Glutathione Peroxidase Induced in Rat Retinas to Counteract Photic Injury

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Purpose. To examine the hypothesis that glutathione peroxidase (GPX) is induced at different time points after retinal exposure to light and localizes in different retinal cells.

Methods. The rats were kept in cyclic light for 2 weeks before the experiments. The animals were maintained in 12-hour light–dark cycles, before and after exposure to intense white fluorescent light, for as long as 24 hours and then returned to cyclic light. Expression of GPX was measured by immunohistocytochemistry and Western and Northern blot analyses. Light-induced retinal damage was determined by the thickness of the outer nuclear layer (ONL) thickness in relation to total retinal thickness.

Results. GPX labeling did not appear in the photoreceptor inner segments, and slight labeling was observed in the photoreceptor outer segments or the retinal pigment epithelial (RPE) cells in the normal retina kept in cyclic light. In retinal specimens maintained in light for 12 and 24 hours, GPX labeling was induced in the photoreceptor outer segments and RPE cells. High expression of GPX in the RPE was sustained until day 7 after challenge. In contrast, GPX expression in the photoreceptor outer segments decreased on day 1 and disappeared on days 3 and 7 after exposure. Intense GPX labeling was seen from the internal limiting membrane to the ganglion cell layer. GPX labeling was constantly localized in both high-intensity white light and cyclic conditions, suggesting no induction of GPX in those areas. In addition, GPX labeling was apparent at the posterior retinal pole but not at the peripheral retina. We observed marked upregulation of GPX mRNA in rats kept in high-intensity white light. One, 3, and 7 days after exposure to high-intensity white light, there was a significant difference ($P < 0.0001$) between the control and experimental groups in the ratio of the outer nuclear layer thickness to the entire retina.

Conclusions. GPX was induced at different time points after exposure to high-intensity white light and localized in different retinal cells. Changes in expression of GPX after exposure to light may be related to the difference in susceptibility of the retina to damage by light. (*Invest Ophthalmol Vis Sci.* 2003;44:1230–1236) DOI:10.1167/iovs.02-0191

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There is increasing evidence that antioxidants play a major role in retinal protection against exposure to light, although the mechanism of light-induced damage in the retina is not understood.[1,2] Reactive oxygen species are believed to be important causative factors underlying damage of the retina by light.[3,4] An imbalance between excess free radical production and the antioxidant defense results in cellular damage leading to lipid peroxidation in the retina.[5] To defend against a reactive oxygen species attack, the retina, like many other organs, has its own defense system. The retina contains high levels of protective antioxidants[6,7] and antioxidative enzymes.[7–10] We reported that an intrinsic superoxide scavenger, mitochondrial manganese superoxide dismutase (Mn-SOD), was induced in the retina after exposure to light.[11] One mechanism involves the action of SODs, which causes dismutation of the superoxide anion to hydrogen peroxide. These enzymes provide an important first line of defense against oxygen injury in the lung, heart, and other organs.[12–14] Glutathione peroxidase (GPX) is also an essential antioxidant enzyme that can function for catalase by removing hydrogen peroxide formed after the SOD-catalyzed dismutation reaction.

Although previous immunohistologic reports have shown that SOD,[15–17] catalase,[18] and GPX[19–21] are found in similar sites in the normal rat retina, we previously reported changes in immunoreactivity of SOD with retinal degeneration,[22] aging,[23] developing,[24] and exposure to light.[11] Antioxidant protein mRNAs, including hemeoxygenase-1, metallothionein, CuZn SOD, and catalase, have been reported to have very different expression patterns in the developing rat retina.[25] Because a variety of antioxidant enzymes and antioxidants exists in the retina, antioxidants must coexist with cellular defenses against oxidative stress. It seems that each antioxidant has a different role under conditions of oxidative stress. We hypothesized that various reactive oxygen species scavengers are induced at different time points after retinal exposure to light, and these enzymes localize in different cell types. To gain deeper insight into antioxidant functions, data are needed on the retinal localization of specific antioxidants. We examined the precise localization of GPX under oxidative stress by studying the results of exposing rat retinas to light compared with those obtained with normal retinas, by using an immunohistochemical technique and Western and Northern blot analyses. We also discussed the possible protective mechanism of GPX.

Materials and Methods

Animals

All procedures adhered to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research, and all protocols were approved by the Shimane Medical University Animal Care Committee. Sprague-Dawley (SD) rats (6-weeks-old) were obtained from Charles River Japan, Inc. (Kanagawa, Japan).

