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Early changes in lipid peroxidation and antioxidative defense in diabetic rat retina: effect of DL-α-lipoic acid

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Abstract

This study was designed to (1) evaluate retinal lipid peroxidation in early diabetes by the method specific for free malondialdehyde and 4-hydroxyalkenals, (2) identify impaired antioxidative defense mechanisms and (3) assess if enhanced retinal oxidative stress in diabetes is prevented by the potent antioxidant, DL-α-lipoic acid. The groups included control and streptozotocin-diabetic rats treated with or without DL-α-lipoic acid (100 mg kg^{-1} day^{-1}, i.p., for 6 weeks). All parameters were measured in individual retinae. 4-Hydroxyalkenal concentration was increased in diabetic rats (2.63 ± 0.60 vs. 1.44 ± 0.30 nmol/mg soluble protein in controls, $P < 0.01$), and this increase was prevented by DL-α-lipoic acid (1.20 ± 0.88, $P < 0.01$ vs. untreated diabetic group). Malondialdehyde, reduced glutathione (GSH) and oxidized glutathione (GSSG) concentrations were similar among the groups. Superoxide dismutase, glutathione peroxidase (GSHPx), glutathione reductase (GSSGRed) and glutathione transferase (GSHTrans) activities were decreased in diabetic rats vs. controls. Quinone reductase was upregulated in diabetic rats, whereas catalase and cytoplasmic NADH oxidase activities were unchanged. DL-α-Lipoic acid prevented changes in superoxide dismutase and quinone reductase activities induced by diabetes without affecting the enzymes of glutathione metabolism. In conclusion, accumulation of 4-hydroxyalkenals is an early marker of oxidative stress in the diabetic retina. Increased lipid peroxidation occurs in the absence of GSH depletion, and is prevented by DL-α-lipoic acid.

Keywords: Antioxidant; Diabetic retinopathy; Glutathione; Lipid peroxidation; Superoxide dismutase

1. Introduction

Chronic hyperglycemia is the major determinant of diabetic vascular complications, including diabetic retinopathy (Kohner et al., 1998; The Diabetes Control and Complications Trial Research Group, 1993). One of the important consequences of ''glucose toxicity'' in the diabetic retina is oxidative stress, i.e. increased production of reactive oxygen species combined with impaired antioxidative defense. Hyperglycemia-induced oxidative stress has been documented in pericytes (Romeo et al., 1999), endothelial (Paget et al., 1998) and Muller (Hammes et al., 1997) cells and has been implicated in pericyte and endothelial cell apoptosis (Romero et al., 1999), formation of pericyte ghosts (Ansari et al., 1998) and acellular capillaries (Hammes et al., 1997), and increased vascular permeability (Ellis et al., 1998). The attempts to identify early markers of retinal oxidative injury resulted in contradictory findings. One group (Kern et al., 1995) has reported a decrease in reduced glutathione (GSH) concentration as early as 2 months after induction of diabetes, whereas others (Agardh et al., 1998) failed to find these changes. In addition, the assay of thiobarbituric acid reactive substances, used in some studies for evaluating retinal lipid peroxidation (Hammes et al., 1997; Kowluru et al., 1996; Simonelli et al., 1989), has a number of serious limitations (Esterbauer and Cheeseman, 1990; Lefevre et al., 1998; Mihara et al., 1980). Thiobarbituric acid forms complexes with many substances, including glucose (Gutteridge, 1981). Less that 40% of the product, measured as thiobarbituric acid reactive substances in whole tissue homogenates, is quantified as malondialdehyde by high-performance liquid chromatography (HPLC) (Esterbauer and Cheeseman, 1990).

The aims of the present study were (1) to estimate whether increased lipid peroxidation occurs in the retina in

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short-term diabetes by assessing the concentrations of 4-hydroxyalkenals and malondialdehyde by the new method, specific for free malondialdehyde and 4-hydroxyalkenals (Erdelmeier et al., 1998), (2) to identify components of antioxidative defense impaired in early diabetes and (3) to assess whether early changes in parameters of oxidative stress and antioxidative defense in the diabetic retina are prevented by DL-α-lipoic acid, the potent antioxidant (Packer et al., 1995) penetrating through the blood–retinal barrier (Block and Schwartz, 1997).

