Arch Toxicol (2001) 75: 150–158
DOI 10.1007/s002040000206

MOLECULAR TOXICOLOGY

Elke Röhrdanz · Gabriele Schmuck · Sandra Ohler
Quynh-Hoa Tran-Thi · Regine Kahl

Changes in antioxidant enzyme expression in response to hydrogen peroxide in rat astroglial cells

Received: 12 October 2000 / Accepted: 4 December 2000 / Published online: 3 April 2001
© Springer-Verlag 2001

Abstract Oxidative stress has been causally linked to a variety of neurodegenerative diseases. To clarify the role of the antioxidant enzyme (AOE) system in oxidative brain damage primary cultures of rat astroglial cells were exposed to hydrogen peroxide (H_2O_2). Expression of AOEs and several parameters for cell viability and functionality were measured. In our experiments astrocytes responded to low concentrations of H_2O_2 exposure with a pronounced generation of ROS which ran parallel with induction of lipid peroxidation. This distinct oxidative stress was not reflected in cell viability or functionality parameters measured. Cytotoxicity, a decrease in glutathione content of astrocytes, and impairment of mitochondrial functions became obvious only for higher concentrations of H_2O_2. After H_2O_2 exposure catalase, manganese superoxide dismutase, and glutathione peroxidase expression levels were found to be increased, whereas copper/zinc superoxide dismutase mRNA expression was not affected. These data indicate that the AOE system of astrocytes can be directly regulated by oxidative stress and may thus contribute to protection of cells against oxidative insults.

Keywords Catalase · Superoxide dismutase · Glutathione peroxidase · Hydrogen peroxide · Astrocytes

E. Röhrdanz (✉)
Institut für Toxikologie, Postfach 101007,
40001 Düsseldorf, Germany
E-mail: roehrdan@uni-duesseldorf.de
Tel.: +49-211-8113003
Fax: +49-211-113013

E. Röhrdanz · G. Schmuck · S. Ohler · Q.-H. Tran-Thi · R. Kahl
Institut für Toxikologie, Heinrich-Heine-Universität Düsseldorf,
Universitätsstrasse 1, 40225 Düsseldorf, Germany

G. Schmuck
Bayer, Pharma Research Centre, Aprather Weg,
42096 Wuppertal, Germany

Introduction

Oxidative stress is believed to be implicated in a wide variety of human degenerative disorders of the central nervous system (CNS), including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, and conditions such as ischemia and excitotoxicity (Halliwell 1992). To prevent injury from reactive oxygen species (ROS), aerobic organisms are endowed with defense systems including both specific enzymes and scavenger molecules, such as glutathione or α-tocopherol. Included among the antioxidant enzymes (AOEs) are catalase, copper/zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD), and the glutathione peroxidases (GPxs). Catalase and GPxs convert H_2O_2 to H_2O and the SODs catalyze the dismutation of the superoxide radical anion (Aebi 1984; McCord 1979; Ursini et al. 1995).

The CNS is generally considered to be especially sensitive to free radical damage due to its high metabolic rate, high levels of lipids, and the comparatively low content of protective systems (Halliwell and Gutteridge 1989). The antioxidant defense via the AOEs is particularly important for the brain. An increased activity of CuZnSOD in the brains of transgenic mice protects neurons from ischemia reperfusion injury and also lessens the cerebral edema caused by traumatic brain injury (Li et al. 1999). Conversely, a deficiency in CuZnSOD or MnSOD worsens the brain damage (Chan 1996; Kondo et al. 1996). Familial amyotrophic lateral sclerosis is associated with mutations in the CuZnSOD gene, but the mechanisms by which these mutations lead to amyotrophic lateral sclerosis are still unknown (Rabizadeh et al. 1995).

The expression of AOEs can be regulated by oxidative stress itself. Exposure to H_2O_2 or hyperoxia led to an increase in catalase mRNA expression in various epithelial cell lines as well as in the rat lung (Clerch et al. 1990; Shull et al. 1991; Tate et al. 1995). Previous results

from our laboratory have shown differential regulation of AOE expression in rat hepatocyte cultures and hepatoma cells (Röhrdanz and Kahl 1998; Röhrdanz et al. 2000). Inflammatory mediators are able to induce selectively MnSOD mRNA expression in different cell types, among others in primary cortical neurons and astrocytes (Dougall and Nick 1991; Kifle et al. 1996; Mokuno et al. 1994; Wong and Goeddel 1984). Since inflammation is assumed to act via generation of ROS, induction of AOEs is thus linked to oxidative stress. Pinteaux et al. (1996) found an increase of MnSOD activity and a decrease in CuZnSOD activity after exposure of primary glial cell cultures to ROS generated by a xanthine/xanthine oxidase mixture. However, if and how the mRNA expression of those enzymes was affected has not been shown.

Astroglial cells represent the major compartment of GPx in the human mesencephalon and hippocampus (Damier et al. 1993; Takizawa et al. 1994). For MnSOD and CuZnSOD a neuronal localization in the human brain is discussed (Akai et al. 1990; Bergeron et al. 1996; Ceballos et al. 1989; Zhang et al. 1994). Changes of the cellular localization of SODs were observed in cases of Alzheimer's disease and Down's syndrome (Ceballos-Picot 1997; Furuta et al. 1995; Pinteaux et al. 1998). In cerebral sites of degeneration MnSOD was more enriched in reactive astrocytes than in neurons. This suggests that MnSOD may be activated by oxidative stress as a defense mechanism against deleterious effects of ROS. Neuroprotection through astrocytes was also shown in primary cultures of astrocytes and neurons. Co-culture with astrocytes attenuated killing by H_2O_2 of striatal neurons (Desagher et al. 1996). This neuroprotective role of astrocytes appears most evident following reactive gliosis, where different antioxidant mechanisms were found to be stimulated.

To elucidate the role of AOEs in oxidative brain damage, we investigated the influence of oxidative stress on AOE expression. In our model system primary cultures of astroglial cells were treated with H_2O_2 as a direct mediator of oxidative stress.

Materials and methods

Preparation of primary rat astroglial cultures

Astrocytes were isolated from the whole rat fetal brain (E19–20). The brains were filtered through two different Nylon meshes (212 and 135 μm pore diameter) as previously described (Schmuck and Haynes 2000). The cells were cultured in DMEM medium supplemented with 10% FCS on Primaria culture plates (Becton and Dickinson, Heidelberg, Germany) in a humidified incubator in 5% CO_2/95% air. The astrocytes consisted of around 95% glial fibrillary acid protein (Roche, Mannheim, Germany)-positive cells, 1% microglia (CD11/B/C-positive), and 1–5% oligodendrocytes (galactocerebroside-positive). The cultures were grown for 10–14 days with two changes of medium in between at 37°C before use. When not stated otherwise astrocytes were treated for 24 h with different concentrations of H_2O_2 to perform the experiments.