Model of Exposure to Light

The animals were exposed to high-intensity white light according to a method described by Yamamoto et al.[11] with minor modification. Young adult male SD rats were maintained on a 12-hour light–dark

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cycle at 20°C for 2 weeks. The illumination intensity during the light phase was 80 lux. Water and rat chow were provided ad libitum. Each cage, which had wire tops and minimal bedding, housed one animal. The animals then were exposed to high-intensity illumination produced by white fluorescent bulbs for 24 hours. The average light intensity at 10 randomly selected points in the cages was 3000 lux. The temperature during the illumination was maintained at 25 ± 1.5°C. After exposure to high-intensity illumination, the animals were returned to a 12-hour light (80 lux)–dark cycle at 20°C during recovery. To obtain tissue for analysis, for Western and Northern blot analyses, the animals were killed by an overdose of pentobarbital (Tanabe Pharmaceutical Company, Osaka, Japan) injected intraperitoneally. For histologic and immunohistologic analyses, deep anesthesia was induced with pentobarbital and the animals were perfused with fixative.

The eyes were enucleated 12 and 24 hours after the start of exposure to light or 1, 3, and 7 days after the completion of the exposure to light. Rats raised under cyclic light without high-intensity exposure to light served as the control.

Antibodies

Rabbit anti-rat GPX was prepared by immunizing rabbits with the purified protein.[26] This antibody recognizes cellular GPX.[27] The specificity of the antiserum was assessed by Western blot analysis.[28]

Fixation of Ocular Tissue for Histologic Examination

After induction of deep anesthesia by intraperitoneal injection of pentobarbital, the rats were perfused through the left cardiac ventricle with 0.1 M phosphate buffer (pH 7.4) at 4°C to wash out the blood before fixation, and then with freshly prepared 2% paraformaldehyde and 0.1% glutaraldehyde and 1% sucrose in the same buffer. The eyes were removed and fixed with the same fixative for 6 hours at 4°C. All tissues were embedded in paraffin with a melting point of 56°C, and cut into 4-μm-thick sections parallel with the sagittal line. Tissues were collected on silanized slides (Dako, Kyoto, Japan).

Immunohistochemical Analysis

The techniques for immunohistochemical staining have been described in detail elsewhere.[11] We used a silver enhancing kit (BB International Inc., Cardiff, Wales, UK) to detect GPX. The antibody to GPX was diluted 1:1000 with 1% bovine serum albumin (BSA) on phosphate-buffered saline containing potassium (KPBS; pH 7.4). Briefly, tissue sections were treated for 30 minutes with xylene and a graded series of alcohol to deparaffinize the sections. To avoid artifacts resulting from tissue processing, the experiments were carefully performed in an identical manner. The sections were incubated overnight at 4°C with rabbit anti-rat GPX antibody or with control antibody (nonimmunized rabbit IgG) 10 pg/mL dissolved in KPBS with 1% BSA. Sections were rinsed with cold KPBS, incubated with gold-conjugated secondary antibody (BB International, Inc.) for 30 minutes, and rinsed again. The antigen–antibody complexes were visualized by immersion in enhancing solution (BB International Inc.) for 5 minutes and examined by light microscopy. We used purified nonimmunized rabbit IgG and KPBS with 1% BSA as negative controls. We also prepared sections of normal rat brain and liver in an identical manner as positive controls. We observed the immunoreactivity in the retinal area located 100 μm superotemporally from the optic nerve head.

For precise comparison, all tissue and sample processing and immunologic staining procedures were performed at the same time under identical conditions.