2. Materials and methods

The experiments were done in accordance with regulations specified by The Guiding Principles in the Care and Use of Animals (DHEW Publication, NIH 80-23) and the University of Michigan Protocol for Animal Studies.

2.1. Animals

Barrier-sustained, Caesarean-delivered male Wistar rats (Charles River, Wilmington, MA, USA), body weight 250–300 g, were fed a standard rat chow diet (ICN Biomedicals, Cleveland, OH) and had access to water ad libitum. Diabetes was induced by a single intraperitoneal injection of streptozotocin (Upjohn, Kalamazoo, MI, USA, 55 mg/kg body weight, i.p., in 0.2 ml of 10 mmol/l citrate buffer, pH 5.5) to animals fasted overnight. Blood samples for measurements of glucose were taken from the tail vein 48 h after the streptozotocin injection and the day before they were killed. The rats with blood glucose of 13.9 mmol/l or more were considered as diabetic. The experimental groups comprised control and untreated diabetic rats as well as diabetic rats treated with DL-α-lipoic acid (Sigma, St. Louis, MO, 100 mg kg^{-1} day^{-1}, i.p., for 6 weeks). DL-α-Lipoic acid (Sigma) was dissolved as described by Cameron et al. (1998). The powder was mixed with sterile saline in the dark bottle, and NaOH was added until the suspension dissolved. The pH was then brought to pH 7.4 with HCl. The treatment was started about 48 h after streptozotocin injection.

2.2. Reagents

Unless otherwise stated, all chemicals were of reagent-grade quality, and were purchased from Sigma. Methanol (HPLC grade), perchloric acid, hydrochloric acid, and sodium hydroxide were purchased from Fisher Scientific, Pittsburgh, PA, USA. Ethyl alcohol (200 proof dehydrated alcohol, USP punctilious) was purchased from Quantum Chemical, Tiscola, IL, USA. β-D-Glucose was purchased from Pfanstiehl Laboratories (Waukegan, IL, USA). Kits for malondialdehyde and 4-hydroxyalkenals as well as glutathione peroxidase (GSHPx) assays were purchased from Oxis International (Portland, OR, USA).

2.3. Experimental procedure

Rats were sedated with carbon dioxide and immediately killed by cervical dislocation. The left retinae from each rat were rapidly (~30 s) dissected, frozen in liquid nitrogen and used for measurements of glucose, GSH, and oxidized glutathione (GSSG). The right retinae were used for assessment of total malondialdehyde plus 4-hydroxyalkenals, malondialdehyde, activities of antioxidative defense enzymes (i.e. superoxide dismutase, catalase, GSHPx, glutathione reductase (GSSGRed), glutathione transferase (GSHTrans)), quinone reductase and cytoplasmic NAD oxidase.

2.4. Biochemical measurements

2.4.1. Preparation of perchloric extract

Retinae were homogenized in 1.5 ml of ice-cold 6% perchloric acid. In separate tubes for GSSG measurements, 0.4 ml of homogenate were mixed with 0.2 ml 0.04 M *N*-ethylmaleimide. *N*-ethylmaleimide complexes with GSH (Hissin and Hilf, 1976) thus preventing interference from GSH oxidation. Both sets of samples were centrifuged at $4000 \times g$ for 10 min. After centrifugation, the samples were immediately neutralized with 5 M K_2CO_3 to pH 6–7, and were centrifuged again at $4000 \times g$ for 5 min, to precipitate insoluble $KClO_4$.