Viability assay

To determine the viability of H_2O_2-treated cells, mitochondrial dehydrogenase activity was measured in the MTT assay as described elsewhere (Carmichael et al. 1985).

Intracellular ATP concentration

The intracellular ATP concentration was determined according to a chemiluminescence reaction in a luciferin/luciferase system (ATP Lite-M; Packard, Groningen, The Netherlands). Astrocytes were treated for 24 h with H_2O_2 before they were tested.

Mitochondrial membrane potential

The membrane potential of the inner mitochondrial membrane was measured by the dye tetramethylrhodamine after treating the astrocyte cultures for 24 h with H_2O_2. The dye was added to the cell culture medium in a concentration of 3.3 μM for 30 min at the end of the incubation period with H_2O_2. The cells were washed twice and the fluorescence was determined with a fluorostar spectrophotometer (SLT, Crailsheim, Germany) at 530/580 nm.

Detection of ROS

The determination of ROS was done according to Oyama et al. (1996). The cells were washed with PBS and loaded with 2,7-dichlorofluorescein diacetate (Sigma, Deisenhofen, Germany) at a concentration of 50 μM in phosphate-buffered saline (PBS) for 1 h. Dichlorofluorescein diacetate crosses cell membranes and then undergoes deacetylation by intracellular esterases. The resulting compound, dichlorofluorescein, is proposed to be trapped within the cell and susceptible to ROS-mediated oxidation to the fluorescent compound, dichlorofluorescein (Buxser et al. 1999). After 1 h the remaining dichlorofluorescein diacetate was removed, the cells were washed with PBS, and then treated with the indicated concentrations of H_2O_2. The fluorescence was determined up to 4 h in a fluorostar spectrophotometer (SLT) at 485/530 nm.

Lipid peroxidation

Lipid peroxidation was measured by quantitation of malondialdehyde (MDA) release of treated cells into the medium. MDA was determined after reaction of cell culture supernatants with thiobarbituric acid and subsequent high-pressure liquid chromatography according to a modified method by Draper et al. (1993).

Intracellular glutathione (GSH)

Intracellular GSH concentration was measured according to Mundi et al. (1997). The cells were washed with PBS and then treated with the indicated concentrations of H_2O_2 for 1 h. Afterwards the cells were loaded with 5-methylchlorofluorescein diacetate (Molecular Probes, Eugene, Ore., USA) in a concentration of 20 μM in PBS for 1 h. Then the fluorescence was determined in a fluorostar spectrophotometer (SLT) at 485/530 nm.

RNA isolation and RNA analysis

Total RNA was isolated from cells using Trizol reagent (Gibco BRL, Eggenstein, Germany). For northern blot analysis, 5 μg total RNA were resolved by electrophoresis in a 1% agarose, 2.25 mM formaldehyde gel in a running buffer containing 20 mM MOPS, pH 7, 0.5 mM sodium acetate, and 1 mM EDTA. RNA was transferred to nylon membrane (Amersham, Arlington Height, Ill., USA) according to Maniatis et al. (1982). Purified cDNAs were labeled with [$\alpha^{32}P$] dCTP (111 TBq/mmol; Hartmann Analytic, Braunschweig, Germany) by random hexamer priming (Roche

Diagnostics, Mannheim, Germany). Blots were prehybridized and hybridized with cDNAs as described previously (Röhrdanz and Kahl 1998). Autoradiographs were made by exposing blots to X-ray film (Kodak XAR) with an intensifying screen at –80°C. Blots were stripped and reprobed with different cDNAs. Autoradiographs were analyzed by densitometric scanning using the Quantity One system from BioRad (Munich, Germany).

Dr. Shuichi Furuta (Sinshu University, Department of Biochemistry, Nagano, Japan) provided the rat catalase cDNA containing plasmid, PMJ1010 (Furuta et al. 1986). We obtained a 1.4 kb rat MnSOD cDNA containing plasmid, pSP65-RMS (Ho and Crapo 1987a), and a 0.6 kb rat CuZnSOD cDNA containing plasmid, pUC-13-RCS (Ho and Crapo 1987b), from Dr. Ye-Shih Ho (Wayne State University, Institute of Chemical Toxicology, Detroit, Mich., USA). For 18 S rRNA detection we used the oligonucleotide probe 5′-GCCGTGCGTACTTAGACATGCATG-3′ (Chan et al. 1984).

PCR following reverse transcription was performed for semiquantitative determination of GPx mRNA. One microgram of total RNA was transcribed into cDNA in a 25-μl final volume of reaction buffer (50 mM TRIS-HCL, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 0.5 mM each dNTP) and 5 μM oligo-d$(T)_{16}$-primer, 1 U RNase inhibitor, and 2.5 U MLV reverse transcriptase by incubation for 1 h at 42°C. The reaction was stopped by incubation at 99°C for 5 min. For rat GPx PCR 2.5 μl of the product of reverse transcription was added to a 22.5-μl PCR mixture containing 10 mM TRIS-HCL, 50 mM KCl, 0.1% Triton X-100, 2 mM $MgCl_2$, 0.2 mM each dNTP, 1 U Taq DNA polymerase (Promega, Mannheim, Germany), and 0.2 μM each PCR primer. The primers were designed for detection of the rat GPx according to the genomic sequence published by Ho and Howard (1992); upstream primer 5′-ATGTCTGCTGCTCGGCTCTC-3′ and downstream primer 5′-GTTGCTAGGCTGCTTGGACAG-3′. Amplification was initiated with 4 min denaturation at 94°C followed by 27 cycles at 94°C, 61°C, and 72°C for 60 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) PCR was performed with 100 ng synthesized cDNA as described elsewhere (El-Bahay et al. 1999). The amplified PCR products were 602 bp for GPx mRNA and 450 bp for GAPDH mRNA. The conditions of PCR were set to be in the linear phase of amplification to allow for semiquantification of mRNA content. Five microliters from each PCR reaction were electrophoresed in an 1% agarose gel in TRIS-borate-EDTA buffer. The cDNA bands were visualized by UV illumination after staining the gels with ethidium bromide. Gels were photographed and scanned densitometrically.

Statistics

Statistical analysis was performed using Student's *t*-test (Sigma Stat; Scientific, Erkrath, Germany).

