glucose and plasma insulin were measured on trunk blood. Comparisons were done by analysis of variance. Due to differences in the age of the rats, activity of STZ, acitivity of ^{32}P at time of use, and duration of exposure on film no comparisons could be done between experiment 1 and experiment 2. mRNA values are normalized with 28S rRNA. [a] $p < 0.001$; [b] $p < 0.005$; [c] $p < 0.01$; [d] $p < 0.025$; [e] $p < 0.05$ vs the respective control

glucose values of diabetic rats were higher in experiment 2 than in experiment 1. During the experiments, hyperglycaemia persisted in both the untreated diabetic rats and in those that received low-dose (2 IU/day) insulin therapy (Table 1), was less severe in the subgroup that received moderate doses (3–8 IU/day) of insulin, and was ameliorated completely in those that received the highest dose (6–10 IU/day) (Table 1). Rats treated with the two highest doses of insulin gained weight rapidly, reaching values not significantly different from control animals at the end of the experimental period (Table 1, Table 2). The kidney weight/body weight ratio was significantly increased in both the untreated diabetic rats and in those that received low-dose insulin therapy, indicating the presence of renal hypertrophy that was prevented by both moderate- and high-dose insulin regimen (Table 2). Plasma insulin levels were reduced significantly in both the untreated and low-dose insulin-treated animals, equivalent to controls in those that received moderate doses, and significantly greater than controls in those that received the highest doses of insulin (Table 2).

Renal Cu,Zn-SOD mRNA levels were significantly greater in untreated diabetic and in low-dose (2 IU/day) insulin-treated rats than in controls (Table 2, Fig. 1). Treatment with a moderate dose (3–8 IU/day) of insulin decreased Cu,Zn-SOD mRNA levels but did not normalize them (Table 2, Fig. 1). The highest insulin regimen (6–10 IU/day), in

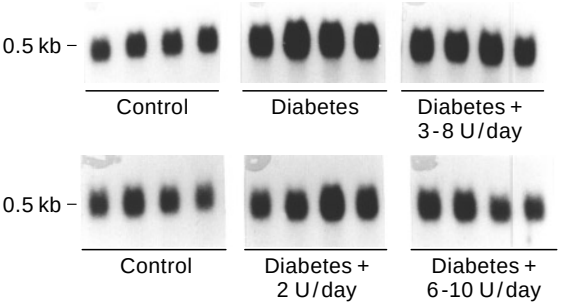

Fig. 1. Representative Northern blot of Cu,Zn-SOD mRNA extracted from kidney of control and diabetic rats treated with different insulin doses in experiment 1 (upper panel) and experiment 2 (lower panel). Total RNA (40 μg) was electrophoresed through a 1 % agarose gel, transferred to a nylon filter, and hybridized with a ^{32}P-labelled Cu,Zn-SOD probe at 65 °C. The probe was synthesized using a *Xho* I linearized SP65 vector containing 450-bp *Alu* I to *Taq* I human Cu,Zn-SOD cDNA insert. Size of bands indicated in kb

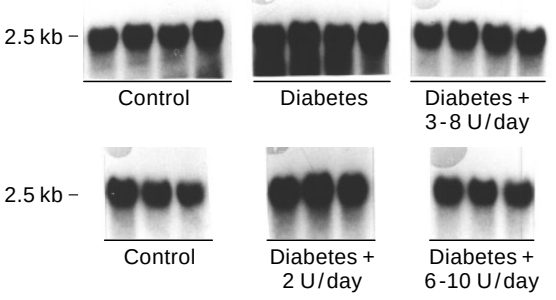

Fig. 2. Representative Northern blot of catalase mRNA extracted from kidneys of control and diabetic rats treated with different insulin doses in experiment 1 (upper panel) and experiment 2 (lower panel). Total RNA (40 μg) was electrophoresed through a 1 % agarose gel, transferred to a nylon filter, and hybridized with a ^{32}P-labelled catalase probe at 65 °C. The probe was synthesized using a *Xho* I linearized SP65 vector containing the 1250-bp *Hind* III t_0 *Pvu* II human catalase cDNA insert. Size of bands is indicated in kb