2.4.2. Glucose, GSH and GSSG

Glucose and GSH were measured in *N*-ethylmaleimide-free set of perchloric extracts (Lowry and Passonneau, 1972). For GSH measurements, 0.1 ml of extract was mixed with 0.89 ml of 20 mM EDTA in 1.0 M Tris–HCI buffer (pH 8.1), and the reaction was initiated by the addition of 0.01 ml *O*-phthaldialdehyde (10 mg/1 ml methanol). The difference in initial and final readings (λ excitation: 345 nm; λ emission: 425 nm) was compared to those in the corresponding GSH standards ($0.5–10 \times 10^{-9}$ M) processed in the same run. GSSG levels were assayed in *N*-ethylmaleimide-containing perchloric extracts spectrofluorometrically, by both enzymatic procedure with GSSGRed (Obrosova and Stevens, 1999) and non-enzymatic procedure with *O*-phthaldialdehyde. The analytical mixture for GSSG assay with GSSGRed contained 0.8 ml 0.1 M buffer, pH 7.6, 0.2 ml of extract, and 0.2–10 μM NADPH. The reaction was started by addition of approximately 0.3 U of GSSGRed (Type IY; Sigma). The difference in initial and final readings (λ excitation: 340 nm; λ emission: 460 nm) was compared to those in the corresponding GSSG standards ($0.1–1 \times 10^{-9}$ M) processed in the same run. The analytical mixture for GSSG assay with *O*-phthaldialdehyde contained 0.89 ml 1.0 M Tris–HCI buffer, pH 12.0 and 0.1 ml perchloric extract. The reaction was initiated by addition of 0.01 ml *O*-phthaldialdehyde (10 mg/1 ml methanol). The difference in initial and final readings (λ excitation: 345 nm; λ

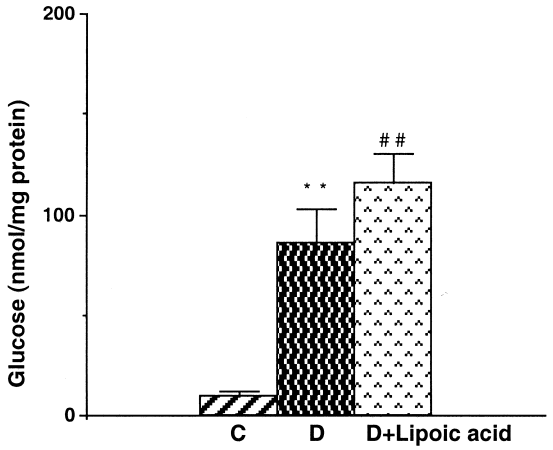

Fig. 1. Retinal glucose concentrations (means ± s.d.) in control and diabetic rats treated with or without lipoic acid. Number of observations: $n = 10$ (controls); $n = 8$ (diabetic); $n = 8$ (diabetic + lipoic acid). $^{**}P < 0.01$ vs. controls. $\#\#P < 0.01$ vs. untreated diabetics.

emission: 425 nm) was compared to those in the corresponding GSSG standards ($0.1–1 \times 10^{-9}$ M) processed in the same run.

2.4.3. Preparation of samples for measurements of lipid aldehydes, antioxidative defense enzymes, quinone reductase and NADH oxidase activities

Retinae were homogenized in 1.5 ml of ice-cold 0.1 M sodium-phosphate buffer, pH 6.5. The homogenate was centrifuged at $3000 \times g$ for 10 min to remove large particles (A). An amount of 0.2 ml of supernatant was used for measurements of total malondialdehyde plus 4-hydroxyalkenal levels, and another 0.2 ml for malondialdehyde levels alone. The rest of the supernatant from step A was centrifuged at $20{,}000 \times g$ (B). The supernatant fractions from step B were used for assays of enzymatic activities.

2.4.4. 4-Hydroxyalkenals and malondialdehyde

Measurements of malondialdehyde plus 4-hydroxyalkenals and malondialdehyde were performed using kits from Oxis International (LPO-586 assay). The method is based on the reaction of a chromogenic reagent, *N*-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals at 45°C, and is specific for free malondialdehyde and 4-hydroxyalkenals. The absorbance of chromogenic products was measured at 586 nm (spectrophotometer Beckman DU 640), and was compared to the absorbance in corresponding standards. 4-Hydroxyalkenal levels have been calculated for individual samples as the difference between total malondialdehyde plus 4-hydroxyalkenal and malondialdehyde levels.