Immunohistocytochemical Analysis by Electron Microscopy

After fixation, eyes were embedded in optimal cutting temperature (OCT) compound (Sakura, Tokyo, Japan) and cut into 20-μm-thick frozen sections. Tissues were collected on silanized slides (Dako, Kyoto, Japan), dried, and washed in KPBS containing 10% sucrose. The antibody to GPX was diluted 1:500 with 1% BSA on KPBS. The sections were incubated with 10% normal goat serum for 10 minutes at room temperature and then with rabbit anti-rat GPX antibody or with control antibody (nonimmune rabbit IgG) overnight at 4°C. Sections were rinsed with cold PBS containing 10% sucrose, incubated with peroxidase-conjugated anti-rabbit IgG (ICN Pharmaceuticals Inc., Aurora, OH) for 4 hours at room temperature, and then rinsed again. Sections were fixed again in 0.5% glutaraldehyde solution for 10 minutes at 4°C. The antigen–antibody complexes were visualized by immersion in 0.05% 3,3′ diaminobenzidine tetrahydrochloride (DAB; Dojindo, Kumamoto, Japan) with 0.01% H_2O_2 in 0.05 M Tris-HCl buffer (pH7.6) for 5 minutes. After a wash in KPBS, the sections were postfixed in 2% osmium tetroxide, dehydrated, and embedded in epoxide. Sections were polymerized in gelatin capsules at 60°C for 3 days. Ultrathin sections (approximately 90 nm) were investigated by transmission electron microscope (model 1200-EX; JEOL, Tokyo, Japan) and photographed.

Quantitation of Histologic Changes

Exposure to high-intensity light results in severe damage to the outer nuclear layer (ONL) of the retina. For statistical analysis of the extent of histologic damage, we compared the thickness of the ONL to the thickness of the entire retina (defined as the internal limiting membrane to the retinal pigment epithelium [RPE]). We also counted the rows of nuclei in the ONL. The thickness of each eye was measured in sagittal sections near the optic nerve ($n = 6$).

For analysis of the morphometry, 4-μm retinal paraffin-embedded sections including the optic disc were stained with hematoxylin-eosin, and digitized color images of four locations in each sections were obtained by a digital imaging system (PDMC le; Olympus, Tokyo, Japan). Four locations including two in the superior retina, 100 to 1600 μm above the optic disc, and two in the inferior retina, 100 to 1600 μm below the optic disc. The number of hematoxylin-positive photoreceptor cell nuclei was counted in each image. All statistical analyses were performed on a personal computer (Macintosh; Apple Computer, Cupertino, CA; StatView software, ver. 5.0; SAS, Cary, NC). $P < 0.05$ was considered significant.

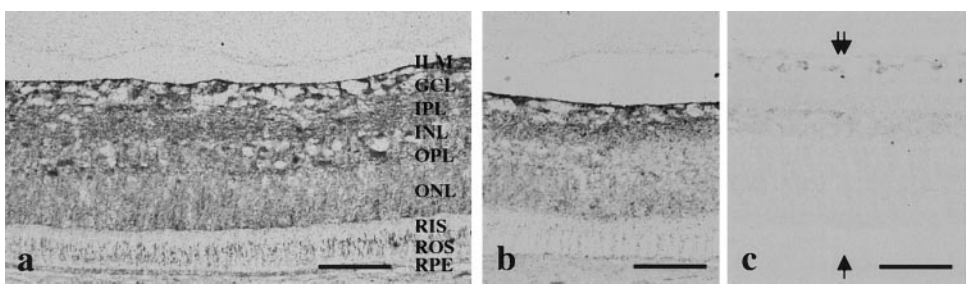

FIGURE 1. Immunohistochemical localization of GPX in the retina. (**a**) GPX labeling was observed in the internal limiting membrane (ILM), ganglion cell layer (GCL), inner plexiform layers (IPLs), and inner nuclear layer (INL) in normal retina kept in cyclic light in the posterior retinal pole. (**b**) GPX labeling was not apparent except in the inner retina in the peripheral retina. (**c**) GPX labeling was not observed in a negative control specimen incubated with nonimmune IgG instead of the primary antibody. *Arrow*: RPE; *double arrows*: ILM. OPL, outer plexiform layer; RIS, rod inner segment; ROS, rod outer segment. Bar, 50 μm.