Results

Cytotoxicity

After H_2O_2 exposure, viability of cells was determined via the MTT assay, which reflects mitochondrial dehydrogenase activity (Fig. 1). According to the MTT assay, H_2O_2 toxicity started to show at concentrations of 0.05–0.1 mM H_2O_2 with around 20% dead astrocytes, increasing to 80% dead cells at 1 mM H_2O_2.

Mitochondrial function

Mitochondrial function was determined by the intracellular ATP concentration and the mitochondrial membrane potential (tetramethylrhodamine) (Fig. 2). There was a reduction in ATP content, which became obvious at a concentration of 0.5 mM H_2O_2. At 0.5 mM H_2O_2 a 30% and at 1 mM H_2O_2 a 40% reduction was detected. These results are confirmed by the loss in membrane potential with increasing concentrations of H_2O_2. Treatment of astrocytes with 0.5 mM H_2O_2 lead to a loss of 50% and with 1 mM H_2O_2 to a loss of 80% in membrane potential compared to controls, which ran parallel with the decrease in cell viability.

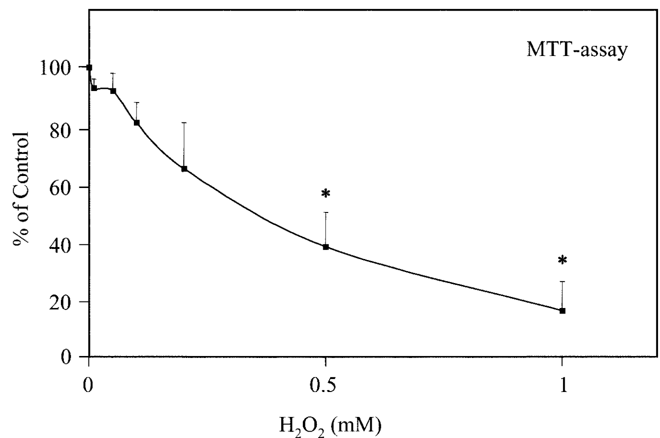

Fig. 1 Viability of astrocytes after treatment with H_2O_2. Astrocytes were incubated with the indicated concentrations of H_2O_2 for 24 h and viability was determined via MTT assay. Values are expressed as percentage of controls (means ± SEM from four to six different experiments). $^*P < 0.05$ vs controls

Generation of ROS

Upon treatment with H_2O_2 a massive production of ROS could be detected in astrocytes (Fig. 3). A sevenfold increase in ROS compared to controls could already be detected for low concentrations of H_2O_2 (≤ 0.1 mM) after treatment for 60 and 120 min, respectively.

Lipid peroxidation

The extent of lipid peroxidation was assessed by measuring the release of the breakdown product MDA. In astrocytes an increase by the factor of 6–7 in MDA levels was obtained after treatment with H_2O_2. This drastic increase in MDA levels already started to show with H_2O_2 concentrations of 0.05 mM and corresponded to the ROS production data (Fig. 4).

Intracellular GSH

With increasing concentrations of H_2O_2, the internal stores of GSH were depleted in astrocytes (Fig. 5). A nearly 20% depletion of GSH was observed after

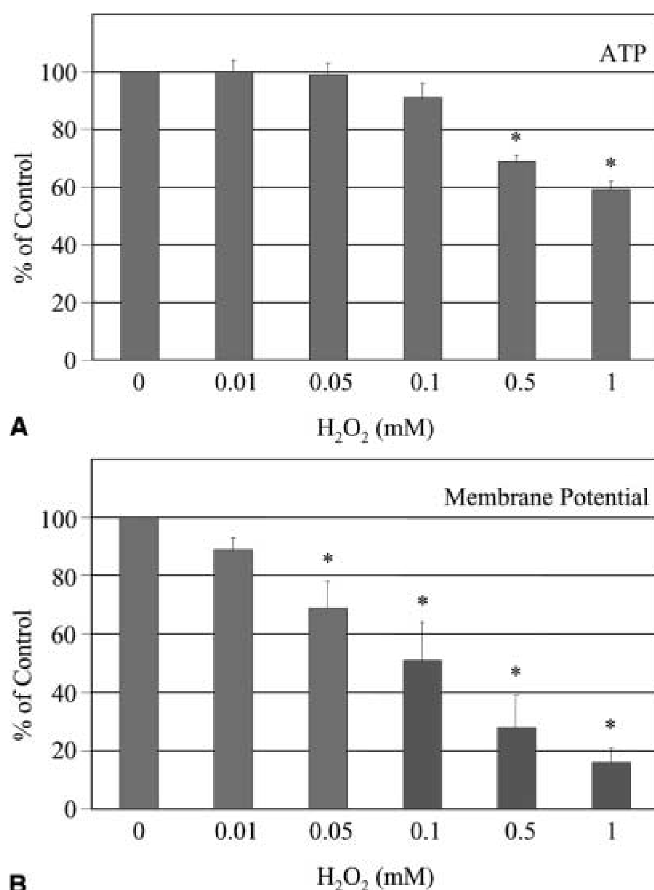

Fig. 2A, B Determination of effects on mitochondrial functions by H_2O_2 in astrocyte cell cultures. Mitochondrial functions were determined after treatment of astrocytes with H_2O_2 for 24 h. **A** Intracellular ATP assessed via chemiluminescence reaction. **B** Mitochondrial membrane potential measured by the dye tetramethylrhodamine. Values are expressed as percentage of controls (means ± SEM from four different experiments). *$P<0.05$ vs controls

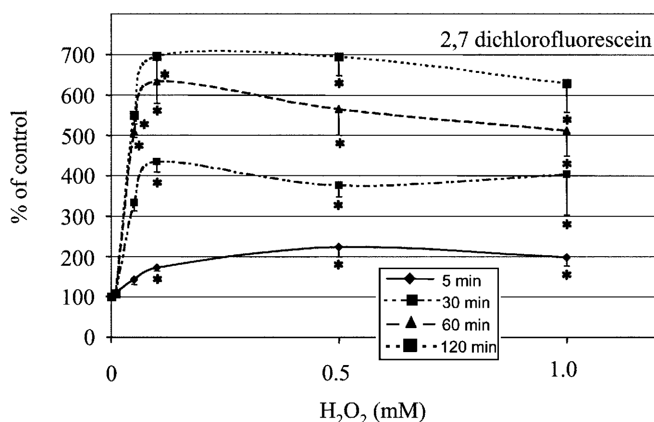

Fig. 3 Production of reactive oxygen species (ROS) by H_2O_2 in astrocyte cell cultures. Astrocytes were exposed to the indicated concentrations of H_2O_2 and ROS production was measured at different times thereafter. Values are expressed as percentage of controls (means ± SEM from four to six different experiments).*$P<0.05$ vs controls