2.4.5. Antioxidative defense enzymes, quinone reductase and cytoplasmic NADH oxidase

Superoxide dismutase, GSHPx, GSSGRed, GSHTrans, quinone reductase and cytoplasmic NADH oxidase activities were assayed as we have described (Obrosova et al., 1999). Catalase activity was measured spectrophotometrically (at 240 nm) by following the decrease in absorbance of hydrogen peroxide after addition of 0.1 ml of supernatant to 0.9 ml of 15 mM H_2O_2 in 50 mM phosphate buffer, pH 6.8 (Aebi, 1974).

2.4.5.1. Protein. The pellets, obtained after the first centrifugation during perchloric acid extraction, were reconstituted in 0.5 ml 0.1 N NaOH, sonicated and left overnight for complete solubilization and measurements of total protein. These were used for normalization of retinal glucose, GSH and GSSG data. Supernatant protein levels, measured in steps 4A and 4B, respectively, were used for normaliza-

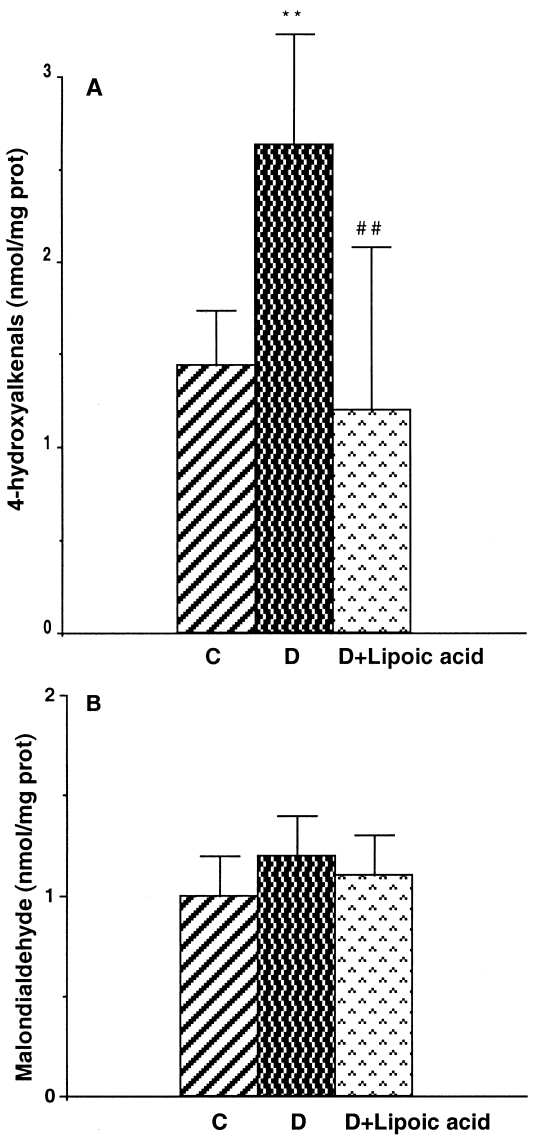

Fig. 2. Retinal 4-hydroxyalkenal (A) and malondialdehyde (B) concentrations (means ± s.d.) in control and diabetic rats treated with or without lipoic acid. Number of observations for 4-hydroxyalkenals: $n = 6$ (controls); $n = 6$ (diabetic); $n = 6$ (diabetic + lipoic acid). Number of observations for malondialdehyde: $n = 8$ (controls); $n = 6$ (diabetic); $n = 8$ (diabetic + lipoic acid). $^{**}P < 0.01$ vs. controls. $\#\#P < 0.01$ vs. untreated diabetics.