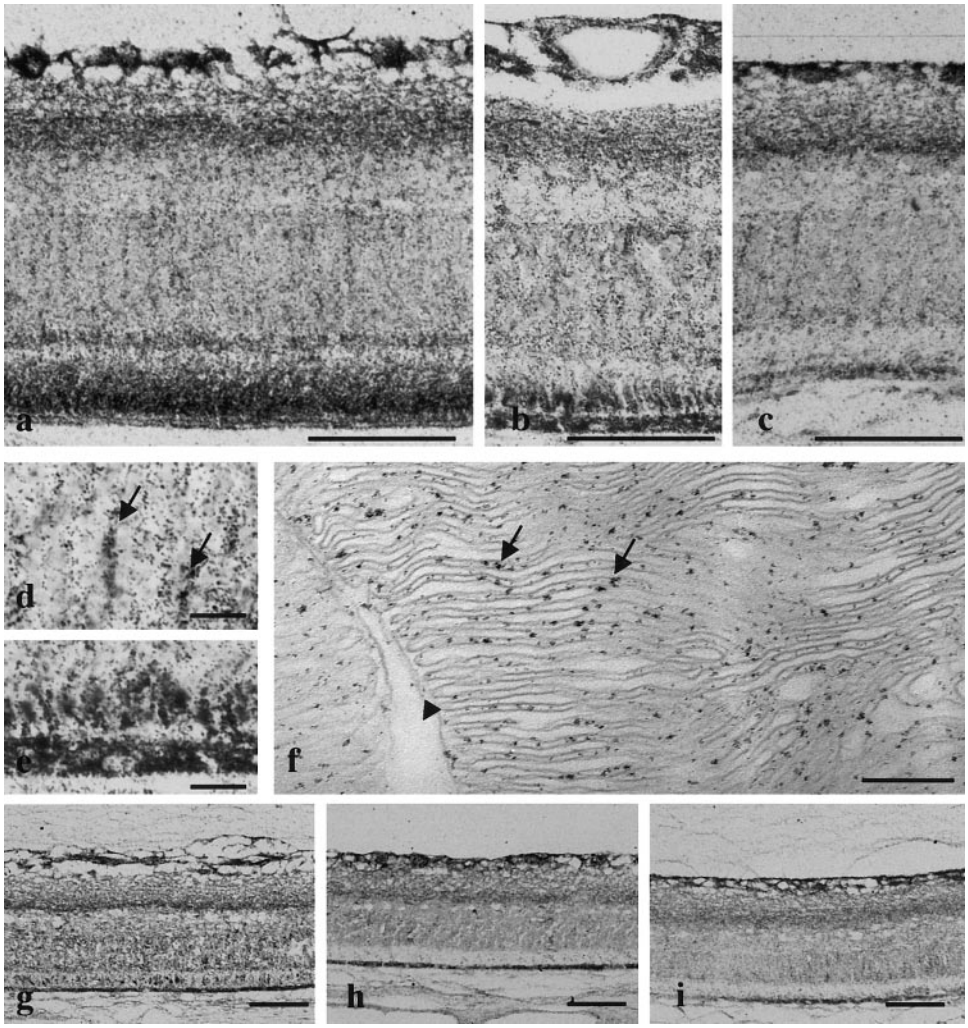

FIGURE 2. Immunohistochemical localization of GPX in a light-exposed retina. (**a**) GPX labeling was clearly visible in the photoreceptor outer segments and RPE at 12 and (**b**) 24 hours during exposure to light. (**c**) GPX labeling was present in the retina; however, labeling decreased in the photoreceptor outer segments and RPE in the peripheral retina. (**d**, **e**) Strong, clear labeling was observed at higher magnification of the Müller cells at 12 hours during exposure to light. (**d**) The photoreceptor outer segments and (**e**) RPE at 24 hours during exposure to light. The longitudinal pattern was consistent with the longitudinal processes of the Müller cells in the ONL (*arrow*). (**f**) Electron immunocytochemical localization of GPX in light-exposed retina at 24 hours during exposure to light. GPX labeling was located on the membrane structure of outer segments (*arrows*) but not in the interphotoreceptor matrix (*arrowhead*). (**g**) In the retinal specimens obtained 1 day after exposure to light, although strong GPX labeling was observed in the RPE, GPX labeling in the photoreceptor outer segments had decreased slightly. In retinal specimens obtained 3 (**h**) and 7 days (**i**) after exposure to light, strong GPX labeling was observed in the RPE. However, GPX labeling was not apparent in the photoreceptor inner and outer segments. Bar: (**a-c**, **g-i**) 50 μm; (**d**, **e**) 10 μm; (**f**) 200 nm.