addition to achieving complete metabolic control, as ascertained by normal weight growth and blood glucose levels, normalized Cu,Zn-SOD mRNA levels. Similar to Cu,Zn-SOD, catalase mRNA levels were significantly greater in untreated diabetic and in low-dose insulin-treated rats than in controls (Table 2, Fig. 2). Treatment with both moderate and high-dose insulin normalized catalase mRNA levels (Table 2, Fig. 2). As shown in Table 2, 28S rRNA levels were not affected by STZ-diabetes and are an appropriate standard by which to normalize mRNA levels in diabetic rats.

In both experiments, no significant correlations between plasma glucose and renal mRNA levels of both Cu,Zn-SOD and catalase where found when only the diabetic groups were considered. When all the rat groups were included, renal mRNA levels of Cu,Zn-SOD and catalase were directly correlated in

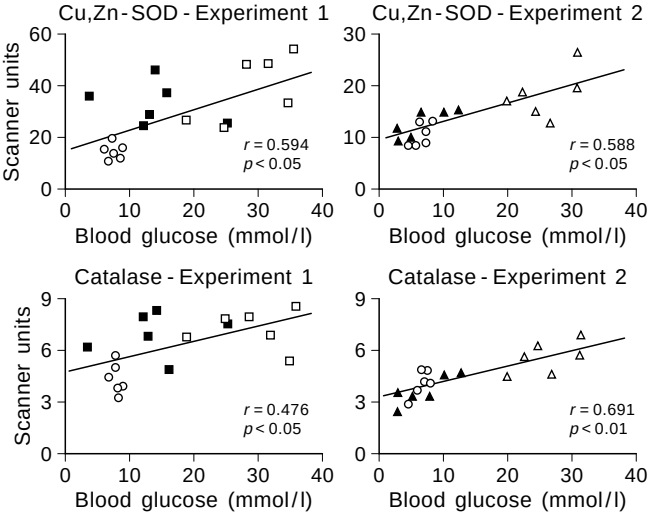

Fig. 3. Relationships between final levels of plasma glucose and the renal, Cu,Zn-SOD mRNA levels (upper panels) and the renal catalase mRNA levels (lower panels) in experiment 1 (left panels) and experiment 2 (right panels). Control rats, ○; untreated diabetic rats, □; diabetic rats treated with moderate insulin dose (3–8 IU/day), ■; diabetic rats treated with low insulin dose (2 IU/day), △; diabetic rats treated with high insulin dose (6–10 IU/day), ▲

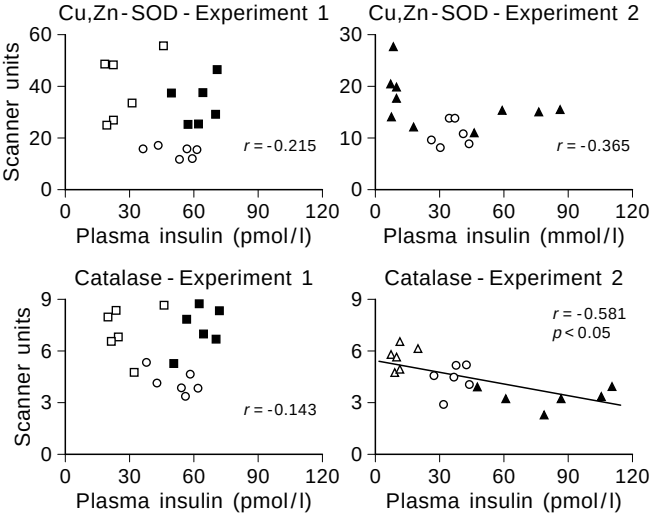

Fig. 4. Relationships between final levels of plasma insulin and the renal Cu,Zn-SOD mRNA levels (upper panels) and the renal catalase mRNA levels (lower panels) in experiment 1 (left panels) and experiment 2 (right panels). Control rats, ○; untreated diabetic rats, □; diabetic rats treated with moderate insulin dose (3–8 IU/day), ■; diabetic rats treated with low insulin dose (2 IU/day), △; diabetic rats treated with high insulin dose (6–10 IU/day), ▲