Table 1
Glutathione redox status in control and diabetic rats treated with or without DL-α-lipoic acid (levels of GSH and GSSG are expressed in nmol/mg prot)

	Control	Diabetic	Diabetic + lipoic acid
GSH	18.7 ± 3.4 ($n = 8$)	17.2 ± 3.5 ($n = 7$)	18.5 ± 3.7 ($n = 8$)
GSSG (GSSGRed)	0.396 ± 0.148 ($n = 9$)	0.300 ± 0.103 ($n = 7$)	0.310 ± 0.085 ($n = 8$)
GSSG (*O*-phthaldialdehyde)	0.528 ± 0.185 ($n = 9$)	0.611 ± 0.110 ($n = 7$)	0.559 ± 0.088 ($n = 7$)
GSSG/GSH[a]	0.021 ± 0.007 ($n = 8$)	0.021 ± 0.009 ($n = 7$)	0.017 ± 0.004 ($n = 8$)
GSSG/GSH[b]	0.031 ± 0.008 ($n = 8$)	0.036 ± 0.004 ($n = 7$)	0.031 ± 0.006 ($n = 7$)

[a] With GSSG measured with GSSGRed.
[b] With GSSG measured with *O*-phthaldialdehyde.

tion of malondialdehyde and 4-hydroxyalkenal concentrations, and enzyme activities. Proteins were quantified with Pierce BCA protein assay kits (Rockford, IL, USA). One milligram of total retinal protein gives a yield of about 0.3 mg of supernatant protein after step 4A and 0.1 mg of supernatant protein after step 4B.

2.5. Statistical analysis

The results are expressed as means ± standard deviation. Data were subjected to equality of variance *F*-test, and then to log transformation, if necessary (datasets for retinal glucose levels and GSHPx and GSHTrans activities), before one-way analysis of variance. Where overall significance ($P < 0.05$) was attained, individual between-group comparisons were made using the Student–Newman–Keuls multiple range test. Significance was defined at $p < 0.05$. When between-group variance differences could not be normalized by log transformation (datasets for body weights, plasma glucose, retinal 4-hydroxyalkenal concentrations, retinal superoxide dismutase and GSSGRed activities), the data were analyzed by the nonparametric Kruskal–Wallis one-way analysis of variance, followed by the Fisher's PLSD test for multiple comparisons.

3. Results

The final body weights were lower in diabetic rats compared with controls (365 ± 24 vs. 478 ± 16 g, $P < 0.01$). The initial body weights were similar in control and diabetic groups (correspondingly, 292 ± 8 g and 275 ± 18 g). No significant difference was found between body weights in diabetic rats treated with DL-α-lipoic acid (350 ± 31 g) and the corresponding untreated group.

Blood glucose concentrations were increased about fivefold in diabetic rats compared with those in control rats (18.4 ± 2.4 vs. 3.7 ± 0.5 mmol/l, $P < 0.01$). DL-α-Lipoic acid treatment had no effect on blood glucose concentrations in diabetic rats (19.6 ± 3.1).

Retinal glucose concentrations (Fig. 1) were 8.4-fold higher in diabetic rats than in control rats ($P < 0.01$), and 1.3-fold higher in diabetic rats treated with DL-α-lipoic acid than in the corresponding untreated group ($P < 0.01$).

4-Hydroxyalkenal concentrations (Fig. 2A) were increased 1.8-fold in the retinae of diabetic rats compared those in controls ($P < 0.01$). This increase was completely prevented by DL-α-lipoic acid ($P < 0.01$ vs. untreated diabetic group). Retinal malondialdehyde concentrations (Fig. 2B) were similar in control and diabetic rats treated with or without DL-α-lipoic acid.

No difference in retinal GSH concentrations was found among control and diabetic groups treated with or without DL-α-lipoic acid (Table 1). Retinal GSSG concentrations measured by non-enzymatic procedure with *O*-phthaldialdehyde were 1.3–2-fold higher ($P < 0.05$) than those assessed in the same samples by enzymatic method with GSSGRed. Both GSSG levels, measured by either method, and GSSG/GSH ratios were similar in control and diabetic rats treated with or without DL-α-lipoic acid.