Western Blot for GPX

The methods of retinal sample preparation and Western blot have been described.[28] Briefly, after deep anesthesia was induced by intraperitoneal injection of pentobarbital, the rats were perfused through the left cardiac ventricle with ice-cold 0.1 M phosphate buffer (pH 7.4) to wash out the blood, and the eyes then were removed ($n = 6$ for each time interval). After the cornea and the lens were removed, the inner retinal layers (neural retina) were separated from the eyecups under a microscope. After perfusion with ice-cold phosphate buffer, the adhesion between the photoreceptor cell layers and RPE cell layers was weakened, and they were easily separated. After the removal of the neural retina, the eyecups were analyzed as an RPE cell fraction, which also contained the choroid and the sclera. Equal amounts of retinal protein (100 μg protein per lane) were electrophoresed on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel and then electrophoretically transferred to a cellulose nitrate membrane (Schleicher & Schuell, Dassel, Germany). After the membranes were blocked, it was incubated with the first antibody and then with the peroxidase-linked second antibody. Chemiluminescence was detected with an ECL Western blot detection kit (Amersham Pharmacia Biotech, Buckinghamshire, UK). The optical density of each band was measured using NIH Image software (version 1.62; by Wayne Rasband, National Institutes of Health, Bethesda, MD; available by ftp from zippy.nimh.nih.gov/or from http://rsb.info.nih.gov/nih-image/) and expressed as the relative intensity compared with that of the control.

Northern Blot Analysis

According to the manufacturer's instructions, total RNA was extracted separately (TRIzol; Invitrogen Corp., Carlsbad, CA) from the neural retinas and the eyecups, including the RPE layer ($n = 4$ for each time interval). RNA samples were separated on a formaldehyde-1.2% agarose gel and transferred to a nylon membrane (Hybond N+, Amersham Pharmacia Biotech). The membrane then was preincubated at 65°C in prehybridization buffer (Rapid-hyb; Amersham Pharmacia Biotech) and hybridized for 12 hours at the same temperature with an [α-32P]dCTP-labeled probe for GPX. The probe for GPX was prepared from plasmids containing rat GPX cDNA. The blot was washed three times and autoradiographed with x-ray film. Quantitative analysis of the Northern blot analysis was performed with NIH Image software.

TABLE 1. GPX Intensity during and after Exposure to Light

	Cyclic Light	12 h	24 h	Day 1	Day 3	Day 7
Photoreceptor inner segment	−	+	+	+	−	−
Photoreceptor outer segment	+	+++	+++	++	−	−
RPE	+	++	+++	+++	++	+

According to the intensity of labeling, the immunoreaction is represented as +++, strongly intense; ++, moderately positive; +, weakly positive; and − negative.

TABLE 2. Ratio of the Thickness of the Outer Nuclear Layer to the Thickness of the Entire Retina

		Probabilities				
	Ratio	**Versus Cyclic Light**	**Versus 12 h**	**Versus 24 h**	**Versus Day 1**	**Versus Day 3**
Normal	29.9 ± 1.1	—	—	—	—	—
12 h	30.0 ± 1.1	NS	—	—	—	—
24 h	29.2 ± 1.9	NS	NS	—	—	—
Day 1	24.6 ± 1.5	<0.0001	<0.0001	<0.0001	—	—
Day 3	24.9 ± 1.1	<0.0001	<0.0001	<0.0001	NS	—
Day 7	19.9 ± 2.3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Data are the mean ± SD. Statistical significance was calculated by one-way ANOVA followed by the Bonferroni/Dunn posthoc test; $n = 6$ in each group. NS, not significant.

RESULTS

GPX Labeling in the Retina in Cyclic Lighting

Retinal micrographs representing each group of animals before and after exposure to high-intensity white light are shown in Figures 1 and 2. Intense GPX labeling was observed in the internal limiting membrane and ganglion cell layer, and moderate labeling was seen in the inner plexiform layer and inner nuclear layer. No labeling was present in the photoreceptor inner segments. Slight labeling was seen in the outer segments or the RPE (Fig. 1a). The labeling in the inner retinal layer was more intense than in the other layers. This labeling pattern may correspond to the end feet of the Müller cells. In addition, GPX labeling was more apparent in the posterior pole (Fig. 1a) than in the periphery (Fig. 1b). Some labeling of GPX was observed in ONL (Fig. 1a).

GPX labeling was not observed in the sections incubated with purified nonimmunized rabbit IgG (Fig. 1c) or GPX-absorbed antisera, or in sections incubated with KPBS in 1% BSA.

