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Expression of antioxidant enzymes in rat retinal ischemia followed by reperfusion

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Abstract

To evaluate the expression and protein levels of antioxidant enzymes in the rat retina exposed to oxidative stress induced by ischemia-reperfusion injury. Retinal ischemia was induced in female Wistar rats by ligation of the optic nerve and vessels behind the left eye bulb, and was followed by reperfusion for 0, 3, 6, or 24 hours. The right eye served as control. RNA and protein were extracted simultaneously from each retina. Expressions of the endogenous antioxidant enzymes glutathione peroxidase (GPx1), catalase (CAT), copper/zinc superoxide dismutase, manganese superoxide dismutase, and the catalytic subunit of glutamylcysteine ligase (GCLc) were analyzed with real-time reverse transcription polymerase chain reaction and related to the endogenous control cyclophilin B. Protein levels were measured with Western blot analysis. During the early phase (0 or 3 hours) of reperfusion, no changes were seen in enzyme expression. After 6 hours, GCLc expression increased by a factor of 1.14 ($P = .034$), followed by a decline of 0.80 after 24 hours ($P = .00004$), according to the comparative Ct method. After 24 hours of reperfusion, GPx1 expression increased by a factor of 1.14 ($P = .028$), and CAT had decreased by 0.82 ($P = .022$). Expressions of copper/zinc superoxide dismutase and manganese superoxide dismutase showed a tendency toward a decrease by factors of 0.86 ($P = .055$) and 0.88 ($P = .053$), respectively, after 24 hours. Protein levels did not differ for any of the antioxidants, regardless of reperfusion time. The slightly increased messenger RNA expression of GPx1 after 24 hours of reperfusion with a concomitant very modest decrease in CAT and GCLc expression and no change in protein levels indicate a very modest, if any, response to oxidative stress generated by ischemia followed by reperfusion in rat retina.

1. Introduction

Ischemia is common in several retinal conditions, such as central and branch retinal artery occlusion, anterior ischemic optic neuropathy, venous occlusive disorders, retinopathy of prematurity, glaucoma, and diabetic retinopathy [1]. Ischemia deprives the retina of oxygen and nourishment, and compromises an efficient removal of waste products. This ultimately disrupts cellular energy metabolism and leads to several harmful events [1], for example, the formation of reactive oxygen species (ROS), degradation of the antioxidant system, induction of cytokine production via transcriptional factors, leukocyte activation triggering an inflammatory response [2-9], and extracellular accumulation of glutamate likely to be excitotoxic to neuronal elements [10-12]. Reperfusion after initial ischemia paradoxically maintains the destruction process, perhaps due to increased levels of extracellular neurotransmitters, ROS, and waste products damaging previously unharmed cells when being reoxidized [1,13]. Any imbalance between ROS and the antioxidant defense system can create a state of oxidative stress, ultimately resulting in DNA strand breakage, damage to membrane ion transporters and other membrane components, depletion of nicotine amide adenine dinucleotide and adenosine triphosphate, and peroxidation of lipids [1]. In response to the exposure to ischemic damage, the organism strives to modulate its gene expression of several antioxidant enzymes [1]. Superoxide dismutases (SODs) convert the superoxide radical to hydrogen peroxide, which in turn is converted to water and oxygen by catalase (CAT) and glutathione peroxidase (GPx1) in the presence of glutathione, and the catalytic subunit of glutamylcysteine ligase (GCLc)

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is needed for the de novo synthesis of glutathione, necessary for the complete antioxidation to take place [14-19].

Animal models make it possible to induce ischemia in the retina and to study the tissue response in a controlled setting. The messenger RNA (mRNA) expression and protein levels of antioxidant enzymes in the rat retina after ischemia-reperfusion injury can be considered as an indirect measure of the response to oxidative stress.

We recently showed that simultaneous extraction of RNA and protein from rat retina allows parsimonious tissue handling as well as direct coupling between an altered mRNA expression and protein levels in the retina by subsequent real-time reverse transcription polymerase chain reaction (RT-PCR) and Western blot analysis [20]. The aim of the present study was to apply those methods to rat retina subjected to ischemia-reperfusion injury to explore the expression and protein levels of retinal endogenous antioxidant enzymes (ie, GPx1, CAT, copper/zinc superoxide dismutase [CuZnSOD], manganese superoxide dismutase [MnSOD], and GCLc) in response to oxidative stress and to relate the results to an internal control, cyclophilin B (Cyp B), for quantification.

