Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 1177–1181, February 1995
Biochemistry

Induction of heme oxygenase 1 in the retina by intense visible light: Suppression by the antioxidant dimethylthiourea

R. KRISHNAN KUTTY*†, GEETHA KUTTY*, BARBARA WIGGERT*, GERALD J. CHADER*, RUTH M. DARROW‡, AND DANIEL T. ORGANISCIAK‡

*Laboratory of Retinal Cell and Molecular Biology, National Eye Institute, National Institutes of Health, Bethesda, MD 20892; and ‡Department of Biochemistry and Molecular Biology, Wright State University, Dayton, OH 45435

Communicated by Rudi Schmid, University of California, San Francisco, CA, November 18, 1994

ABSTRACT **The effect of intense visible light (light damage) on the expression of heme oxygenase 1 (HO-1), a protein induced by oxidative stress, was investigated in the rat retina. A sensitive reverse transcription–PCR assay demonstrated the expression of mRNA for HO-1 as well as HO-2, the noninducible HO form, in the normal retina. As analyzed by Northern blotting, however, HO-1 mRNA was barely detectable under normal circumstances. After exposure to intense visible light, retinas had markedly higher HO-1 mRNA levels than unexposed controls, with increases up to 52- and 98-fold at 12 and 24 hr of exposure, respectively. Intense light exposure also resulted in an increase in HO-1 protein. In contrast, no appreciable change in HO-2 mRNA or protein was observed. The increase in HO-1 message was more pronounced in rats previously reared in the dark than in those reared in a weak cyclic-light environment. A marked decrease from the high level of HO-1 mRNA induced by light insult was observed when the animals were allowed to recover in the dark for 24 hr after light exposure. Most important, treatment of animals with 1,3-dimethylthiourea, a synthetic antioxidant, prior to light exposure effectively blocked the increase in HO-1 mRNA. Thus, HO-1 is a sensitive marker for assessing light-induced insult in the retina. Since increased expression of HO-1 is thought to be a cellular defense against oxidative damage, its expression may play an important role in protecting the retina against light damage.**

Oxidative damage has been implicated as a causative agent in several degenerative diseases loosely associated with aging (1). Since the neural retina is in a particularly oxygen-rich environment, several model systems have been established to examine the possible induction of oxidative damage in the retina by environmental factors. A rat model system in which the animals are exposed to intense light of 490–580 nm was originally described by Noell *et al.* (2) and has been employed to study the effects of light on photoreceptor cell degeneration in the retina (2–7). In this model, the interaction of light with visual pigments has been postulated to result in the production of reactive oxygen species which are injurious to the photoreceptors (2, 8). Conversely, high levels of chemical antioxidants such as ascorbic acid and antioxidative enzymes such as glutathione peroxidase present in retina may work in concert to protect it from the toxic effect of light (9–12). Although the mechanism of light damage is not understood, the loss of ascorbic acid from the light-exposed retina (4) and the fact that exogenously administered ascorbic acid and the synthetic antioxidant 1,3-dimethylthiourea (DMTU) protect against light damage are evidence in support of the oxidative hypothesis (3, 5–7).

Heme oxygenase (HO; EC 1.14.99.3), the rate-limiting enzyme in the heme degradative pathway first described by Schmid and coworkers (13), is induced in many cell types in response to a variety of stimuli (reviewed in ref. 14). HO consists of two isozymes: an inducible form, HO-1, and a constitutive form, HO-2 (15). The specific induction of HO-1 is thought to be a cellular response to oxidative stress (16). HO activity has been detected in bovine retina (17). Previously, we have found that HO-1 mRNA is expressed in human retina as well, and that its expression in cultured human retinoblastoma cells is increased in response to oxidative insult (18).

In the present study, we have investigated the effect of intense light exposure *in vivo* on the expression of HO-1 and HO-2 in the rat retina. It was reasoned that HO-1, if induced, could be a part of the retinal antioxidative defense mechanism and an ideal marker to obtain an insight into the mechanism of retinal light damage. Specifically, we found that not only was HO-1 induced to high levels following light exposure but that the increase was much less pronounced when animals were pretreated with the antioxidant DMTU, suggesting that HO-1 induction in retina is oxidatively driven.