GPX Labeling in the Light-Exposed Retinal Tissue

In retinal specimens obtained at 12 and 24 hours during exposure to light, GPX labeling was clearly seen in the photoreceptor outer segments and RPE at the posterior retinal pole (Figs. 2a, 2b, 2e), but the labeling was not substantial in the peripheral retina just behind the iris (Fig. 2c). Labeling was present from the internal limiting membrane to the ONL. Those layers had slightly increased labeling in the Müller cells, indicating a longitudinal pattern (Fig. 2d). The electron microscopic finding indicated labeling in the membrane structure of the outer segments of photoreceptors (Fig. 2f).

In the retinal specimens obtained 1 day after exposure to light, no GPX immunolabeling was apparent in the photoreceptor inner segments, although GPX immunolabeling was visible in the photoreceptor outer segments; however, the intensity was slightly decreased (Fig. 2g). Strong GPX labeling was observed in the RPE until 7 days after exposure to light. In retinal specimens obtained 3 and 7 days after exposure to light, strong GPX labeling was observed in the RPE cells (Figs. 2h, 2i). However, GPX labeling was not apparent in the photoreceptor inner and outer segments.

Intense GPX labeling was observed from the internal limiting membrane to ganglion cell layer under both normal and experimental conditions. GPX induction was seen at the posterior pole, but this induction was not substantial in the peripheral retina in the experimental group. Table 1 shows GPX labeling in the photoreceptor inner and outer segments and RPE cells during and after exposure to light.

Histologic Changes

One, 3, and 7 days after exposure to light, there was a significant difference between the control and experimental groups in the ratio of the thickness of the ONL to the entire retina. These ratios did not change during exposure to light, but the ratio gradually decreased after exposure. In the control retinas, the ONL occupied approximately 29.9% ± 1.1% of the entire retina, whereas the ONL occupied only 19.9% ± 2.3% of the entire retina 7 days after exposure to light (Table 2). Seven days after exposure, the number of nuclei per row in the ONL also gradually decreased from 12.0 ± 0.6 in the control to 5.2 ± 0.8 (Table 3).

Western Blot for GPX

GPX labeling increased slightly during and after exposure to high-intensity white light. Strong labeling was observed after exposure for 24 hours (Fig. 3a). Compared with the retinas not exposed to light, the relative labeling intensities after exposure

TABLE 3. Number of Nuclei in the Outer Nuclear Layer

		Probabilities				
	Number	**Versus Cyclic Light**	**Versus 12 h**	**Versus 24 h**	**Versus Day 1**	**Versus Day 3**
Normal	12.0 ± 0.6	—	—	—	—	—
12 h	13.0 ± 0.6	NS	—	—	—	—
24 h	12.2 ± 1.3	NS	NS	—	—	—
Day 1	10.2 ± 0.8	=0.0005	<0.0001	=0.0002	—	—
Day 3	8.7 ± 0.5	<0.0001	<0.0001	<0.0001	=0.0033	—
Day 7	5.2 ± 0.8	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Data are the mean ± SD. Statistical significance was calculated by one-way ANOVA followed by the Bonferroni/Dunn posthoc test. $n = 6$ in each group. NS, not significant.

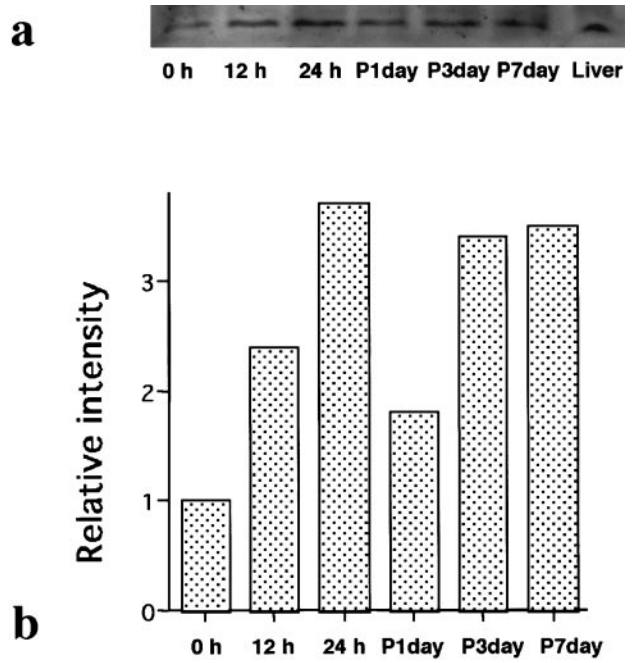

FIGURE 3. Western blot for GPX and histogram of the relative intensity of labeling. (**a**) GPX labeling increased slightly during and after exposure to light. Sections of rat liver served as a positive control. (**b**) The relative intensities of labeling during exposure to light at 12 and 24 hours and at 1, 3, and 7 days after exposure were 2.4, 3.7, 1.8, 3.4, and 3.5, respectively ($n = 6$ for each time interval).

to high-intensity white light for 12 and 24 hours and 1, 3, and 7 days were 2.4, 3.7, 1.8, 3.4, and 3.5, respectively (Fig. 3b).