2. Materials and methods

2.1. Animals

Female Wistar rats (body weight, 200-250 g) were from Taconic (Lille Skensved, Denmark). The animals were housed in a temperature-controlled environment with free access to food and water and a 12-hour light-dark cycle. All animals were treated according to the Principles for the Care and Use of Animals in Ophthalmic and Vision Research approved by the Association for Research in Vision and Ophthalmology. The Animal Ethics Committee of Malmö/Lund approved the study.

2.2. Induction of transient retinal ischemia

The animals were anesthetized intraperitoneally (0.33 mL per 100 g body weight) with a mixture of pentobarbital (9.72 mg/mL, Apoteket, Malmö, Sweden), chloral hydrate (42.5 mg/mL, Merck, Sollentuna, Sweden), magnesium sulfate (86.25 mmol/L), ethanol (10% v/v), and propylene glycol (40% v/v). The pupils were dilated with 1% Cyclogyl (cyclopentolate, Alcon, Stockholm, Sweden) and a local anesthetic; 1% Tetrakain Chauvin (Novartis Ophthalmics, Copenhagen, Denmark) was applied. Retinal blood flow was observed using a corneal contact lens and a stereomicroscope (Wild M650, Wild Heerbrugg, Heerbrugg, Switzerland). Retinal ischemia was induced by ligation of the vessels and the accompanying optic nerve behind the left eye bulb [21] using a 5-0 silk suture (Ethicon, Sollentuna, Sweden). The ligature was gently tightened until complete cessation of the retinal blood flow was observed and maintained for 45 minutes. Reperfusion was established by removing the ligature, resulting in a visibly restored blood flow; anesthesia was disconnected; and no analgesics were administered. The animals were euthanized with CO_2 after 0, 3, 6, or 24 hours of reperfusion. After euthanization, each eye was immediately enucleated, the lens was removed, and the retina was gently peeled off from the pigment epithelium, snap frozen on dry ice, and stored at −80°C until use. All groups comprised 16 animals with an even spread in body weight and time of surgery during the day. The right eye served as control.

2.3. Simultaneous extraction of RNA and protein

The protein and RNA extraction protocol has previously been described in detail [20]. In short, the retinas were homogenized in TRI Reagent (Molecular Research Center, Cincinnati, OH), phases separated, the RNA extracted as described by Chomczynski [22], the DNA discarded, and the protein was kept in solution and washed with a wash buffer and concentrated with an ultrafiltration device. RNA and protein samples were stored at −80°C.

2.4. Real-time RT-PCR

cDNA was synthesized from 2 μg of RNA using the SuperScript II RNase H^- RT (Invitrogen Life Technologies, Paisley, UK) protocol as described [20]. mRNA levels were analyzed with the real-time RT-PCR 7900HT system (Applied Biosystems, Stockholm, Sweden) using 5 ng of cDNA as described by Applied Biosystems. PrimerExpress 2.0 software (Applied Biosystems) was used for the design of primers and TaqMan probes (FAM-TAMRA). Each primer and probe set were selected to span over an intron-exon boundary and blasted for specificity for the rat genome against the total genome data base [23]. Relative expression levels were calculated using the comparative Ct method [24,25] with Cyp B as endogenous control [26]. If the standard deviation of the duplicate Ct value differed by more than 0.16, the sample was rerun. All sequences are 5′ → 3′ :

1. GPx1 exons 1 and 2
   Forward: CTC GGT TTC CCG TGC AAT
   Reverse: CAT ACT TGA GGG AAT TCA GAA TCT CTT
   Probe: ATT CTT GCC ATT CTC CTG ATG TCC GAA CT
2. CAT exons 12 and 13
   Forward: CCC GAG TCC AGG CTC TTC T
   Reverse: CGG CCT GTA CGT AGG TGT GA
   Probe: ACC AGT ACA ACT CCC AGA AGC CTA AGA ATG CA
3. CuZnSOD exons 3 and 4
   Forward: GCG GTC CAG CGG ATG A
   Reverse: GTC CTT TCC AGC AGC CAC AT
   Probe: AGG CAT GTT GGA GAC CTG GGC
4. MnSOD exons 4 and 5
   Forward: TCA GGA CCC ACT GCA AGG A
   Reverse: GCG TGC TCC CAC ACA TCA
   Probe: CCA CAG GCC TTA TTC CAC TGA TGG G