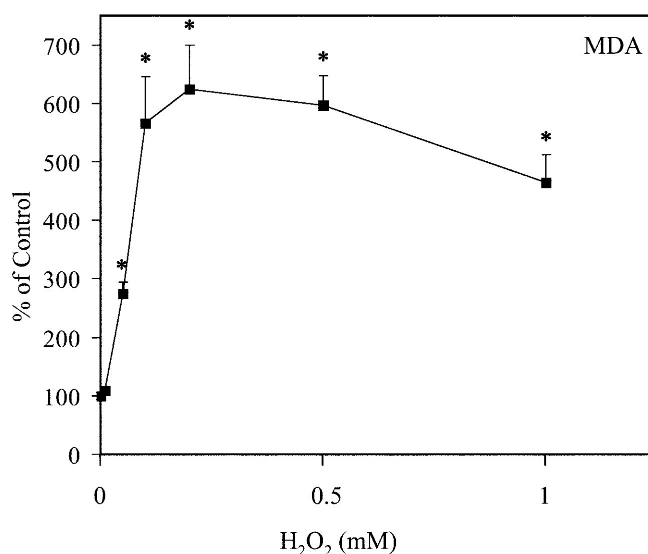

Fig. 4 Formation of malondialdehyde (MDA) after treatment with H_2O_2. Astrocyte cultures were incubated with the indicated concentrations of H_2O_2 for 24 h and formation of MDA was assessed. The results are expressed as percentage of controls (means ± SEM from five or six different experiments). *$P<0.05$ vs controls

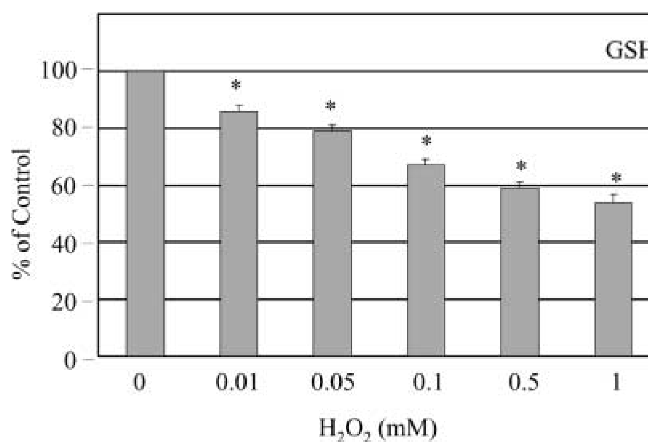

Fig. 5 Determination of intracellular glutathione (GSH) concentration. Rat astrocytes were treated with the indicated concentrations of H_2O_2 for 1 h. GSH concentrations were determined using a fluorescent dye. The results are expressed as percentage of controls (means ± SEM from four different experiments). *$P<0.05$ vs controls

treatment with 0.01 mM H_2O_2 increasing to nearly 50% of GSH depletion at 1 mM H_2O_2.

Expression of AOEs in astrocytes during cell culture

The basal expression levels of catalase, MnSOD, and CuZnSOD during culturing are shown in Fig. 6A. The amount of 18 S rRNA serves as internal loading control. mRNA expression of GPx was determined via semi-quantitative PCR with GAPDH mRNA expression as

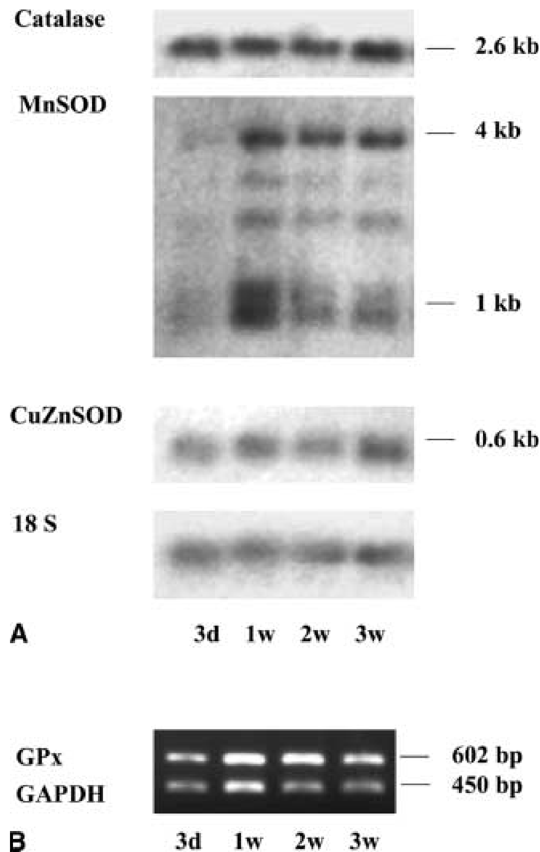

Fig. 6A, B Basal expression levels of catalase, manganese superoxide dismutase (MnSOD), copper/zinc superoxide dismutase (CuZnSOD), and glutathione peroxidase (GPx) during culturing in primary astrocyte cell cultures. **A** Shown is a representative northern blot analysis for catalase, MnSOD, and CuZnSOD, and as internal loading control the 18 S rRNA expression. **B** Shown is a representative PCR analysis for GPx expression with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression as internal standard

internal standard (Fig. 6B). In astrocytes expression of catalase, CuZnSOD, and GPx did not change during cell culture. Expression of MnSOD showed some changes during culturing; whereas at day 3 MnSOD expression was barely detectable, it shows a peak after 1 week of culturing and a stable expression pattern thereafter. Accordingly astrocytes were used for experiments at 2 weeks of culturing when a stable expression pattern of all AOEs was reached.

Effect of H_2O_2 on AOE expression in astrocytes

Astrocytes were cultured for 2 weeks and then exposed to different concentrations of H_2O_2. Figure 7A shows the northern analysis and Fig. 7B the densitometric data relative to 18 S rRNA. In Fig. 8A the PCR for detection of GPx mRNA expression is depicted and in Fig. 8B the densitometric data relative to GAPDH mRNA expression. To obtain densitometric data for MnSOD mRNA expression all five bands of MnSOD detected by northern blotting were quantitated together, because no changes in between this expression pattern was observed (Hurt et al. 1992). There was an increase in MnSOD, catalase, and GPx mRNA expression levels after treatment with H_2O_2. A twofold increase of MnSOD expression levels could be shown starting at a concentration of 0.05 mM H_2O_2. Catalase mRNA expression was elevated around 2 times only at the highest concentration of 0.2 mM H_2O_2. The same was found for GPx mRNA expression, a slight but significant increase of 1.5 times after treatment with 0.2 mM H_2O_2. No changes in CuZnSOD mRNA expression was detected after H_2O_2 exposure.