Northern Blot for GPX

In samples of neural retina and RPE, GPX messenger RNA was upregulated by exposure to high-intensity white light for 24 hours and further upregulated 1 day after 24-hours of exposure (Figs. 4a, 4b). Compared with eyes not exposed to light, the relative intensities of GPX labeling, when adjusted by the relative intensities of 18s rRNA with ethidium bromide stain, after exposure to high-intensity white light for 6, 12, and 24 hours and in eyes enucleated 1 day after exposure to high-intensity white light for 24 hours were 1.2, 1.2, 1.9, and 3.4 in the neural retina, respectively, and 1.2, 1.2, 1.7, and 2.2 in the RPE, respectively (Figs. 4c, 4d).

DISCUSSION

Morphometric examination showed that exposure to light caused significant loss of photoreceptor nuclei. Retinal photic damage progressed over the course of the study. This result is consistent with those in previous reports.[1,2,5–11] Reactive oxygen species are believed to be important causative factors underlying retinal damage by light.[3] We examined the changes in GPX during this process and confirmed them by immunohistochemical and Western and Northern blot analyses.

There is an apparent discrepancy in quantitative evaluation of GPX in the retina at different time intervals between the data obtained by the immunohistochemical and Western blot methods. Because phototoxicity has been shown to lead to a breakdown of the blood–retinal barrier at the RPE,[29] one possibility is that leakage from choroidal blood vessels after exposure to high-intensity light results in high GPX levels in the retina, particularly in the RPE and rod outer segments. In fact, a fluorophotometric study revealed significant leakage of fluorescein into the vitreous, demonstrating a temporary dysfunction of the blood–retinal barrier after exposure to white light in the rabbit eye.[30] However, this is speculative and is difficult to show in this study. Further studies are needed to test this hypothesis.

Most stresses lead to the development of oxidative stress. Recent studies recognized the importance of an inducible pathway for the antioxidant defense.[31,32] An acute stress response induced by diverse stresses, including exposure to light, leads to the expression of a variety of antioxidant genes and proteins. Mittag et al.[33] reported that SOD knockout mice are more damaged by light. Northern and Western blot data in the present study indicate that GPX mRNA and GPX were upregulated by exposure to light in the neural retina and the RPE. Histochemical examination showed that GPX was significantly upregulated during exposure to light in both the photoreceptor outer segments and the RPE, suggesting that GPX was a light inducible endogenous molecule. Lipid hydroperoxides, which play an important role in light-induced damage in the retina, form in the photoreceptor outer segments soon after exposure to light.[34] These findings may be the initial phase of retinal protection against oxidative insult by exposure to light.

Previous studies have shown that exposure to light increases phagocytosis of the rod outer segments[35] and produces superoxide anion in RPE cells,[36] which is eliminated by SOD. Hydrogen peroxide then accumulates in the cell if the second protective line does not function well. GPX and catalase are important enzymes for eliminating hydrogen peroxide. We previously reported that Mn-SOD localized in the RPE cells and rod inner segment in the normal rat retina. Mn-SOD was lost 3

