Histol Histopathol (2007) 22: 593-601
DOI: 10.14670/HH-22.593

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Short-term exposure of mice to gasoline vapor increases the metallothionein expression in the brain, lungs and kidney

D. Grebić[1], H. Jakovac[1], I. Mrakovčić-Šutić[1], J. Tomac[2], A. Bulog[3], V. Mićović[3] and B. Radošević-Stašić[1]
Departments of [1]Physiology and Immunology, [2]Histology and Embryology and
[3]Public Health, Medical Faculty of Rijeka Rijeka, Croatia

Summary. Environmental airborne pollution has been repeatedly shown to affect multiple aspects of brain and cardiopulmonary function, leading to cognitive and behavioral changes and to the pronounced inflammatory response in the respiratory airways. Since in the cellular defense system the important role might have stress proteins-metallothionein (MT)-I and MT-II, which are involved in sequestration and dispersal of metal ions, regulation of the biosynthesis and activities of zinc-dependent transcription factors, as well as in cellular protection from reactive oxygen species, genotoxicity and apoptosis, in this study we investigated their expression in the brain, lungs and kidney, following intermittent exposure of mice to gasoline vapor. Control groups consisted of intact mice and of those closed in the metabolic chamber and ventilated with fresh air. The data obtained by immunohistochemistry showed that gasoline inhalation markedly upregulated the MTs expression in tissues which were directly or indirectly exposed to toxic components, significantly increasing the number of MT I+II positive cells in CNS (the entorhinal cortex, ependymal cells, astroglial cells in subventricular zone and inside the brain parenchyma, subgranular and CA1-CA3 zone of the dentate gyrus in hippocampus and macrophages-like cells in perivascular spaces), in the lungs (pneumocytes type I and type II) and in the kidneys (parietal wall of Bowman capsule, proximal and distal tubules). The data point to the protective and growth-regulatory effects of MT I + II on places of injuries, induced by inhalation of gasoline vapor.

Key words: Gasoline inhalation, Metallothionein I+II expression, Brain, Lungs, Kidney

Offprint requests to: Biserka Radošević-Stašić, Medical School, University of Rijeka, B. Branchetta 22, 51000 Rijeka, Croatia. e-mail: biserr@medri.hr

Introduction

The metallothionein (MT) family is a class of low-molecular-weight, cysteine-rich proteins with high affinity for metal ions, which are involved in many physiological and pathological processes, including cell proliferation and apoptosis, metal ion homeostasis and detoxification, protection against oxidative damage, drugs and radiotherapy resistance, as well as in several aspects of the carcinogenic process (Moffatt and Denizeau, 1997; Miles et al., 2000; Coyle et al., 2002; Sato and Kondoh, 2002; Theocharis et al., 2004). The extensive data show that these proteins confer a protective effect also within the mammalian CNS, where the MT expression dramatically increases in response to many types of CNS and spinal cord injury or ischemia, as well as in senescence and in several neurodegenerative diseases such as Alzheimer's or Pick's disease and amyotrophic lateral sclerosis (Choudhuri et al., 1995; Nakajima and Suzuki, 1995; Aschner et al., 1997; Hidalgo et al., 2001; Giralt et al., 2002; West et al., 2004; Mocchegiani et al., 2005; Dittmann et al., 2005; Streit, 2005; Penkowa, 2006). Thus, human, rat and mouse brain contains three different isoforms of MTs (MT I, -II, and -III, which might have specific localization and function. Constitutive expression of MT-I is predominantly localized in glial cells of the Purkinje cell layer of the cerebellum and in ependymal cell of lateral ventricles, whereas MT-III mRNA expression is preferentially found in the granular layer of the dentate gyrus of hippocampus, and in many layers of the neocortex, particularly in neurons that sequester zinc in synaptic vesicles (Choudhuri et al., 1995). The most potent inducers of MTs are heavy metals such as cadmium, mercury, zinc, but also the physical and oxidative stressors, glucocorticoids, anticancer drugs, radiation etc, indicating that MTs have cytoprotective effects that might be related to their ability to act as scavengers of hydroxyl and superoxide radicals, or as agents which

protect against the cytotoxic and DNA-damaging effects of various agents (Moffatt and Denizeau, 1997; Aschner et al., 1997; Hidalgo and Carrasco, 1998; Miles et al., 2000; Hidalgo et al., 2001; Coyle et al., 2002; Giralt et al., 2002; Sato and Kondoh, 2002; West et al., 2004; Theocharis et al., 2004; Penkowa, 2006).

Extensive data point also to the possibility that MTs have a special function in sensory organs (Aschner et al., 1997; Shimada et al., 2005) particularly in the olfactory mucosa and bulb, where all three MT isoforms were found, indicating that they might have a protective role on places which provide a direct route of entry into the CNS for agents that are normally excluded by blood-brain barrier (Hasting and Evans, 1991; Thorne et al., 2004). Moreover, in the olfactory system, MT expression might be induced by intranasal instillation of cadmium (Tallkvist et al., 2002), or zinc (Persson et al., 2003), as well as by the exposure of mice to mercury vapor (Yasutake et al., 2004; Shimada et al., 2005), or various toxic agents, present in the air, owing to external pollution, occupational exposure or volatile substance abuse (Chang, 1990; Flanagan and Ives, 1994; Goodheart and Dunne, 1994; Hong et al., 1997).