MATERIALS AND METHODS

Animals and Treatment. Weanling male albino Sprague–Dawley rats were obtained from Harlan Laboratories (Indianapolis) and maintained in clear polycarbonate cages in a weak cyclic-light environment (20–40 lx, measured at the cage floor) for 12 hr/day (lights on at 08:00, off at 20:00) or in darkness for 40 days. The animals had free access to rat chow (Teklad, Madison, WI) and water. At age 60 days, the rats were dark-adapted overnight and then exposed to intense visible light for 12 or 24 hr. Light exposures were started at 09:00 and were performed in 6-inch (o.d.) × 22-inch green Plexiglas 2092 cylinders (Dayton Plastics, Dayton, OH) surrounded by six circular 12-inch (o.d.) 32-W Cool White fluorescent tubes (General Electric FC 12T/CW). Because the green Plexiglas has an effective bandpass of 490–580 nm (2), the animals received only green light. The animals were unrestrained; food and water were available during exposure. Body temperature was normal during light exposure (4). During exposure, the light intensity, measured inside the chamber, was 750–850 $\mu W/cm^2$ (Radiometer model 6SA, Yellow Springs Instruments). Rats were killed in carbon dioxide-saturated chambers and retinas were excised and immediately frozen on dry ice and stored in liquid nitrogen. Before sacrifice, some animals were allowed to remain in the dark for 24 hr, following the light exposure.

Prior to light treatment, some animals were injected intraperitoneally with DMTU (Aldrich) dissolved in saline. Doses of 500 mg/kg of body weight were administered 24 hr before and just prior to light exposure (6). The use of animals in this investigation conformed to the Association for Research in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HO, heme oxygenase; RT, reverse transcription; DMTU, 1,3-dimethylthiourea.

†To whom reprint requests should be addressed.

Vision and Ophthalmology Resolution on the Use of Animals in Research.

Reverse Transcription (RT)–PCR. Poly(A)$^+$ RNA preparations were isolated from retina, liver, or brain of rat and were reverse transcribed with an oligo(dT) primer. The reagents were obtained from Invitrogen. The first-strand cDNA preparations were used as templates for PCR (19). The primers, 5′-AAG-GAG-GTG-CAC-ATC-CGT-GCA-3′ (sense) and 5′-ATG-TTG-AGC-AGG-AAG-GCG-GTC-3′ (antisense) were designed from the published cDNA sequence of rat HO-1 (20) to amplify the region from bp 64 to bp 632. Similarly, 5′-ATG-GCA-GAC-CTT-TCT-GAG-CTC-3′ (sense) and 5′-CTT-CAT-ACT-CAG-GTC-CAA-GGC-3′ (antisense) were designed from the published cDNA sequence of rat HO-2 (21) to amplify the bp 264–818. The oligodeoxynucleotides were synthesized on a PCRmate DNA synthesizer and purified on oligonucleotide purification cartridges (Applied Biosystems). A reaction mixture (100 μl) consisting of a cDNA preparation [equivalent to 200 ng of poly(A)$^+$], 50 mM Tris·HCl (pH 9.0), 20 mM ammonium sulfate, 1.5 mM $MgCl_2$, deoxynucleoside triphosphates (200 μM each), sense primer (1 μM), antisense primer (1 μM), and 5 units of AmpliTaq DNA polymerase (Perkin–Elmer/Cetus) was overlaid with 70 μl of mineral oil and subjected to 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min. The reaction mixture was kept at 72°C for 10 min. An aliquot (20 μl) was analyzed for amplification product by agarose gel electrophoresis followed by ethidium bromide staining.

Northern Blot Analysis. Total RNA was extracted from tissue samples with RNAzol B (Tel-Test, Friendswood, TX). The ethanol-precipitated RNA was dissolved in diethyl pyrocarbonate-treated water and subjected to agarose gel electrophoresis in the presence of formaldehyde. The RNA from the gel was then capillary blotted onto an Immobilon N membrane (Millipore), UV crosslinked, and hybridized with the rat HO-1, rat HO-2, or a β-actin probe.