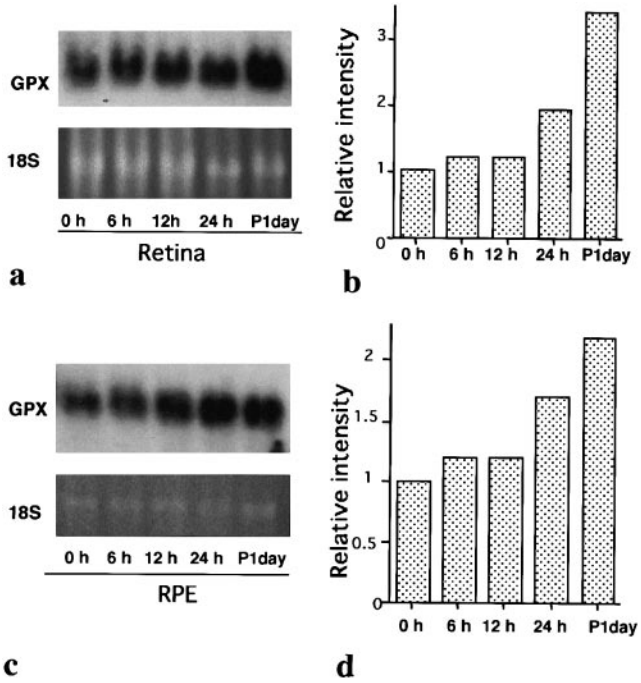

FIGURE 4. Northern blot for GPX and histogram of the relative intensity. (**a**) In the neural retina, GPX mRNA was upregulated by exposure to light for 24 hours. (**b**) Compared with the unexposed neural retina, the relative intensities of the GPX labeling in the retina exposed for 6, 12, and 24 hours and 1 day after exposure were 1.2, 1.2, 1.9, and 3.4, respectively. (**c**) GPX mRNA was also upregulated in the RPE by exposure to light for 24 hours. (**d**) Compared with the unexposed RPE, the relative intensities of GPX labeling after exposure to light for 6, 12, and 24 hours and 1 day were 1.2, 1.2, 1.7, and 2.2, respectively ($n = 4$ for each time interval).

hours and 1 day after light challenge, and then it reappeared in the RPE cells on days 3, 7, and 14.[11] In contrast, expression of GPX was not seen in the RPE cells in the normal retina. We found that GPX labeling also occurred in the RPE cells after exposure to light. The expression of GPX in the RPE was sustained at a high level until day 7 after exposure. Our findings may be consistent with a previous report that each antioxidant has a different role in oxidative stress.[37] GPX in RPE cells after exposure to light may have a protective role.

GPX labeling was present in the inner retinal layers but weak in other layers of the neural retina. The present study indicated that distribution of GPX ranged from weak to strong in the rat retina, which is similar to the pattern in neurons and glial cells in the brain.[38] One possible explanation for the sensitivity of the retina to oxidative stress is that antioxidant proteins are widely distributed in the retina. Morphometry of the retina showed a decrease in the photoreceptor cell layer after exposure to light in this experiment. Our results suggest that selective vulnerability may be attributable to the absence of GPX, which prevents oxidative stress in the retinal cells. In contrast, we observed that GPX is enriched in the posterior pole, which is constantly exposed to light, but is not enriched in the peripheral retina, indicating that GPX is not as necessary under normal conditions in the retina and the peripheral retina.

Previous studies of localization of GPX in rat retina have reported results contradictory to ours[19,20]—that is, that GPX was localized in the photoreceptor inner segments.[19,20] The reason for this discrepancy is unknown but may have been due to differences in procedures, antisera, or rat strains. Those studies mainly focused on the antioxidant sites in the retina, whereas we examined the changes in GPX during exposure to light on the retina and considered a time element as well as location. Akeo et al.[21] reported GPX labeling in the photoreceptor outer segments in degenerating rat retinas, which agreed with our results.

The severity of retinal damage may depend on a balance between the degree of the light challenge, such as the light intensity or the frequency of exposure, and the degree of physiologic protection against damage. The intensity of the challenge beyond the protective capabilities of the retina may result in irreversible damage, which supports our hypothesis that low expression of GPX may be related to the vulnerability of the outer retina.

The present study indicated that GPX labeling is induced in the photoreceptor outer segments and RPE cells at 12 and 24 hours during exposure to high-intensity white light. We recently reported that thioredoxin,[39] involved in the scavenging activity against hydrogen peroxidase in association with peroxiredoxin, differed from GPX in distribution in normal and light-exposed retinas. We also reported that Mn-SOD is localized at different sites compared with other antioxidants.[11] The results of the present study seem to support our hypothesis that various reactive oxygen species scavengers are induced at different time points after exposure to high-intensity white light, and these enzymes localize in different cell types. Organisciak et al.[40] reported that both catalase and GPX appear to be localized in the inner retinal layers. This pattern may help to explain why the inner retinal neurons are not damaged by high-intensity white light, whereas the photoreceptors undergo massive structural and functional changes. We concluded that differences in GPX distribution may be one cause of the selective vulnerability of retinal cells after photic retinal damage. Changes in GPX may have important implications in the defense against oxidative stress in the eye.

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