both experiment 1 ($r = 0.55$, $p < 0.05$) and experiment 2 ($r = 0.47$, $p < 0.05$). In both experiments, a significant positive correlation was observed between the level of renal Cu,Zn-SOD (experiment 1: $r = 0.59$, $p < 0.05$; experiment 2: $r = 0.59$, $p < 0.05$) and catalase (experiment 1: $r = 0.48$, $p < 0.05$;

experiment 2: $r = 0.69$, $p < 0.01$) mRNA and the blood concentration of glucose obtained at the time of death (Fig. 3). A significant inverse correlation was observed between catalase mRNA and plasma insulin only in experiment 2 (experiment 1: $r = -0.14$; experiment 2: $r = -0.58$, $p < 0.05$) (Fig. 4). No significant correlation was found between Cu,Zn-SOD and insulin (experiment 1: $r = -0.22$; experiment 2: $r = -0.37$) (Fig. 4). In both experiments, no correlations were observed between renal mRNA levels of both enzymes and the kidney weight/body weight ratios.

Discussion

The results of this study indicate that the steady-state mRNA levels of Cu,Zn-SOD and catalase are increased in the kidneys of rats with STZ-induced diabetes in comparison to control rats. In diabetic rats, treatment with an insulin dose designed to achieve euglycaemia normalized the mRNA levels of both antioxidant enzymes. A significant direct correlation was observed between the levels of blood glucose at the end of the experiments and renal mRNA levels of both Cu,Zn-SOD and catalase, suggesting that high glucose concentration in plasma and/or tissue affects the gene expression of renal AOEs. Treatment with a moderate dose of insulin normalized catalase but not Cu,Zn-SOD mRNA levels, suggesting a different threshold in the response of these genes to glucose concentration. Exposure to high glucose concentrations increases both the activity and the mRNA levels of Cu,Zn-SOD and catalase in cultured endothelial cells, suggesting a compensatory effect to counter increased free radical production [19]. The present findings extend this observation to an in vivo setting showing that the gene expression of antioxidant enzymes is associated with greater glucose concentration also in animal tissues.

Increased formation of reactive oxygen metabolites occurs in diabetes for reasons related to the presence of increased glucose concentration in plasma and tissues [4]. It is known that glycated proteins, which are increased in diabetes as a result of nonenzymatic glycation, may form superoxide anions under physiological conditions [5]. In addition to inducing direct glycation reactions, monosaccharides can enolize and thereby reduce molecular oxygen, yielding hydrogen peroxide and free radicals in a reaction known as "auto-oxidative glycation" [6]. Further hypothetical mechanisms that might account for increased free radical generation in diabetic tissues are the hyperglycaemia-induced activation of protein kinase C [7, 20] and activation of the polyol pathway [8]. Because excessive generation of reactive oxygen metabolites is the most frequent condition in which the activity and gene expression of free radical scavenging enzymes is increased [10] and greater levels of antioxidant enzymes are strong indicators of increased formation of reactive oxygen species [21], the increase in AOE mRNA levels that we found in kidneys of diabetic rats might well occur as a result of an excessive production of free radicals.

The activity of AOEs has been measured in the renal tissue of rats with chemically induced diabetes. Cu,Zn-SOD has been found to be decreased [22, 23] or comparable to normal controls [24–26], whereas catalase has been found to be decreased [22, 27], increased [23], or comparable to normal [26]. The basis for the discrepancies of these studies is not clear, although differences in severity, duration, and treatment of diabetes may be indicated. In addition, analysis of data from various investigators suggests the possibility that there may be transient changes in AOE activity after induction of diabetes. For instance, decrease in tissue AOEs may occur as a result of the acute toxicity of STZ or acute hyperglycaemia [24], whereas, after prolonged hyperglycaemia, induction of AOEs might occur to meet the oxidant stress [28]. The findings on AOE activity also appear to be in conflict with the observation of an increased mRNA level of both Cu,Zn-SOD and catalase in diabetic kidneys. In this respect, it is important to note that, in addition to the already mentioned differences in the experimental protocols, other factors might be involved. First, AOEs might undergo nonoxidative glycation and this could impair their activity when this activity is assayed in vitro. Second, increased oxidative stress, as indicated by increased levels of lipid peroxides and 8-hydroxydeoxiguanosine in plasma, urine [29–31], and renal tissue [32, 33] of diabetic rats, might interfere with the in vitro assessment of AOE activity. Finally, an increase in AOEs has been shown to be the commonest response to an increased oxidative stress in other rat models of kidney disease characterized by early and massive proteinuria [9, 34, 35] and therefore it might be reasonable to expect a similar change in diabetes.