Probes for HO-1 and HO-2 were generated by RT–PCR from spleen and brain poly(A)$^+$ RNA preparations, respectively, as described earlier in this section, and purified by agarose gel electrophoresis. Human β-actin probe was obtained from Clontech. All the probes were labeled with ^{32}P by random priming (22). Hybridization was carried out with the HO-1 probe at 42°C in the presence of 50% formamide. The blots were then washed under stringent conditions and exposed to Kodak X-Omat AR film. The intensities of individual bands on the film were estimated with an LKB Ultroscan XL laser densitometer. Membranes were then stripped and reprobed with the β-actin probe or the HO-2 probe.

Western Blot Analysis. Retinas were homogenized in a buffer (200 μl) consisting of 20 mM Tris·HCl (pH 7.4), 0.25 M sucrose, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, leupeptin (50 μg/ml), and aprotinin (100 μg/ml). After centrifugation at 10,000 × *g* for 10 min, the supernatant was stored at −80°C until use. The extracts (25 μg of protein) were subjected to SDS/polyacrylamide gel electrophoresis using 10–20% Tricine gels (NOVEX, Encinitas, CA) and the proteins were transferred to a nitrocellulose membrane (Schleicher & Schuell) (23, 24). The membrane was blocked with a 5% solution of Carnation dried milk in Tris-buffered saline and then incubated at 0–4°C overnight with HO-1- or HO-2-specific antiserum. HO-1 antiserum was diluted 3000-fold and that for HO-2 was diluted 200-fold. The blot was washed with Tris-buffered saline and incubated with an alkaline phosphate-conjugated goat F(ab′)$_2$ fragment against rabbit IgG (Cappel). The blot was rewashed and the immunoreactive bands were visualized with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium.

The multiple antigenic peptide (MAP) system described by Posnett *et al.* (25) was employed for the production of rabbit antibodies against HO-1, as described (26). A peptide, MERPQLDSMSQDLSEALKEATKEVHIRAEN, consisting of residues 1–30 from the sequence reported for rat HO-1 by Shibahara *et al.* (20) was used as the antigen. The peptide, synthesized as a four-branch MAP (custom synthesis by Applied Biosystems), was used for the immunization of rabbits. A specific antibody preparation against rat HO-2 was a generous gift from M. D. Maines (27).

RESULTS

Expression of HO-1 and HO-2 mRNAs. Because the level of HO-1 mRNA is very low in normal retina (see below), we first developed a specific and sensitive RT–PCR technique to demonstrate the expression of mRNA for HO-1 and HO-2 in small samples of rat retina. As shown in Fig. 1, PCR using primers specific for HO-1 amplified a 568-bp DNA fragment from RNA preparations isolated from pooled retinas from normal rats. A product of similar size was also amplified when an RNA preparation from spleen, a known source of HO-1, was employed. The retinal RNA preparation also yielded a product of expected size when subjected to RT–PCR with HO-2-specific primers. These primers also yielded a product of similar size when the RNA preparation used was that from brain, a standard source of HO-2. The authenticity of PCR products were verified by DNA sequencing. We thus conclude that mRNAs for both HO-1 and HO-2 are expressed in normal rat retina.

Light Damage and Expression of HO-1 and HO-2 mRNAs. The effect of intense light exposure on the retinal expression of HO-1 and HO-2 mRNAs was investigated by Northern blot analysis of RNA preparations obtained from control and light-exposed rats. Rats reared in weak cyclic-light as well as dark-reared rats were used for this experiment because the degree of morphological damage imparted by intense light is known to be different in these two groups (28). A representative Northern blot is shown in Fig. 2. HO-1 signal was barely detectable in animals reared in either light environment prior to intense-light exposure (Fig. 2 *Top*, lanes 1 and 5). There were no differences in HO-1 message in the retinas of various control animals tested, including ones sacrificed at different times of the day (data not shown). However, HO-1 message greatly increased following light exposure (Fig. 2 *Top*, lanes