Table 2
Activities of antioxidative defense enzymes and NADH oxidase in control and diabetic rats treated with or without DL-α-lipoic acid[a]

	Control	Diabetic	Diabetic + lipoic acid
Superoxide dismutase	3.30 ± 1.35 ($n = 7$)	1.98 ± 0.64* ($n = 8$)	3.79 ± 1.33** $n = 7$)
Catalase	69.5 ± 9.9 ($n = 7$)	88.9 ± 8.3 ($n = 7$)	78.4 ± 24.3 ($n = 7$)
GSHPx	39.5 ± 15.2 ($n = 7$)	24.2 ± 9.8* ($n = 8$)	25.9 ± 10.2* ($n = 8$)
GSSGRed	43.6 ± 24.2 ($n = 8$)	16.7 ± 8.4*** ($n = 8$)	22.9 ± 6.7* ($n = 8$)
GSHTrans	154.4 ± 82.4 ($n = 8$)	76.4 ± 28.1* ($n = 6$)	106.9 ± 35.7 ($n = 8$)
Quinone reductase	22.0 ± 13.5 ($n = 8$)	51.5 ± 16.6*** ($n = 8$)	15.9 ± 7.3** ($n = 8$)
NADH oxidase	3.26 ± 1.69 ($n = 8$)	2.94 ± 1.33 ($n = 7$)	2.67 ± 1.60 ($n = 7$)

[a] Superoxide dismutase activity is expressed in μmol/mg prot/min, other enzymes in nmol/mg prot/min.
* Significantly different from those in controls ($P < 0.05$).
** Significantly different from those in untreated diabetic rats ($P < 0.01$).
*** Significantly different from those in controls ($P < 0.01$).

Retinal superoxide dismutase activity was 1.7-fold lower in diabetic rats than in the control group (Table 2). Catalase activities were similar among control and diabetic rats treated with or without DL-α-lipoic acid. GSHPx, GSSGRed and GSHTrans activities were decreased 1.6-, 2.6- and 2-fold, respectively, in the retinae of diabetic rats compared with those in the non-diabetic group, and none of these activities in the diabetic rats were affected by DL-α-lipoic acid. Quinone reductase activity was increased 2.3-fold in the diabetic rats compared with control rats, and this increase was completely prevented by DL-α-lipoic acid. No difference in cytoplasmic NADH oxidase activities were found among control and diabetic rats treated with or without DL-α-lipoic acid.

4. Discussion

Accumulation of α,β-unsaturated lipid aldehydes, 4-hydroxyalkenals, occurs in the retina as early as 6 weeks after induction of diabetes. 4-Hydroxyalkenals are the most toxic products of lipid peroxidation. The most abundant 4-hydroxyalkenal, 4-hydroxy-*trans*-2-nonenal (Esterbauer, 1993), binds to lysine, histidine and cysteine residues of proteins (Hartley et al., 1997),including enzymes (Uchida and Stadtman, 1993; Chen et al., 1998), with the formation of aldehyde protein adducts. It activates phospholipases C and D (Esterbauer, 1993; Natarajan et al., 1997), affects ion homeostasis through modification of ion-channel activity and the function of ATPases (Mattson, 1998), causes genotoxicity (Esterbauer, 1993) and apoptosis (Kruman et al., 1997).

In contrast to 4-hydroxyalkenals, malondialdehyde levels were similar in the retinae of control and diabetic rats. The differential response of malondialdehyde and 4-hydroxyalkenals is not surprising considering that, in addition to lipid peroxidation, malondialdehyde is formed as a side-product of prostaglandin metabolism (Bose et al., 1989; Naveh-Floman et al., 1984). Retinal arachidonic acid metabolism via the cyclooxygenase pathway is decreased in diabetes (Naveh-Floman et al., 1984) although the expression of constitutive cyclooxygenase is unchanged (Fang et al., 1997). Numerous findings (Chemtob et al., 1995; Hardy et al., 1994; Mochizuki, 1987; Nuhrich et al., 1992; Tanaka et al., 1994a,b; Tokyay et al., 1999; Watson, 1998) indicate that cyclooxygenase inhibitors decrease malondialdehyde levels although some observations (Misselwitz et al., 1989; Morisaki et al., 1984) do not support this point.