Discussion

The brain is particularly vulnerable to oxidative stress because of its high rate of oxygen consumption, the high levels of polyunsaturated fatty acids as substrates for lipid peroxidation, and the essentially non-regenerative nature of neurons (Halliwell 1992; Halliwell and Gutteridge 1989). The AOE system is very important for the defense against such oxidative damage. Changes in AOE expression caused by ROS may be crucial for cell survival.

After treatment of astrocytes with H_2O_2, a massive increase in intracellular oxidation paralleled by lipid peroxidation could be detected. The ability of astrocytes to respond toward an oxidative insult by the production of MDA was also described by Schroeter et al. (1999), who detected an increase in MDA levels after hyperoxia/reoxygenation. Café et al. (1995) found lipid peroxidation products in astroglial cell cultures after iron-stimulated oxidative stress. We detected high levels of ROS and MDA already at low concentrations of H_2O_2.

Viability was only slightly impaired at a concentration of 0.1 mM H_2O_2, when ROS concentration and MDA release were increased sevenfold. Cell damage became more severe at much higher concentrations. Treatment with 1 mM H_2O_2 resulted in 60% dead cells. Several groups tested the neurotoxic potential of H_2O_2, mostly with emphasis on differences in cytotoxicity between neuronal and astroglial cultures (Abe and Saito 1998; Desagher et al. 1996; Langeveld et al. 1995). Astrocytes were always detected as the more resistant cell type toward H_2O_2 toxicity. Therefore a neuroprotective role was attributed to astrocytes (Desagher et al. 1996; Wilson 1997). Similar toxicity data like ours were obtained in those studies. Abe and Saito (1996) found a steeper dose response curve probably resulting from different conditions in cell culturing.

Mitochondrial functions, as measured by ATP consumption and the mitochondrial membrane potential, decreased in parallel with cell viability. The same was observed for GSH depletion in astrocytes after H_2O_2 exposure. Impairment of mitochondrial functions result in deficiencies of energy metabolism thus contributing to cell death. The importance of the GSH/GSSG ratio in

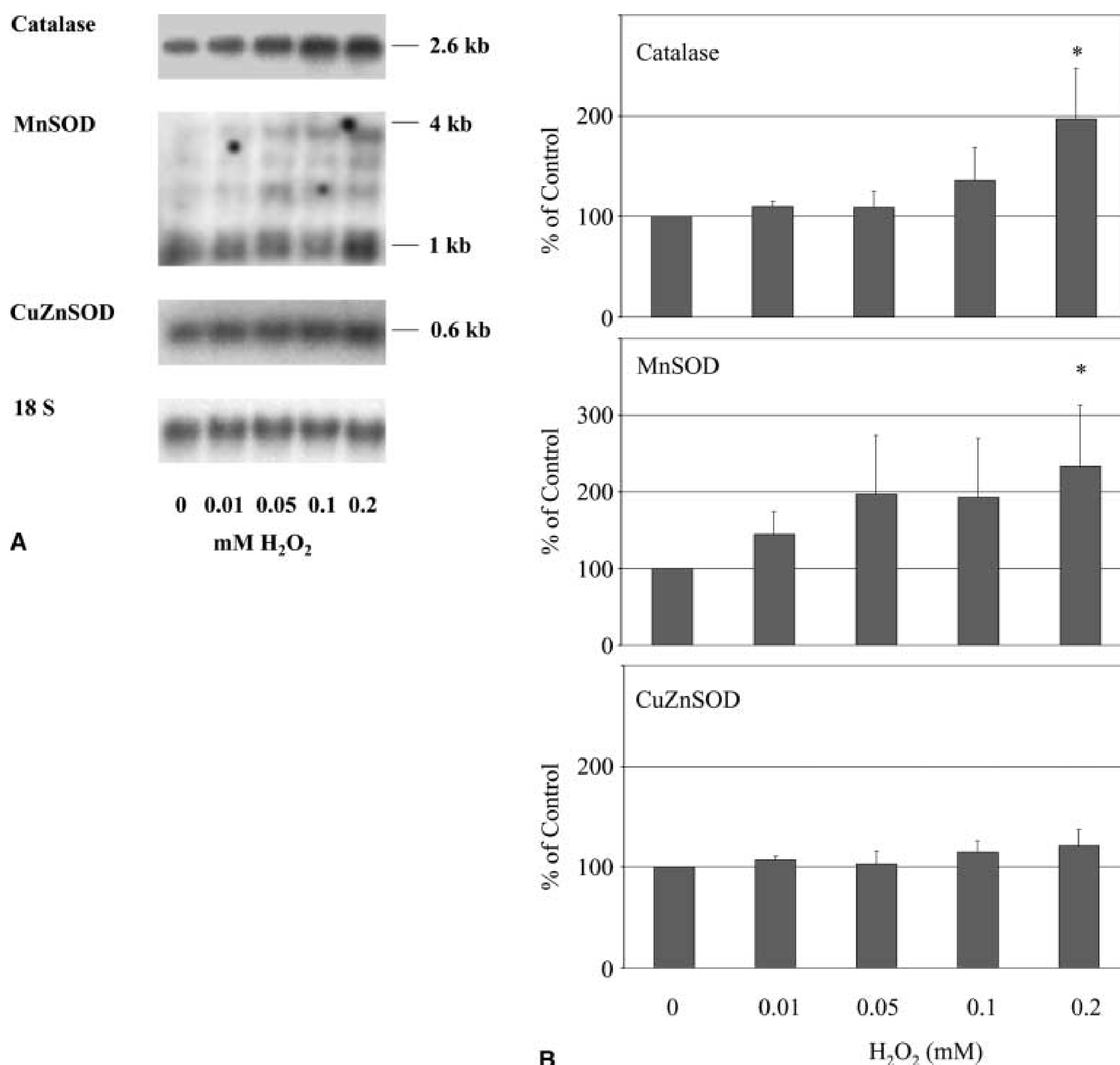

Fig. 7A, B Gene expression of catalase, MnSOD, and CuZnSOD in primary astrocyte cultures after treatment with H_2O_2. **A** Northern blot analysis of catalase, MnSOD, and CuZnSOD mRNAs. Two-week cultures of astrocytes were exposed to the indicated concentrations of H_2O_2 for 24 h. Northern analysis was carried out using rat catalase, MnSOD, or CuZnSOD cDNA as a probe. The northern analysis shown is representative of three to six individual experiments. **B** Quantitative densitometric data for catalase, MnSOD, and CuZnSOD mRNA expression levels. mRNA levels of catalase, MnSOD, and CuZnSOD were quantitated relative to 18 S rRNA levels shown in the *lowest panel* of **A**. Values are expressed as percentage of controls (means ± SEM from three to six different experiments). $*P < 0.05$ vs controls