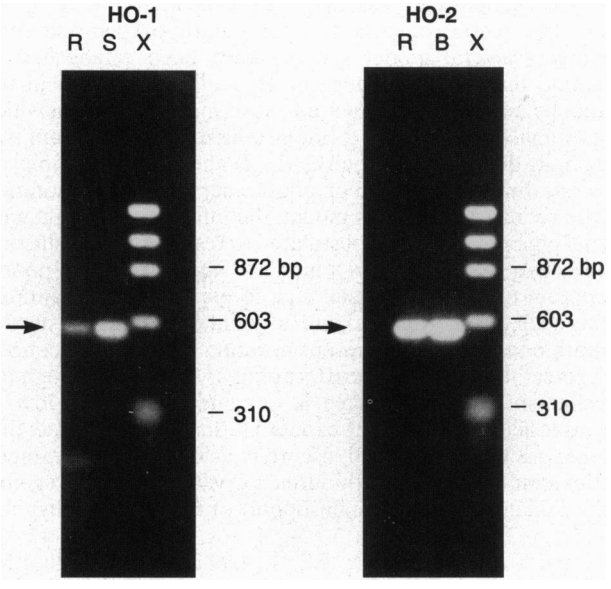

FIG. 1. RT–PCR analysis of HO-1 and HO-2 mRNAs in retina. A retinal RNA preparation pooled from at least 12 retinas was reverse transcribed and subjected to PCR using primers specific for HO-1 or HO-2. Lanes: R, retina; S, spleen; B, brain; X, molecular size marker (*Hae* III fragments of phage φX174 replicative-form DNA).

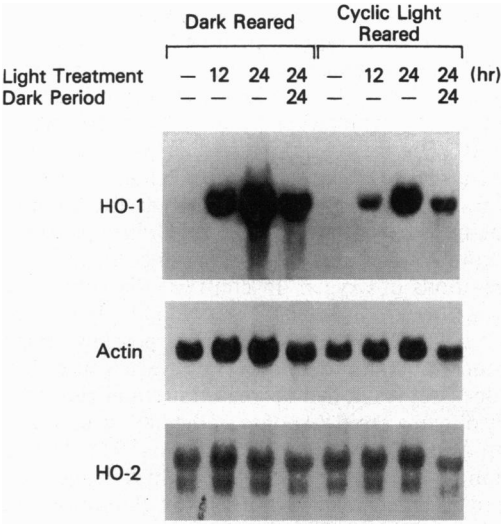

FIG. 2. Effect of intense visible light on the expression of HO-1 and HO-2 mRNAs in rat retina. Retinal RNA preparations (15 μg) pooled from two retinas each were subjected to Northern blot analysis using the HO-1 probe. The blot was stripped and reprobed for HO-2 or β-actin message.

2–4, and 6–8). The HO-1 signals from four different experiments were normalized by using the corresponding actin signals (Fig. 2 *Middle*) as controls for RNA loading and used for calculating the fold increase in HO-1 message induced by intense light (Table 1). By 24 hr of exposure, HO-1 mRNA was increased almost 100-fold in dark-reared animals; this effect was generally somewhat less in animals previously reared in weak cyclic light. Keeping the animals in darkness for 24 hr following 24 hr of light damage reduced the HO-1 signal from the elevated level in both dark- and cyclic light-reared rats.

Two bands (1.9 kb and 1.3 kb) were observed when retinal RNA preparations were analyzed by Northern blot using the HO-2 cDNA probe (Fig. 2 *Bottom*). Although significant HO-2 mRNA was present in control retinas (lanes 1 and 5), neither of the HO-2 mRNA species responded to light exposure as did HO-1 mRNA.

DMTU Effects on Light-Induced Expression of HO-1 mRNA. The effect of pretreating rats with the synthetic antioxidant DMTU on the light-induced increase in expression of retinal HO-1 message was studied. Fig. 3 shows three such separate experiments (*A–C*). In each experiment, rats previously reared in weak, cyclic light were divided into two groups. One group was treated with DMTU before exposure to intense light, while the other group served as a control. Retinal RNA samples were prepared from both groups after light treatment and analyzed for HO-1 expression by Northern blotting. In these experiments, a marked increase in HO-1 mRNA was observed following light exposure in nontreated animals (Fig. 3, lanes 1–4), but rats pretreated with DMTU (lanes 5–8)

Table 1. Light-induced expression of HO-1 mRNA

Treatment	Fold increase in HO-1 mRNA from control (mean ± SD)		
	Dark-reared	Cyclic light-reared	Cyclic light-reared and DMTU-treated
12 hr light	52 ± 5 [a]	27 ± 12 [b]	2 ± 1 [c]
24 hr light	98 ± 24 [d]	70 ± 17 [e]	12 ± 3 [f]
24 hr light + 24 hr dark	61 ± 2 [g]	37 ± 6 [h]	28 ± 2 [i]

Data from four Northern blots were analyzed. The differences between a and b ($P = 0.019$), b and e ($P = 0.009$), e and h ($P = 0.034$), g and h ($P = 0.006$), b and c ($P = 0.023$), and e and f ($P = 0.007$) were significant when analyzed by *t* test.