Owing to frequent urban air pollution with toxic polycyclic aromatic hydrocarbons (PAH) and diesel exhaust particles, in this study we attempted to investigate the effects of short-term exposure of mice to vapor containing gasoline on MT I+II protein expression in the brain and lungs, as the organs, which were inevitably exposed to air contaminated with pollutants, as well as in the kidney, which might be involved in the elimination of the metabolized products of these agents. The data have shown that gasoline vapor increased the expression of MT I+II in the lungs and in the kidney, but even more in the brain areas receiving a strong olfactory input (dentate gyrus of the hippocampus) as well as on astroglial and ependymal cells, suggesting that MTs upregulation was connected with repair mechanisms on sites of injuries.

Materials and methods

Animals

Mice of strain C57/BL6 aged 2-3 months were used for the experiment. They were housed in the colony room in groups 6-8 and kept under controlled conditions (temperature 20±2°C; relative humidity 50-60%; 12-h light-dark cycle, lights on 7:00 a.m.). Animals had free access to standard laboratory food (Pliva, Zagreb) and water. All animal experiments had been approved by a local ethics committee and were done in accordance to National Institutes of Health regulations and guidelines on animal experimentation. Efforts were made throughout the study to minimize the animal suffering and to use the minimal number of animals required.

Exposure to gasoline vapor

Mice were randomly assigned into three groups, each consisting of 6 mice: 1) untreated mice, 2) mice exposed to gasoline vapor in metabolic chamber and 3) mice treated with fresh air in the same conditions. To expose multiple animals to the same protocol, mice from group 2 or 3 were all together put in a 1L metabolic cage, which was ventilated by small animal ventilator (SRI 5056, Ugo Basile, Milano, Italy), performing the timed ventilation of the chamber, using the constant volume, pressure (20 cm H_2O, respiration rate (60/min) and total air flow (adjusted to 0.4 L/min). The quantity of gasoline (Euro super 95, INA, Rijeka) was determined by Floutec vaporizer (concentration 0.5-5%). The temperature and humidity in the chamber was maintained constant by cold water circulating between the two external layers of the metabolic chamber, which contained also a soda lime for the absorption of CO_2 from exhaled air. Treatment with gasoline vapor or fresh air lasted 1 h/day and the protocol was repeated for 10 subsequent days. One day after the last treatment the anesthetized mice were sacrified by cervical dislocation.

Tissue preparation

The brain, lungs and kidneys were rapidly removed from three mice in each group and all tissue samples were fixed in 10% buffered formalin solution, for a minimum of 24h. Tissue was then embedded in paraffin wax and sections were cut at 4 μm using HM 340E microtome, Microtom, Germany. The plane of sectioning in the brain was perpendicular to the base of the brain, so that the frontal sections of brain were obtained. Heat induced epitope retrival was done prior to staining procedures by heating tissue slides in boiled citrate buffer pH 6.0 four times, each 5 minutes, using a microwave steamer.

Immunohistochemistry

Immunohistochemical studies were performed on paraffin embedded tissues using DAKO EnVision+ System, Peroxidase (DAB) kit according to the manufacturer's instructions (DAKO Corporation, USA). Briefly, slides were incubated with peroxidase block to eliminate endogenous peroxidase activity. After washing, monoclonal anti-MT I+II antibody (clone E9; DakoCytomation, USA) diluted 1:50 in phosphate-buffered saline supplemented with bovine serum albumin was added to tissue samples and incubated overnight at 4°C in a humid environment, followed by 45 minutes incubation with peroxidase labeled polymer conjugated to goat anti-mouse immunoglobulins containing carrier protein linked to Fc fragments to prevent nonspecific binding. The immunoreaction product was visualized by adding substrate-chromogen (DAB) solution. Tissues were counterstained with hematoxylin and 37 mM ammonia water, dehydrated in gradient of alcohol and mounted with mounting medium. The specifity of the reaction was confirmed by substitution of anti-MT I+II antibody with mouse irrelevant IgG1 kappa immunoglobulin (clone DAK-

G01; Dako, USA), used in the same conditions and dilutions as a primary antibody.

Semiquantitative analysis of MT I+II expression

To estimate the relative number of cells that express MT I+II, after photographing in the stained sections of brain, lungs and kidney under a light microscope we counted the total number of cells and the number of cells stained by anti-MT I+II antibody in 5 randomly chosen fields (magnification 400x, Olympus BX51 Microscope). The analysis was made in 3 mice per experimental group in an evaluator-blinded manner, and the data were expressed as percentage of MT I/II positive cells, calculated by dividing the MT-expressing cells by the number of total cells. Staining intensity in specific tissue areas was also recorded as weakly/sparsely/moderately/highly positive by an ordered metric scale.