astrocytes for detoxification of H_2O_2 was investigated by Dringen and Hamprecht (1997). Immediately after administration of H_2O_2 to astroglial cells GSH was found to be rapidly oxidized indicating that GPx and GSH are involved in astroglial detoxification of exogenously applied H_2O_2. The pentose phosphate pathway seemed to be responsible for re-establishing the high ratio of GSH and GSSG levels, probably by the provision of NADPH for GSH regeneration. Ben-Yoseph et al. (1994) showed that the activity of the pentose phosphate pathway can directly be stimulated by H_2O_2 in a concentration-dependent fashion. As long as glucose is not the limiting factor, astrocytes should thus be able to cope with GSH depletion by H_2O_2. It can be assumed that at least for the lower concentrations of H_2O_2 in our experiments GSH is probably restored later on.

The AOE system confers protection against oxidative damage. Indeed, overexpression of catalase in immortalized neural cells protected against H_2O_2 toxicity (Mann et al. 1997). On the other hand overexpression of MnSOD or CuZnSOD in cells of neuronal origin afforded no protection (Mann et al. 1997; Zhong et al. 1996) against oxidative damage caused by radiation or BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) treatment. Probably overexpression of MnSOD can cause an imbalance of AOEs, which results in an elevation of

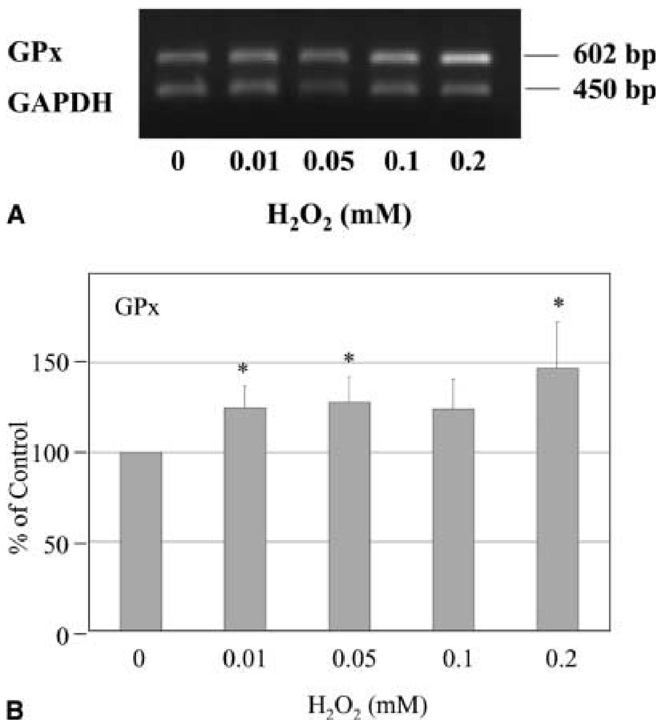

Fig. 8A, B Gene expression of GPx in primary astrocyte cell cultures after treatment with H_2O_2. **A** PCR blot of GPx and GAPDH mRNAs obtained by RT-PCR after exposure of 2-week cultures of astrocytes to the indicated concentrations of H_2O_2 for 24 h. **B** GPx/GAPDH quotient after densitometric scanning of PCR blots obtained after RT-PCR shown in **A**. Values are expressed as percentage of controls (means ± SEM from three different experiments). $^*P < 0.05$ vs controls

intracellular H_2O_2. If the expression levels of the H_2O_2 scavenging enzymes, catalase and GPx, are not elevated accordingly oxidative damage will be the consequence. We found basal mRNA expression of catalase, MnSOD, CuZnSOD, and GPx in astrocyte cell cultures. Astrocytes were treated with H_2O_2 after 2 weeks of culturing, when a stable expression pattern of AOEs was observed. H_2O_2 exposure lead to a 2–3 times increase in catalase and MnSOD mRNA expression in astrocytes. GPx mRNA levels increased about 1.5 times, whereas CuZnSOD mRNA expression was not influenced by H_2O_2 treatment. Obviously the upregulation of AOEs in our system strongly depends on the presence of ROS. The mechanisms residing behind the induction of catalase and MnSOD by oxidants are still under investigation. In the promoter region of the MnSOD gene consensus sequences for NF-κB and AP1 binding sites can be found by computer analysis (Darville et al. 2000; Ho et al. 1991). The transcription factor NF-κB as well as AP1 have been extensively studied as transcriptional activators of genes in stress response (Baeuerle 1991; Curran and Franza 1988). In accordance, the involvement of NF-κB and AP1 in activating MnSOD genes in response to TNF-α, TPA, H_2O_2, or thiol-reducing agents has been suggested in human and rat cells (Darville et al. 2000; Kuo et al. 1999; Yoshioka et al. 1994). Recently induction of the rat MnSOD gene by inflammatory mediators was also found to be regulated through some intronic sequences (Darville et al. 2000; Kuo et al. 1999; Rogers et al. 2000). While MnSOD seems to be regulated on the transcriptional level, for catalase a post-transcriptional regulation can be assumed. Stabilization of catalase mRNA could be shown through a redox-sensitive mRNA binding protein in lungs of neonatal rats exposed to hyperoxia (Clerch and Massaro 1992). A similar mechanism may be important for upregulation of catalase gene expression in astrocytes.

In our experiments also GPx mRNA expression was influenced by H_2O_2 treatment. In mice with a homozygous null mutation for Gpx1 an increased susceptibility to the oxidative stress inducing agent paraquat and H_2O_2 could be shown (de Haan et al. 1998). Emphasis was laid on the importance of Gpx for protection of neurons against those oxidative stressors. In the 5′ flanking region of the human Gpx gene two cis acting elements were characterized which are of importance for gene regulation in an oxidative environment (Cowan et al. 1993). It can be assumed that the rat gene is regulated similarly.

Our data show, that astrocytes are able to respond to oxidative stress via their AOE system. This upregulation of the AOE system may be crucial for cell survival after oxidative brain damage and may contribute to the important role for astrocytes in the antioxidant defense of the brain.