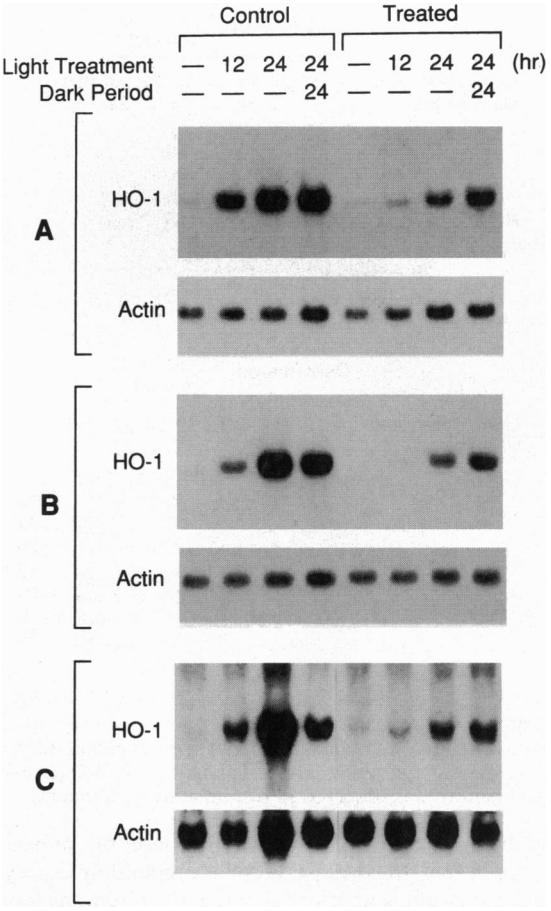

FIG. 3. Effect of DMTU on the intense visible light-mediated increase in HO-1 mRNA in the retina. Retinal RNA preparations (15 μg) from two eyes of individual animals were analyzed for HO-1 and β-actin mRNAs by Northern blotting. *A–C* represent three different experiments.

showed a much lower induction than those in the untreated group (Table 1). Interestingly, HO-1 expression continued to rise in the DMTU-treated animals in the 24-hr dark period after light damage.

Effects of Light Damage on Expression of HO-1 and HO-2 Proteins. We felt it of importance to determine the relative levels of actual HO-1 and HO-2 proteins in retina and their responses to intense light exposure. Retinal extracts were analyzed by Western blot using specific antibody preparations against HO-1 and HO-2. For dark-reared animals (Fig. 4 *Upper*, lanes 1–4), increased HO-1 immunoreactivity was observed in the retinas of animals exposed to intense light for 12 and 24 hr and remained high in the dark period after exposure. In rats reared in cyclic light (Fig. 4 *Upper*, lanes 5–8), HO-1 protein was barely detectable in unexposed control retinas, with little increase observed in those exposed to intense light for 12 hr. However, a prominent HO-1 band was detected following 24 hr of light exposure; this band decreased somewhat when the rats were subsequently kept in darkness for 24 hr after the 24-hr period of intense light treatment.

The rat retinas also contained the HO-2 protein when tested with a specific antibody for this protein (Fig. 4 *Lower*). However, no appreciable differences in the intensity of the immunoreactive band were found in any of the samples tested.