5. GCLc exons 3 and 4
   Forward: AGG AGA ACA TCA GGC TCT TTG C
   Reverse: GTG CTC TGG CAG TGT GAA TCC
   Probe: CGA TAA CTT CAT TTC CCA GGC TAG GCT GC
6. Cyp B exons 3 and 4
   Forward: GGA GAT GGC ACA GGA GGA AA
   Reverse: CCA TAG TGC TTC AGC TTG AAG TTC T
   Probe: AGC ATC TAT GGT GAG CGC TTC CCA GA

2.5. Western blot

Gel electrophoresis and Western blot procedures were performed as previously described [20]. For immunoprobing, Tris-Buffered Saline (ph 7.6) with 0.1% Tween 20 (TBS-T) with 0.5% gelatine was used as blocking buffer. All primary and horseradish peroxidase–conjugated secondary antibodies were commercially available (Table 1).

2.6. Pooling and concentration of sample extracts

It was not possible to detect GPx1 in one single retina, as the limit for detection turned out to be 12.5 to 25 μg [20]. This necessitated pooling of extracts from 4 individuals, and to get comparable results, analyses on all enzymes were performed on pooled retinas. A volume corresponding to 50 μg total protein for each of 4 single-extracted samples was mixed to give a total volume of approximately 200 to 250 μL. The pooled sample volume was transferred to a Microcon YM-3, 3000 MWCO (Millipore, Molsheim, France) centrifugal filter device and inserted into a Microcon vial (Millipore), then centrifuged at 14000 × *g* for 45 minutes at room temperature to receive approximately 80 to 120 μL of retentate. The sample reservoir containing the retentate was placed upside down in a new vial, centrifuged at 1000 × *g* for 3 minutes at room temperature to collect the retentate, and stored at −20°C for later use.

Table 1
Antibodies and dilutions for the Western blot multiplex assays

	Primary or secondary antibody	Dilution
Assay 1	Rabbit antihuman MnSOD (S8060-10A)[a]	1/1000
	Sheep antihuman CuZnSOD (S8060-15)[a]	1/1500
	Rabbit antihuman CAT (LF-PA0060)[b]	1/2000
	Rabbit antihuman Cyp B (PAI-027)[c]	1/100000
	Sheep antirabbit IgG (H&L) HRP (I1964-41P)[a]	1/100000
	Rabbit antisheep IgG (H&L) HRP (I1904-59C)[a]	1/100000
Assay 2	Rabbit antirat GCLc (RB-1697-PI)[d]	1/1000
	Rabbit antihuman GPx1 (LF-PA0019)[b]	1/2000
	Rabbit antihuman Cyp B (PAI-027)[c]	1/750000
	Sheep antirabbit IgG (H&L) HRP (I1904-41P)[a]	1/50000
Assay 3	Rabbit antirat GCLc (RB-1697-PI)[d]	1/1000
	Rabbit antihuman Cyp B (PAI-027)[c]	1/750000
	Sheep antirabbit IgG (H&L) HRP (I1904-41P)[a]	1/50000

H&L indicates heavy and light chain.
[a] US Biological (Swampscott, Mass).
[b] Lab Frontiers (Seoul, Korea).
[c] Affinity Bio Reagents (Sydney, Australia).
[d] NeoMarkers, Lab Vision (Newmarket, Suffolk, UK).

2.7. Quantification of proteins

Quantification of proteins was based on a fixed concentration (yielding 50 μg) from each extract regardless of volume. After pooling and concentrating 4 extracts from each experimental group, the gel was loaded with a fixed volume (20 μL) from each pool. Extracts from 16 retinas in each group resulted in 4 samples (n = 4) for further statistical analysis. Three multiplexed assays were run to identify the antioxidants (Table 1). The results of the chemiluminescent detection of the membranes were measured using MultiGauge v 2.2 software (Fujifilm, Stockholm, Sweden). Signal strength was given as arbitrary units (AU) for each target as well as for the endogenous control of a pooled sample. The coefficient of variation was calculated for each duplicate, and if the coefficient of variation was 15% or higher, the sample was rerun. Each pooled sample was normalized against the endogenous control by calculating the ratio between the AU value of the target and the AU value of the endogenous control. Thereafter, the relative quantity for each target was obtained by calculating the ratio between the normalized value of the ischemic eye and the control eye. For quantification, we used the same comparative Ct method as for mRNA, but because Western blot results cannot be expressed exponentially, we used ratios instead of delta (Δ) values. The relative quantity for Cyp B was calculated to evaluate its reliability as an endogenous control.