Acknowledgements We thank Mrs. Susanne Groneberg and Mrs. Nicole Groß-Toll for their excellent technical assistance. This work was performed in accordance with German legislation on animal welfare.

References

Abe K, Saito H (1996) Menadione toxicity in cultured rat cortical astrocytes. Jpn J Pharmacol 72:299–306

Abe K, Saito H (1998) Characterization of t-butyl hydroperoxide toxicity in cultured rat cortical neurones and astrocytes. Pharmacol Toxicol 83:40–46

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Akai F, Maeda M, Suzuki K, Inagaki S, Takagi H, Taniguchi N (1990) Immunocytochemical localization of manganese superoxide dismutase (MnSOD) in the hippocampus of the rat. Neurosci Lett 115:19–23

Baeuerle PA (1991) The inducible transcription activator NF-κB: regulation by distinct protein subunits. Biochim Biophys Acta 1072:63–80

Ben-Yoseph O, Boxer PA, Ross BD (1994) Oxidative stress in the central nervous system: monitoring the metabolic response using the pentose phosphate pathway. Dev Neurosci 16:328–336

Bergeron C, Petrunka C, Weyer L (1996) Copper/zinc superoxide dismutase expression in the human central nervous system. Am J Pathol 148:273–279

Buxser SE, Sawada G, Raub TJ (1999) Analytical and numerical techniques for evaluation of free radical damage in cultured cells using imaging cytometry and fluorescent indicators. Methods Enzymol 300:256–275

Café C, Torri C, Bertorelli L, Tartara F, Tancioni F, Gaetani P, Rodriguez y Baena R, Marzatico F (1995) Oxidative events in

neuronal and glial cell-enriched fractions of rat cerebral cortex. Free Radic Biol Med 19:853–857

Carmichael J, Mitchell JB, Degraff WG, Gamson J, Gazdar AF, Jonson BF, Gatstein E, Minna JD (1985) Chemosensitivity testing of human cancer cell lines using the MTT assay. Br J Cancer 57:540–547

Ceballos-Picot I (1997) Biology of oxidative stress. In: Ceballos-Picot I (ed) The role of oxidative stress in neuronal death. Springer, Berlin Heidelberg New York, pp 5–38

Ceballos I, Javoy-Agid F, Hirsch EC, Dumas S, Kamoun PP, Sinet PM, Agid Y (1989) Localization of copper-zinc superoxide dismutase mRNA in human hippocampus by in situ hybridization. Neurosci Lett 105:41–46

Chan PH (1996) Role of oxidants in ischemic brain damage. Stroke 27:1124–1129

Chan Y-L, Gutell R, Noller HF, Wool IG (1984) The nucleotide sequence of a rat 18 S ribosomal ribonucleic acid gene and a proposal for the secondary structure of 18 S ribosomal ribonucleic acid. J Biol Chem 259:224–230

Clerch LB, Massaro D (1992) Oxidation-reduction-sensitive binding of lung protein to rat catalase mRNA. J Biol Chem 267:2853–2855

Clerch LB, Iqbal J, Massaro D (1990) Perinatal rat lung catalase gene expression: influence of corticosteroid and hyperoxia. Am J Physiol 260:L428–L433

Cowan DB, Weisel RD, Williams WG, Mickle DAG (1993) Identification of oxygen responsive elements in the 5′-flanking region of the human glutathione peroxidase gene. J Biol Chem 268:26904–26910

Curran T, Franza BR Jr (1988) Fos and jun: the AP-1 connection. Cell 55:395–397

Damier P, Hirsch EC, Zhang P, Agid Y, Javoy-Agid F (1993) Glutathione peroxidase, glial cells and Parkinson's disease. Neuroscience 52:1–6

Darville MI, Ho Y-S, Eizirik DL (2000) NF-κB is required for cytokine-induced manganese superoxide dismutase expression in insulin-producing cells. Endocrinology 141:153–162

Desagher S, Glowinski J, Premont J (1996) Astrocytes protect neurons from hydrogen peroxide toxicity. J Neurosci 16:2553–2562

Dougall WC, Nick HS (1991) Manganese superoxide dismutase: a hepatic acute phase protein regulated by interleukin-6 and glucocorticoids. Endocrinology 129:2376–2384

Draper HH, Squires EJ, Mahmoodi H, Wu J, Agarwal S, Hadley M (1993) A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. Free Radic Biol Med 15:353–363

Dringen R, Hamprecht B (1997) Involvement of glutathione peroxidase and catalase in the disposal of exogenous hydrogen peroxide by cultured astroglial cells. Brain Res 759: 67–75

El-Bahay C, Gerber E, Horbach M, Tran-Thi Q-H, Rördanz E, Kahl R (1999) Influence of tumor necrosis factor-α and silibin on the cytotoxic action of α-amanitin in rat hepatocyte culture. Toxicol Appl Pharamcol 158:253–260

Furuta S, Hayashi H, Hijikata M, Miyazawa S, Osumi T, Hashimoto T (1986) Complete nucleotide sequence of cDNA and deduced amino acid sequence of rat liver catalase. Proc Natl Acad Sci USA 83:313–317

Furuta A, Price DL, Pardo CA, Troncoso JC, Xu Z-S, Taniguchi N, Martin LJ (1995) Localization of superoxide dismutases in Alzheimer's disease and Down's syndrome neocortex and hippocampus. Am J Pathol 146:357–367

Haan JB de, Bladier C, Griffith P, Kelner M, O'Shea RD, Cheung NS, Bronson RT, Silvestro MJ, Wild S, Zheng SS, Beart PM, Hertzog PJ, Kola I (1998) Mice with a homozygous null mutation for the most abundant glutathione peroxidase, Gpx1, show increased susceptibility to the oxidative stress-inducing agents paraquat and hydrogen peroxide. J Biol Chem 273:22528–22536

Halliwell B (1992) Reactive oxygen species and the central nervous system. J Neurochem 59:1609–1623

Halliwell B, Gutteridge JM (eds) (1989) Free radicals in biology and medicine, 2nd edn. Clarendon Press, Oxford

Ho Y-S, Crapo JD (1987a) Nucleotide sequences of cDNAs coding for rat manganese-containing superoxide dismutase. Nucleic Acids Res 15:10070

Ho Y-S, Crapo JD (1987b) cDNA and deduced amino acid sequence of rat copper-zinc-containing superoxide dismutase. Nucleic Acids Res 15:6746

Ho Y-S, Howard AJ (1992) Cloning and characterization of the rat glutathione peroxidase gene. FEBS Lett 301:5–9