DISCUSSION

Although ostensibly having similar functions, HO-1 and HO-2 have markedly different patterns of expression in the retina. HO-1 message and protein are barely detectable in normal

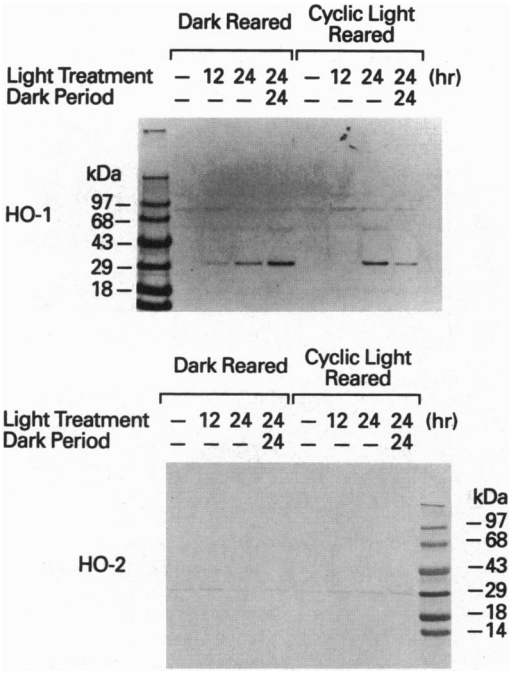

FIG. 4. Effect of intense visible light on the expression of HO-1 and HO-2 proteins in retina. Retinal extracts (25 μg of protein) from at least two eyes each were analyzed by Western immunoblotting using antisera specific for either HO-1 (*Upper*) or HO-2 (*Lower*). An identical pattern was observed in two separate experiments.

retina but are overexpressed in response to intense light exposure *in vivo*. In contrast, HO-2 is moderately expressed in normal retina but is unaffected under all conditions tested. In other tissues, induction of HO-1 has been described as a response to oxidative stress (29). Thus, HO-1 provides an effective marker to study the effects of intense light in the retina and the mechanism by which damage is effected. The induction of HO-1 in the light-exposed rat retina lends support to the hypothesis that light-induced retinal damage results from the formation of reactive oxygen species. In this study, we also found that pretreatment of rats with the antioxidant DMTU effectively reduced the induction of HO-1 mRNA. This not only agrees with the idea that oxidative insult is at least part of the light-induced damage process but, to our knowledge, is the first demonstration that an antioxidant can effectively block the induction of HO-1 *in vivo*.

Although it is not clear whether HO-1 induction is a beneficial or a detrimental cellular response, most investigators have assumed that HO-1 induction is a protective response against oxidative damage (16). Enzymatically, increased HO activity is thought to effectively degrade heme, a prooxidant, to biliverdin (13). This product is readily converted into bilirubin (30, 31), which possesses excellent antioxidant properties (32). Increased HO-1 protects human skin fibroblasts against oxidative stress (33) and decreases the incidence of kidney damage in a rat model system for rhabdomyolysis (34). The situation is complicated, however, since HO activity also leads to the formation of chelatable iron and carbon monoxide. Iron ions can produce oxidative damage in cells, but studies have shown that the HO induction is associated with an induction of ferritin, a protein that can effectively remove free iron (34–36). The second product, carbon monoxide, is now thought to be a second messenger in the central nervous system. It activates soluble guanylate cyclase, leading to an increase in cellular cGMP (37, 38). It is not clear how this response could affect cellular function in the retinas of light-exposed rats, although a constitutive increase in cGMP could mimic the situation in the *rd* mouse, where abnormal cGMP metabolism leads to rapid retinal degeneration (39).

The induction of heme oxygenase has been demonstrated in numerous systems (14, 26). Human skin fibroblasts in culture respond to UV irradiation by increasing the expression of HO-1 (40). The increase in HO-1 has also been shown to be often associated with the depletion of cellular glutathione (29). However, in the *in vitro* fibroblast system, the observed effect could be mediated by factors such as photosensitizers present in the culture medium and the presence of artificially high concentrations of oxygen. In contrast, the present study describes an *in vivo* system where retinal photoreceptor-cell damage is induced by visible light, possibly mimicking a longer-term human condition. The effect is also different in that it does not result in a decrease in retinal glutathione (10), possibly due to a rapid response of the hexose monophosphate shunt to oxidative insult in the rat retina (41). Therefore, the induction of HO-1 in the retina by light damage seems to be mediated by a mechanism not involving glutathione depletion. This is not surprising, since the HO-1 gene is known to contain a variety of regulatory elements (42, 43).