2.8. Statistics

Statistical differences were evaluated using 2-tailed paired Student *t* test for real-time RT-PCR and Wilcoxon's signed rank test for 2 related samples for Western blot. For PCR, the comparative Ct method was used to calculate the relative quantity ($2^{-\Delta\Delta Ct}$) as well as the standard deviation ($\Delta\Delta$Ct SD) of the mRNA expression. Expression and protein levels of each enzyme at each time point were considered exploratory, and, hence, *P* values were not adjusted for multiple comparisons in this study.

3. Results

3.1. Expression of antioxidant enzymes

The results of real-time RT-PCR are shown in Table 2. All expression values given are related to the endogenous control Cyp B and then compared to the control eye.

3.1.1. Glutathione peroxidase

No change in GPx1 mRNA expression levels could be observed after 0, 3, or 6 hours of reperfusion. After 24 hours of reperfusion, there was an increase by a factor of 1.14 (P = .028) compared to the control eye.

3.1.2. Catalase

No change in CAT expression levels could be observed after 0, 3, or 6 hours of reperfusion. After 24 hours of reperfusion, a decline by a factor of 0.82 (P = .022) was seen.

Table 2
RNA expression of antioxidant enzymes

	Reperfusion time (h)	Control eyes		Ischemic eyes		P
		$2^{-\Delta\Delta CtL}$	ΔΔCtL SD range	$2^{-\Delta\Delta CtR}$	ΔΔCtR SD range	
GPx1	0	1	1.14/0.88	1.05	1.25/0.87	.514
	3	1	1.14/0.87	1.06	1.19/0.94	.327
	6	1	1.16/0.86	1.03	1.17/0.91	.643
	24	1	1.14/0.88	1.14	1.28/1.01	.028
CAT	0	1	1.27/0.79	1.04	1.16/0.93	.785
	3	1	1.32/0.76	0.92	1.06/0.81	.285
	6	1	1.12/0.89	0.88	1.10/0.70	.191
	24	1	1.20/0.89	0.82	0.98/0.70	.022
CuZnSOD	0	1	1.15/0.87	0.93	1.26/0.78	.350
	3	1	1.08/0.93	0.97	1.11/0.84	.535
	6	1	1.17/0.85	0.99	1.15/0.85	.868
	24	1	1.20/0.84	0.86	1.02/0.73	.055
MnSOD	0	1	1.19/0.84	0.95	1.11/0.81	.444
	3	1	1.13/0.88	0.99	1.11/0.88	.789
	6	1	1.27/0.79	1.27	1.17/0.82	.106
	24	1	1.14/0.87	0.88	1.03/0.74	.053
GCLc	0	1	1.16/0.86	1.00	1.17/0.85	.963
	3	1	1.17/0.85	1.11	1.30/0.96	.165
	6	1	1.15/0.87	1.14	1.28/1.01	.034
	24	1	1.13/0.89	0.80	0.89/0.71	.00004

Relative quantity for each value is given as $2^{-\Delta\Delta Ct}$ value and range of ΔΔCt SD according to the comparative Ct method. Ischemic eyes were compared to control eyes. CtL indicates left (contrlol) eye; CtR, right (ischemic) eye. *P* value according to Student paired *t* test.

3.1.3. Copper/zinc superoxide dismutase

For CuZnSOD, there were no significant changes in mRNA expression for any of the groups.

3.1.4. Manganese superoxide dismutase

For MnSOD, there were no significant changes in mRNA expression for any of the groups. The analysis for the 6-hour group (factor, 1.27; $P = .11$) was run twice on different occasions with identical results.

3.1.5. Glutamylcysteine ligase

For GCLc, there was an increase in mRNA expression levels after 6 hours of reperfusion with a factor of 1.14 ($P = .034$), which turned into a decrease by a factor of 0.80 ($P = .00004$) after 24 hours of reperfusion. No other changes were observed.