In contrast to other target tissues for diabetic complications (Lee and Chung, 1999; Nagamatsu et al., 1995; Obrosova et al., 1998b, 1999) where increased lipid peroxidation parallels depletion of the major biological antioxidant, GSH, the increased lipid peroxidation in the retina in early diabetes occurs in the absence of GSH depletion. Our results are consistent with findings (Agardh et al., 1998) of the lack of GSH depletion in the retinae of rats with 2-month streptozotocin-diabetes. The GSH concentrations measured spectrofluorometrically with *O*-phthaldialdehyde in our study, are very similar to those obtained by HPLC in the aforementioned report. They are, however, about threefold lower than in another study (Kern et al., 1995) that also used the *O*-phthaldialdehyde assay, but different preparation and extraction of the samples. Based on the report (Kern et al., 1995), one could challenge the applicability of the *O*-phthaldialdehyde assay for retinal GSH measurements. However, several studies, including those from our laboratory are indicative of an agreement between the GSH values in a number of mammalian tissues, i.e. heart (Floreani et al., 1997), lens (Reddy and Giblin, 1984; Lou et al., 1988; Obrosova and Stevens, 1999; Obrosova et al., 1998a), peripheral nerve (Cameron et al., 1999; Carroll et al., 1986; Nagamatsu et al., 1995; Van Dam et al., 1999) and now retina, obtained with the *O*-phthaldialdehyde assay and other procedures. In our GSSG measurements, *N*-ethylmaleimide was added as early as at the step of retinal extraction. The *N*-ethylmaleimide concentration was sufficient to complex GSH concentrations exceeding 10-fold those in the retinal samples. GSSG concentrations measured with the *O*-phthaldialdehyde assay in our study appeared about 12 lower than those in the report (Kern et al., 1995), but also about 1.3–2-fold higher compared to those obtained with enzymatic method. Thus, our findings are consistent with conclusions of others (Floreani et al., 1997) regarding inapplicability of the *O*-phthaldialdehyde assay for GSSG measurements in mammalian tissues.

Decreased superoxide dismutase activity in the diabetic retina is consistent with other reports (Nishida et al., 1984; Kowluru et al., 1997). In contrast to findings (Kowluru et al., 1997), retinal catalase activity in diabetic rats was not decreased at all, and, on the contrary, tended to increase although the difference with corresponding controls did not achieve statistical significance. The decrease of both GSSGPx and GSSGRed activities is indicative of diabetes-induced slowing of glutathione redox cycle and, therefore, impairment of hydrogen peroxide-neutralizing mechanisms. Decreased GSHTrans activity in the diabetic rats is probably one of the factors contributing to 4-hydroxyalkenal accumulation because GSHTrans plays a major role in neuralization of 4-hydroxyalkenals by their conjugation with GSH. Quinone reductase activity was upregulated in diabetic rats compared with controls. This enzyme metabolises quinones, xenobiotics, and a great variety of toxic compounds (Ernster, 1967). Quinone reductase has been reported to protect against increased lipid peroxidation (Romero et al., 1991), but also to produce reactive oxygen species due to redox cycling of quinones through one electron reduction, with formation of semiquinone, superoxide anion, hydrogen peroxide and hydroxyl radicals (Fisher and Gutierrez, 1991; Tampo and Yonada, 1996). It is unclear if quinone reductase upregula-

tion in the diabetic retina is a protective phenomenon or a manifestation of oxidative injury. Cytoplasmic NADH oxidase activity in the retinae of rats with 6-week diabetes was similar to those in controls. Another study (Ellis et al., 1998) applying immunocytochemical approach has reported increased NADH oxidase activity in the retinae of obese, non-insulin dependent diabetic bio-breeding Worcester rats. The apparent discordance between the two studies could be attributed to differences in animal models and duration of diabetes, as well as assessment of total (mitochondrial and cytoplasmic (Ellis et al., 1998)) vs. cytoplasmic enzymatic activity only.