Light damage is known to cause a reduction in cellular ascorbate, another antioxidant (4). Furthermore, ascorbate supplementation reduces the level of retinal light damage in rats (4) as does the synthetic antioxidant DMTU (3, 6, 7). This suggests, as originally proposed by Noell *et al.* (2), that oxidation occurs in the rat retina in response to prolonged light exposure. Green light (490–580 nm) preferentially excites rhodopsin, and the action spectrum for retinal damage is the same as the rhodopsin absorption spectrum (2, 44). Retinal damage has also been found to be more severe in rats previously reared in darkness than in those reared in weak, cyclic light (6, 28). This may be related to the higher rhodopsin levels found in dark-reared rats (28) or to different levels of other transduction proteins in the photoreceptor cells (45). In our study, HO-1 induction was more pronounced in the dark-reared rats than in the cyclic light-reared animals. Thus, HO-1 induction parallels the differential susceptibility of the dark- and cyclic light-reared rats to retinal light damage.

HO-2 is expressed in brain, where it may contribute to neurotransmission by generating carbon monoxide (37, 38, 46). In our study, HO-2 mRNA was detected as two species in the retina (Fig. 1), in good agreement with the findings in brain (46). It is interesting that identical results were obtained even though we employed a much smaller cDNA probe. The reason for the occurrence of two different-size messages is not clear. However, only one immunoreactive HO-2 protein band was detected in the retina, also in agreement with observations in other tissues (14, 15, 27, 46). In sharp contrast to HO-1, HO-2 expression was not altered by intense light exposure, indicating the probability of different regulation and different functions of the two enzyme forms in the retina.

In conclusion, this study has shown a dramatic and selective overexpression of HO-1 in the retina in response to light stress *in vivo*. Whether this response is a protective mechanism or not and whether HO-1 plays a role in other retinal degenerative disorders—such as age-related macular degeneration in the human—where light has been implicated remains to be elucidated. In any event, the present rat model should serve as a useful system to better define the role of HO-1 in oxidative damage in general and its specific role in light damage.

We thank Prof. Mahin Maines (University of Rochester School of Medicine, Rochester, NY) for a generous gift of antibody preparation against HO-2. The study was supported in part by National Eye Institute Grant EY01959 to D.T.O.