3.1.6. Cyclophilin B

The mRNA expression levels for Cyp B were monitored during all assays in parallel to the gene of interest. No significant changes could be observed for Cyp B in any of the reperfusion groups (data not shown).

3.2. Protein levels of antioxidant enzymes

We used a new protocol for measuring total protein concentration as it was not possible to measure the pooled and concentrated sample extracts according to the BCA standard method, as the colorimetric response was too high. Dilution of the sample retentate did not affect the results, nor was there any evidence of protein in the filtrate. We assume that concentrating the samples resulted in too high concentrations of sodium dodecyl sulfate (SDS), exceeding the 5% limit of the bicinchoninic acid method (The BCA Protein Asssay, Pierce Biotechnology, Rockford, IL), or that interference was caused by SDS micelles formed during the concentration process. The critical SDS level for formation of 18-kd micelles is 0.23% [27].

Antibodies and dilutions used for the multiplex detection of the targets on Western blot are shown in Table 1. The membrane detection of multiplexed assays 1 and 3 is illustrated in Figs. 1 and 2. No significant changes in protein levels between ischemic and control eyes were observed for

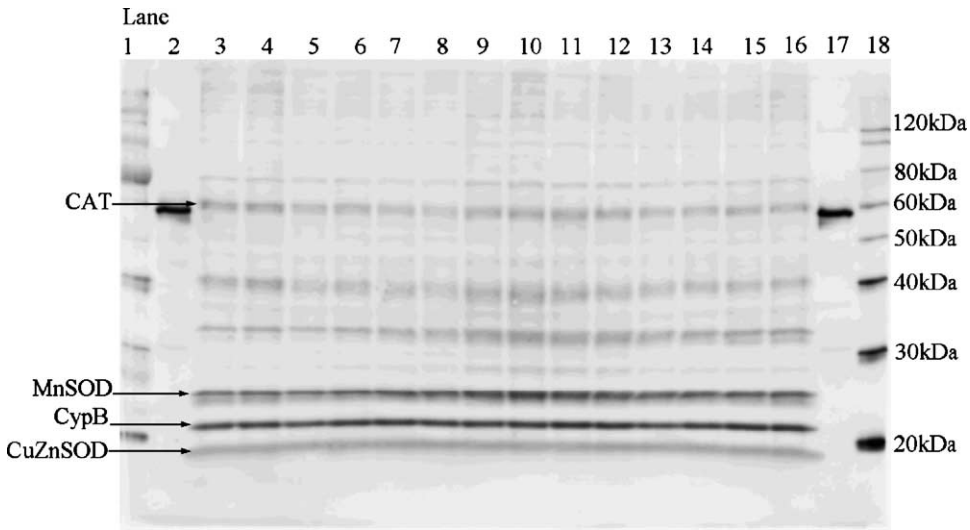

Fig. 1. Multiplexed assay 1: membrane detection of CAT, MnSOD, CuZnSOD, and Cyp B (endogenous control). Lanes 1 and 18 contained protein standards; lanes 2 and 17 contained a positive bovine CAT control; lanes 3 and 4 (0 hour), 7 and 8 (3 hours), 11 and 12 (6 hours), and 15 and 16 (24 hours) were pooled ischemic eyes; and lanes 5 and 6 (0 hour), 9 and 10 (3 hours), and 13 and 14 (6 hours) were pooled control eyes.