Neither the present study nor other reports answer the important question of which mechanism(s) contributes to hyperglycemia-induced increase in lipid peroxidation and impairment of antioxidative defense in the retina in short-term diabetes. Evaluation of the role for glucose autoxidation would require specific studies with metal chelators. It remains to be established if formation of Amadori products affects antioxidative defense enzymes in the diabetic retina, like it does in the diabetic lens (Arai et al., 1987; Takata et al., 1996). The findings of (1) a pivotal role for aldose reductase in oxidative stress in other targets for diabetic complications (Lee and Chung, 1999; Lou et al., 1988; Obrosova et al., 1998b, 1999), (2) implication of both AR and oxidative stress in diabetes-induced pericyte apoptosis (Romeo et al., 1999; Sato et al., 1999) and formation of pericyte ghosts and acellular capillaries (Ansari et al., 1998; Frank et al., 1997; Hammes et al., 1997; Kador et al., 1990; Neuenschwander et al., 1997), and (3) involvement of both aldose reductase- and oxidative stress-mediated mechanisms in increased vascular permeability in the diabetic retina (Ellis et al., 1998; Lightman et al., 1987; Vinores et al., 1990) imply that the role for aldose reductase in retinal oxidative injury deserves a thorough consideration.

Increased retinal lipid peroxidation in early diabetes is completely prevented by DL-α-lipoic acid. In biological tissues, DL-α-lipoic acid is reduced to dihydrolipoate, the potent reductant that combines free radical (hydroxyl, superoxide, peroxyl, singlet oxygen) scavenging and metal chelating properties with ability to regenerate levels of other antioxidants (Packer et al., 1995). In contrast to findings in L-buthionine (*S,R*)-sulphoximine model of oxidative stress in the lens (Maitra et al., 1995) as well as in diabetic lens (Obrosova et al., 1998a) and nerve (Nagamatsu et al., 1995), DL-α-lipoic acid did not increase the GSH concentrations as well as GSHPx and catalase activities in the diabetic retina. Therefore, the effects of this antioxidant in the diabetic model are tissue specific. DL-α-Lipoic acid facilitates translocation of glucose transporters towards the plasma membrane (Bashan et al., 1993; Low et al., 1997) and exacerbates accumulation of both glucose and sorbitol pathway intermediates (Obrosova, unpublished) in the diabetic retina. Thus, the agent acts as an antioxidant without interference with potential aldose reductase or initial phase glycation mechanisms. The lack of any effect on GSHTrans suggests that the antioxidant arrests formation rather than stimulates neutralization of 4-hydroxyalkenals. In addition to free radical scavenging and metal chelating effects (Packer et al., 1995), DL-α-lipoic acid could counteract oxidative stress by upregulating production of nerve growth factor (Garrett et al., 1997). Nerve growth factor, which is intrinsic for retinal neurons (Rickman and Brecha, 1995), induces expression of the superoxide dismutase gene (Nistico et al., 1992) and acts as superoxide dismutase mimic (Santos et al., 1995) in certain models of oxidative injury. Taking into consideration that the vasculature constitutes only 1% of the whole retina, it would be reasonable to suggest that the changes in the parameters of lipid peroxidation and antioxidative defense in diabetic rats treated with or without DL-α-lipoic acid, reflect those present in the neural retina.

In conclusion, accumulation of 4-hydroxyalkenals, but not malondialdehyde, is an early marker of oxidative stress in the diabetic retina. Increased lipid peroxidation occurs in short-term diabetes in the absence of GSH depletion. It is prevented by DL-α-lipoic acid, potentially due to its free radical scavenging and metal chelating properties as well as upregulation of superoxide dismutase, without participation of the glutathione system. DL-α-lipoic acid can be recommended for further studies in experimental diabetic retinopathy. It would be of particular interest to establish whether pericyte degeneration, increase in vascular permeability, vascular endothelial growth factor production and other abnormalities in the diabetic retina can be prevented by an agent exacerbating glucose and sorbitol accumulation.

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