1. Ames, B. N., Shigenaga, M. K. & Hagen, T. M. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 7915–7922.

2. Noell, W. K., Walker, V. S., Kang, B. S. & Berman, S. (1966) *Invest. Ophthalmol.* **5,** 450–473.
3. Organisciak, D. T., Darrow, R. M., Bicknell, I. R., Jiang, Y.-L., Pickford, M. & Blanks, J. C. (1991) in *Retinal Degenerations*, eds. Anderson, R. E., Hollyfield, J. G. & LaVail, M. M. (CRC, Boca Raton, FL), pp. 189–201.
4. Organisciak, D. T., Wang, H.-M., Li, Z.-Y. & Tso, M. O. M. (1985) *Invest. Ophthalmol. Visual Sci.* **26,** 1580–1588.
5. Organisciak, D. T., Jiang, Y.-L., Wang, H.-M. & Bicknell, I. (1990) *Invest. Ophthalmol. Visual Sci.* **31,** 1195–1202.
6. Organisciak, D. T., Darrow, R. M., Jiang, Y.-L., Marak, G. E. & Blanks, J. C. (1992) *Invest. Ophthalmol. Visual Sci.* **33,** 1599–1609.
7. Lam, S., Tso, M. O. M. & Gurne, D. H. (1990) *Arch. Ophthalmol.* **108,** 1751–1757.
8. Wiegand, R. D., Giusto, N. M., Rapp, L. M. & Anderson, R. E. (1983) *Invest. Ophthalmol. Visual Sci.* **24,** 1433–1435.
9. Penn, J. S., Naash, M. I. & Anderson, R. E. (1987) *Exp. Eye Res.* **44,** 779–788.
10. Organisciak, D. T., Wang, H.-M. & Kou, A. L. (1984) *Curr. Eye Res.* **3,** 257–267.
11. Hunt, D. F., Organisciak, D. T., Wang, H. M. & Wu, R. L. C. (1984) *Curr. Eye Res.* **3,** 1281–1288.
12. Penn, J. S. & Anderson, R. E. (1987) *Exp. Eye Res.* **44,** 767–778.
13. Tenhunen, R., Marver, H. S. & Schmid, R. (1969) *J. Biol. Chem.* **244,** 6388–6394.
14. Maines, M. D. (1992) *Heme Oxygenase* (CRC, Boca Raton, FL).
15. Maines, M. D., Trakshel, G. M. & Kutty, R. K. (1986) *J. Biol. Chem.* **261,** 411–419.
16. Stocker, R. (1990) *Free Radical Res. Commun.* **9,** 101–112.
17. Schwartzman, M. L., Masferrer, J., Dunn, M. W., McGiff, J. C. & Abraham, N. G. (1987) *Curr. Eye Res.* **6,** 623–630.
18. Kutty, G., Hayden, B., Osawa, Y., Wiggert, B., Chader, G. J. & Kutty, R. K. (1992) *Curr. Eye Res.* **11,** 153–160.
19. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239,** 487–491.
20. Shibahara, S., Muller, R., Taguchi, H. & Yoshida, T. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7865–7869.
21. Rotenberg, M. O. & Maines, M. D. (1990) *J. Biol. Chem.* **265,** 7501–7506.
22. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
23. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
24. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
25. Posnett, D. N., McGrath, H. & Tam, J. P. (1988) *J. Biol. Chem.* **263,** 1719–1725.
26. Kutty, R. K., Nagineni, C. N., Kutty, G., Hooks, J. J., Chader, G. J. & Wiggert, B. (1994) *J. Cell. Physiol.* **159,** 371–378.
27. Kutty, R. K. & Maines, M. D. (1989) *Arch. Biochem. Biophys.* **268,** 100–107.
28. Noell, W. K., Delmelle, M. C. & Albrecht, R. (1971) *Science* **172,** 72–76.
29. Lautier, D., Luscher, P. & Tyrrell, R. M. (1992) *Carcinogenesis* **13,** 227–232.
30. Tenhunen, R., Ross, M. E., Marver, H. S. & Schmid, R. (1970) *Biochemistry* **9,** 298–303.
31. Kutty, R. K. & Maines, M. D. (1981) *J. Biol. Chem.* **256,** 3956–3962.
32. Stocker, R., Yamamoto, Y., McDonagh, A. F., Glazer, A. N. & Ames, B. N. (1987) *Science* **235,** 1043–1046.
33. Vile, G. F., Basu-Modak, S., Waltner, C. & Tyrrell, R. M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2607–2610.
34. Nath, K. A., Balla, G., Vercelloti, G. M., Balla, J., Jacob, H. S., Levitt, M. D. & Rosenberg, M. E. (1992) *J. Clin. Invest.* **90,** 267–270.
35. Eisenstein, R. S., Garcia-Mayol, D., Pettingell, W. & Munro, H. N. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 688–692.
36. Vile, G. F. & Tyrrell, R. M. (1993) *J. Biol. Chem.* **268,** 14678–14681.
37. Verma, A., Hirsch, D. J., Glatt, C. E., Ronnett, G. V. & Snyder, S. H. (1993) *Science* **259,** 381–384.
38. Maines, M. D., Mark, J. A. & Ewing, J. F. (1993) *Mol. Cell. Neurosci.* **4,** 398–405.
39. Farber, D. B. & Lolley, R. N. (1976) *J. Cyclic Nucleotide Res.* **2,** 139–148.
40. Keyse, S. M. & Tyrrell, R. M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 99–103.
41. Winkler, B. S. & Giblin, F. J. (1983) *Exp. Eye Res.* **36,** 287–297.
42. Lavrovsky, Y., Schwartzman, M. L., Levere, R. D., Kappas, A. & Abraham, N. G. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 5987–5991.
43. Alam, J., Cai, J. & Smith, A. (1994) *J. Biol. Chem.* **269,** 1001–1009.
44. Williams, T. P. & Howell, W. L. (1983) *Invest. Ophthalmol. Visual Sci.* **24,** 285–287.
45. Organisciak, D. T., Xie, A., Wang, H.-M., Jiang, Y.-L., Darrow, R. M. & Donoso, L. A. (1991) *Exp. Eye Res.* **53,** 773–779.
46. Sun, Y., Rotenberg, M. O. & Maines, M. D. (1990) *J. Biol. Chem.* **265,** 8212–8217.