It is important to note that, in this study, renal mRNA levels of AOEs have been measured in whole tissue extracts. One might then argue that, since diabetic nephropathy involves mainly the renal glomeruli, the increased AOE mRNA levels that we found in diabetic rats might reflect glucose-mediated processes unrelated to glomerulopathy. With regard to this observation, we want to point out that glomerular abnormalities found in early diabetic nephropathy are characterized mainly by an increased renal and glomerular blood flow occurring as a consequence of reduced arteriolar resistance [36]. Therefore, the abnormality resides outside the glomerulus per se, most likely in vascular structures, such as afferent and efferent arterioles, but also in larger vessels that are ubiquitous in the kidney. Moreover, renal tubules are not completely spared by the pathological process [37] and could either be the source or the target of

biochemical damage due to high glucose. Finally, although AOEs are reasonably compartmentalized, biological membranes are highly permeable to oxidants [38] and thus the site of oxidant injury may be distant from the site of oxidant generation.

Excessive amounts of reactive oxygen species may be generated as a consequence of metabolic adaptation of surviving nephrons to loss of renal mass. Indeed, there are studies in the remnant kidney model in which increased urinary excretion of lipid peroxidation products has been shown as a likely result of increased generation of reactive oxygen species [39]. Although it is possible that the changes in AOE mRNA levels that we have observed in this study are a consequence of renal hypertrophy rather than being due to diabetes per se, substantial biochemical [40, 41], morphological [42, 43], and functional [36, 44] differences between compensatory renal hypertrophy and diabetic renal hypertrophy argue against this possibility. Moreover, although the present study does not clarify the role of diabetic renal hypertrophy in the induction of AOE gene expression, absence of correlations between the mRNA levels of Cu,Zn-SOD and catalase and the kidney weight/body weight ratios makes this role unlikely.

Most of the previous studies concerning the regulation of SOD activity and gene expression focused on the Mn-dependent isoenzyme since this was considered to be the inducible enzyme. The synthesis of the Cu,Zn-dependent isoenzyme was considered to be largely constitutive [45]. In contrast with this view some studies have shown that the activity of Cu,Zn-SOD is regulated in lung tissue as well as that of Mn-SOD [46]. The present findings in a rat model of renal disease confirm that the expression of Cu,Zn-SOD gene can be regulated.

There is evidence that active oxygen metabolites are involved in the toxic action of STZ on the pancreatic islets [47]. It might then be argued that a similar effect might cause activation of expression of AOEs in the kidney of rats injected with STZ, independent of blood glucose concentration. This possibility is unlikely for three reasons. First, there is evidence that STZ is cleared rapidly from the body after accumulation in the pancreas [47] and therefore it is difficult to believe that its effects persist for 2 weeks. Second, the levels of AOE mRNA were normalized by treatment with high doses of insulin in rats that received identical doses of STZ, and this argues strongly against the possibility of an STZ-mediated effect. Third, we observed a significant correlation between blood glucose concentration at the end of the experiment and Cu,Zn-SOD and catalase mRNA levels, suggesting that the expression of AOE genes is under the influence of glucose.

In conclusion, Cu,Zn-SOD and catalase renal mRNA levels are associated with higher blood glucose levels in rats with STZ-induced diabetes. It is possible that an increased production of reactive oxygen metabolites accounts for this increase in AOE gene expression. Reactive oxygen metabolites might be involved in the pathophysiology of diabetic nephropathy similar to other renal pathological conditions.

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