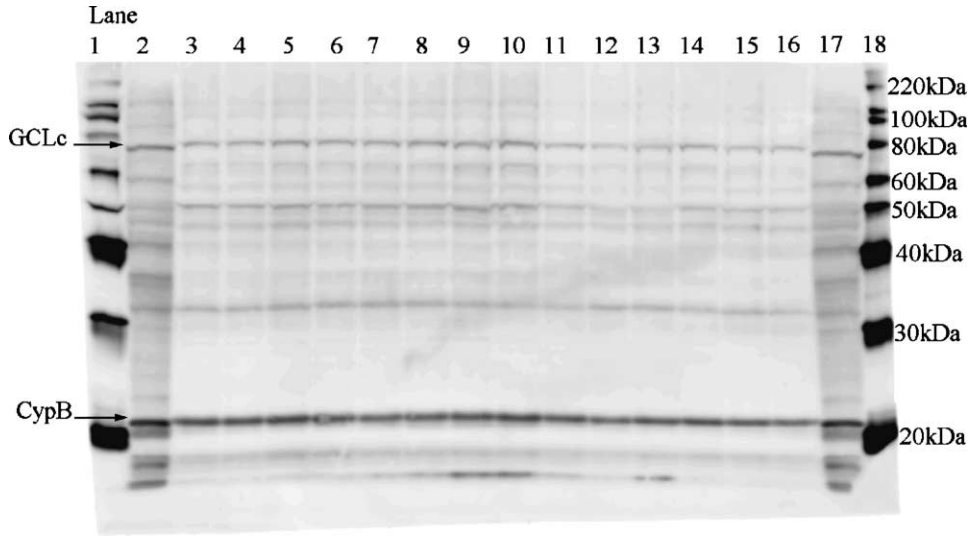

Fig. 2. Multiplexed assay 3: membrane detection of GCLc and Cyp B (endogenous control). Lanes 1 and 18 contained protein standards; lanes 2 and 17 contained a positive human GCLc cell lysate control; lanes 3 and 4, 7 and 8, and 11 and 12 were pooled (24 hours) ischemic eyes and lanes 5 and 6, 9 and 10, 13 and 14, and 15 and 16 were pooled (24 hours) control eyes.

any of the antioxidants, regardless of group (Table 3). Omitting one CuZnSOD outlier in the ischemia followed by the 3-hour reperfusion group did not change the results. The endogenous control was stable except for one outlier in the ischemia without a reperfusion group. Omitting this outlier did not change the results.

4. Discussion

Previous studies have demonstrated an elevated presence of free radicals in ischemic/reperfused rat retina, either directly by electron paramagnetic resonance [28] or indirectly by showing diminished damage after administering antioxidant drugs such as SOD, EGB 761 extracted from *Ginkgo biloba*, vitamin E, mannitol, CAT, and several other compounds [2,29,30]. Several studies have demonstrated the ischemic effect on the retina as shown by electroretinogram (ERG), either alone [31] or by modulation by administration of antioxidant drugs [31-35]. However, only a few studies have dealt with the endogenous antioxidant system in the retina, for example, in Muller cells subjected to oxidative stress [36] and in retina subjected to ischemia-reperfusion injury [37-39]. In the present study, we demonstrate that retinal ischemia followed by reperfusion induces only a slight change in endogenous antioxidant enzyme expressions. Real-time RT-PCR revealed the mRNA expression of GCLc after 6 hours of reperfusion to be modestly but significantly increased, as was the expression of GPx1 after 24 hours of reperfusion with a concomitant decline of CAT and GCLc. In this study, Cyp B was used as an endogenous control, and no changes were observed in any of the reperfusion groups. This is in line with previous studies [40-42].

Our results indicate that the endogenous antioxidant system in the rat retina does not respond particularly well to ischemia/reperfusion induced by temporary ligation of the vessels along the optic nerve. We applied 45 minutes of ischemia, which should be sufficient to induce substantial ischemic damage. Osborne et al [1] claimed that 20 minutes of ischemia was required to cause irreversible functional ischemic injury in Wistar rat retinas as demonstrated with ERG, and 45 minutes was needed to produce histopathological changes. Block and Schwarz [31] demonstrated reversible ERG changes within 30 minutes regardless of

Table 3
Protein levels of antioxidant enzymes

	Reperfusion time (h)	Control eyes		Ischemic eyes		*P*
		Median	Range	Median	Range	
GPx1	0	1.08	0.92	1.28	1.68	.465
	3	0.92	0.89	0.64	0.87	.715
	6	0.81	1.29	0.68	1.68	1.000
	24	0.85	0.79	1.86	1.12	.144
CAT	0	0.79	1.18	0.88	1.32	1.000
	3	1.05	0.45	1.00	3.00	.715
	6	0.99	0.69	1.25	1.40	.144
	24	0.58	2.01	0.84	0.71	1.000
CuZnSOD	0	0.36	2.77	1.06	1.25	.715
	3	0.98	0.32	1.14	6.46	.144
	6	0.34	2.80	1.12	0.53	.715
	24	0.41	2.78	0.90	0.99	.715
MnSOD	0	1.00	0.23	1.01	0.31	.715
	3	1.04	0.27	1.11	1.30	.465
	6	0.28	2.92	1.02	1.11	.715
	24	0.25	3.07	1.07	0.50	.715
Cyp B	0	1.10	1.30	0.95	16.07	.401
	3	0.99	0.73	0.97	1.76	.889
	6	1.08	1.45	1.00	1.21	.575
	24	1.00	1.69	1.01	0.97	.937
GCLc	24	0.93	0.43	0.98	0.20	1.000