Ho Y-S, Howard AJ, Crapo JD (1991) Molecular structure of a functional rat gene for manganese-containing superoxide dismutase. Am J Respir Cell Mol Biol 4:278–286

Hurt J, Hsu J-L, Dougall WC, Visner GA, Burr IM, Nick HS (1992) Multiple mRNA species generated by alternate polyadenylation from the rat manganese superoxide dismutase gene. Nucleic Acids Res 20:2985–2990

Kifle Y, Monnier J, Cheswron SE, Raizada MK, Nick HS (1996) Regulation of the manganese superoxide dismutase and inducible nitric oxide synthase gene in rat neuronal and glial cells. J Neurochem 66:2128–2135

Kondo T, Kinouchi H, Kawase M, Yoshimoto T (1996) Differential response in the release of hydrogen peroxide between astroglial cells and endothelial cells following hypoxia/reoxygenation. Neurosci Lett 215:103–106

Kuo S, Chesrown SE, Mellott JK, Rogers RJ, Hsu J-L, Nick HS (1999) In vivo architecture of the manganese superoxide dismutase promoter. J Biol Chem 274:3345–3354

Langeveld CH, Jongenelen CAM, Schepens E, Stoof JC, Bast A, Drukarch B (1995) Cultured rat striatal and cortical astrocytes protect mesencephalic dopaminergic neurons against hydrogen peroxide toxicity independent of their effect on neuronal development. Neurosci Lett 192:13–16

Li Y, Carlson E, Murakami K, Copin J-C, Luche R, Chen SF, Epstein CJ, Chan PH (1999) Targeted expression of human CuZn superoxide dismutase gene in mouse central nervous system. J Neurosci Methods 89:49–55

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, New York

Mann H, McCoy MT, Subramaniam J, Remmen H van, Cadet JL (1997) Overexpression of superoxide dismutase and catalase in immortalized neural cells: toxic effects of hydrogen peroxide. Brain Res 770:163–168

McCord JM (1979) Superoxide, superoxide dismutase and oxygen toxicity. In: Hodgson E, Bend JR, Philpot RM (eds) Reviews in biochemical toxicology. Elsevier, Amsterdam, pp 109–124

Mokuno K, Ohtani K, Suzumura A, Kiyosawa K, Hirose Y, Kawai K, Kato K (1994) Induction of manganese superoxide dismutase by cytokines and lipopolysaccharide in cultured mouse astrocytes. J Neurochem 63:612–616

Mundi WR, Freudenrich TM, Kodavanti PRS (1997) Aluminium potentiates glutamate-induced calcium accumulation and iron-induced oxygen free radical formation in primary neuronal cultures. Mol Chem Neuropathol 32:41–57

Pinteaux E, Copin J-C, Ledig M, Tholey G (1996) Modulation of oxygen-radical-scavenging enzymes by oxidative stress in primary cultures of rat astroglial cells. Dev Neurosci 18:397–404

Pinteaux E, Perraut M, Tholey G (1998) Distribution of mitochondrial manganese superoxide dismutase among rat glia cells in culture. Glia 22:408–414

Oyama Y, Okazaki E, Chikahisa L, Nagano T, Sadakata C (1996) Oxidative stress-induced increase in intracellular Ca^{2+} and Ca^{2+}-induced increase in oxidative stress: an experimental model using dissociated rat brain neurons. Jpn J Pharmacol 72:381–385

Rabizadeh S, Butler Gralla E, Borchelt DR, Gwinn R, Selverstone Valentine J, Sisodia S, Wong P, Lee M, Hahn H, Bredesen DE (1995) Mutations associated with amyotrophic lateral sclerosis convert superoxide dismutase from an antiapoptotic gene to a proapoptotic gene: studies in yeast and neural cells. Proc Natl Acad Sci USA 92:3024–3028

Rogers RJ, Cheswron SE, Kuo S, Monnier JM, Nick HS (2000) Cytokine-inducible enhancer with promoter activity in both the rat and human manganese-superoxide dismutase genes. Biochem J 347:233–242

Röhrdanz E, Kahl R (1998) Alterations of antioxidant enzyme expression in response to hydrogen peroxide. Free Radic Biol Med 24:27–38

Röhrdanz E, Obertrifter B, Ohler S, Tran-Thi Q-H, Kahl R (2000) Influence of adriamycin and paraquat on antioxidant enzyme expression in primary rat hepatocytes. Arch Toxicol 74:231–237

Schmuck G, Haynes RK (2000) Establishment of an in vitro screening model for neurodegeneration induced by antimalarial drugs of the artemisinin-type. Neurotoxicol Res 2:37–49

Schroeter ML, Mertsch K, Giese H, Müller S, Sporbert A, Hickerl B, Blasig IE (1999) Astrocytes enhance radical defence in capillary endothelial cells constituting the blood-brain barrier. FEBS Lett 449:241–244

Shull S, Heintz NH, Periasamy M, Manohar M, Janssen YMW, Marsh JP, Mossman BT (1991) Differential regulation of antioxidant enzymes in response to oxidants. J Biol Chem 266:24398–24403

Takizawa S, Matsushima K, Shinohara Y, Ogawa S, Komatsu N, Utsunomiya H, Watanabe K (1994) Immunohistochemical localization of glutathione peroxidase in infarcted human brain. J Neurol Sci 122:66–73

Tate DJ Jr, Miceli MV, Newsome DA (1995) Phagocytosis and H_2O_2 induce catalase and metallothionein gene expression in human retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 36:1271–1279

Ursini F, Maiorino M, Brigelius-Flohé R, Aumann KD, Roveri A, Schomgurg D, Flohé L (1995) Diversity of glutathione peroxidases. Methods Enzymol 252:38–114

Wilson JX (1997) Antioxidant defense of the brain: a role for astrocytes. Can J Physiol Pharmacol 75:1149–1163

Wong GHW, Goeddel DV (1984) Induction of manganous superoxide dismutase by tumor necrosis factor: possible protective mechanism. Science 242:941–944

Yoshioka T, Homma T, Meyrick B, Takeda M, Moore-Jarrett T, Kon V, Ichikawa I (1994) Kidney Int 46:405–413

Zhang P, Anglade P, Hirsch EC, Javoy-Agid F, Agid Y (1994) Distribution of manganese-dependent superoxide dismutase in the human brain. Neuroscience 61:317–330

Zhong W, Oberley LW, Oberley TD, Yan T, Domann FE, St. Clair DK (1996) Inhibition of cell growth and sensitization to oxidative damage by overexpression of manganese superoxide dismutase in rat glioma cells. Cell Growth Differ 7:1175–1186