Relative quantity for each target given as median value and range. Ischemic eyes were compared to control eyes. *P* value according to Wilcoxon signed ranks test.

occlusion method, but 60 minutes or more was necessary to induce irreversible changes using central retinal artery occlusion, and Hayreh et al [35] found irreversible ERG changes to occur sometime between 97 and 105 minutes. After more than 90 to 100 minutes of ischemia, cell death may occur [2,43-45], precluding further analyses of processes in viable cells. Because the goal of the present study was to measure cellular expression changes in response to ischemia-reperfusion damage, it was desirable not to exceed the time limit for viable retinal cells.

Various experimental designs have been set up for the induction of ischemia-reperfusion damage in the retina, that is, 2-vessel occlusion by bilateral clamping of the common carotid arteries [31], 4-vessel occlusion by clamping of the vertebral arteries as well as the common carotid arteries [31], central retinal artery occlusion by ligation of the optic nerve [46,47], raised intraocular pressure above the arterial opening pressure [45,46], microembolization [46,47], and laser coagulation of peripheral retinal arteries [31,48,49]. In the present study, we used the method of optic nerve ligation [21], including the central artery and vein. This method enables easy reversible occlusion, a visually controlled blood flow, and prevents additional injury caused by raised intraocular pressure as well as a global cerebral damage by occlusion of major cerebral vessels.

The various expression scenarios for the antioxidant targets involved in the present study may be the result of different turnover rates for the various enzymes and different feedback mechanisms in the biosynthetic pathways, as autoregulation by negative feedback is a key mechanism for the cellular enzyme cascades. It may well be that the enzymes are in the process of normalization after 24 hours of reperfusion, and it cannot be excluded that an altered mRNA expression actually culminates either before 3 hours or sometime between 6 and 24 hours of reperfusion, which would explain the modest expression alterations. There is also a possibility that very short or longer reperfusion periods might have triggered further gene expression. However, there are no consensual data as to which reperfusion time is the most appropriate. It seems that longer reperfusion periods are necessary to induce macroscopic and histological damage [21,30,31,38,41,45], but this does not exclude that alterations in gene expression may occur much earlier; the peak intensity of free radical production in the study of Szabo et al [2] was observed after only 3 minutes of reperfusion.

We have previously shown that all of the antioxidant enzymes measured except for GPx1 were detectable by Western blot [20]. Our intention was to analyze each retina separately. However, as we observed a slightly increased expression of GPx1 after 24 hours of reperfusion, we found it important to include also this enzyme in our Western blot analyses, and hence we decided to pool retinas. Despite this procedure, we found no significant changes in protein levels between ischemic and control eyes in any of the groups. Whereas real-time RT-PCR is a highly sensitive method enabling the detection of very small changes in mRNA expression, Western blot analysis is less sensitive and requires larger quantities of enzymes. Thus, there might have been undetected small alterations in enzyme production, unrevealed by Western blot.

In the present exploratory study, we were not able to demonstrate a clearly altered mRNA expression of endogenous antioxidant enzymes in response to ischemia and subsequent reperfusion in rat retina and there were no correlated protein level changes. After a Bonferroni correction, only one of the observations, the decreased GCLc expression after 24 hours of reperfusion, remained significant. For the other enzymes, it remains an open question whether the small differences in expression could be real. It has been argued that the retina is comparatively insensitive to ischemic injury compared to the closely related brain tissue, which could depend on intravitreous glucose and intraretinal glycogen storage, the ability of photoreceptors to exploit energy sources anaerobically, and the expandable nature of the retinal tissue in an edematous state [1]. Moreover, because the retina is a tissue with a mixture of different cell types, the mRNA expression is necessarily a summation response. There is a possibility for one cell type being more vulnerable to oxidative stress than others, and narrowing the process down to one particular cell type may perhaps reveal a more specific response of oxidative stress effects in the retina.

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