Statistical analysis

All values were shown as means ± SEM. Differences between groups were assessed by Friedman one-way analysis of variance (ANOVA) and by Mann-Withney U test. P values less than 0.05 were considered statistically significant.

Results

Inhalation of gasoline vapor upregulates the MT I-II expression in the brain, lungs and kidney

In the brain the MT-I and II immunoreactivity was

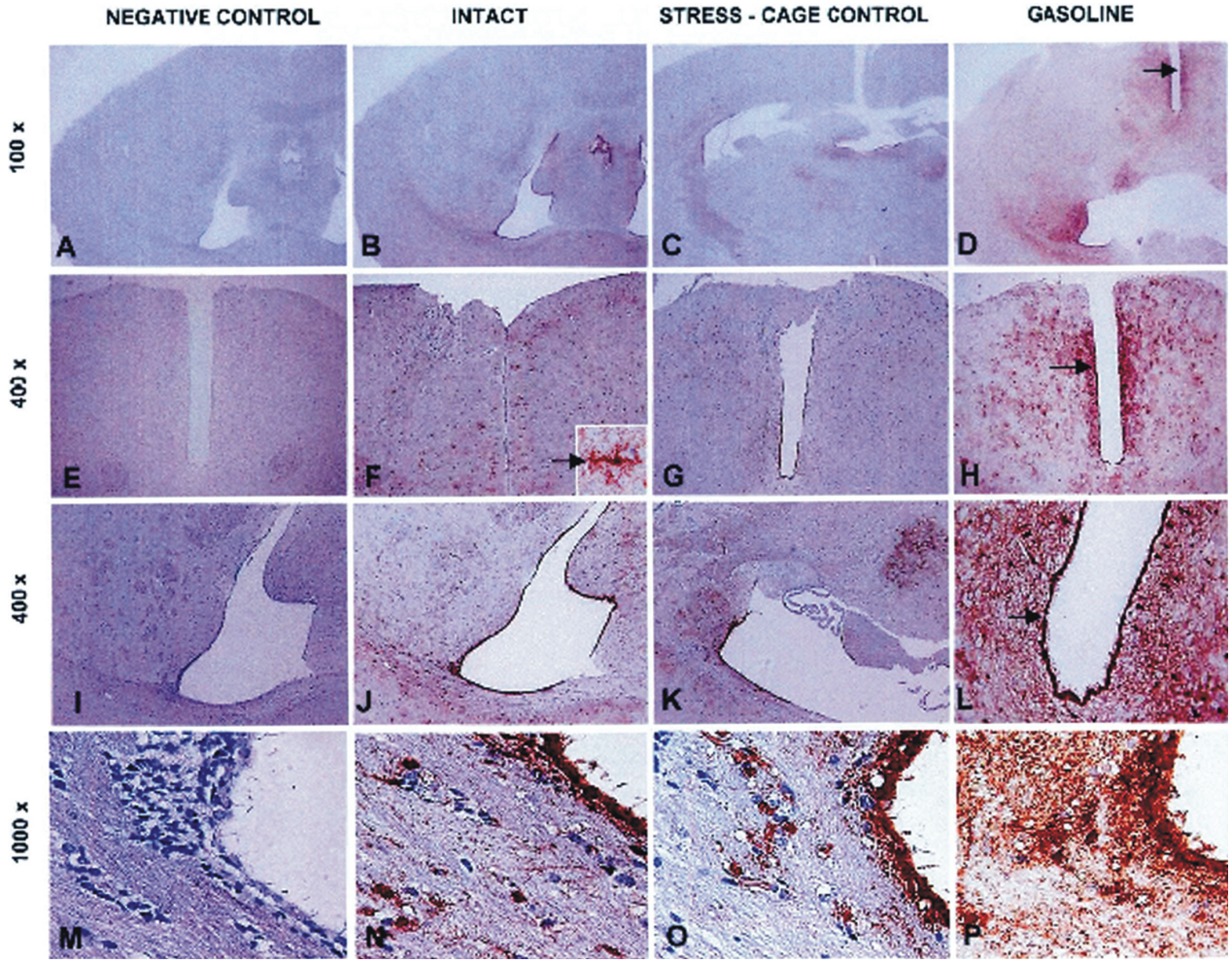

Fig. 1. Immunohistological staining for MT I+II proteins in frontal sections of brain made approximately at -2.12 mm posterior to the bregma in three groups of mice: 1) untreated mice ("intact mice"), 2) mice closed in the metabolic chamber, ventilated with fresh air ("stress-cage control") and 3) mice closed in the metabolic chamber, ventilated with gasoline vapor ("gasoline"). The specificity of the reaction was confirmed by substitution of anti-MT I+II antibody with mouse irrelevant IgG1 kappa immunoglobulin ("negative control"). (**D, H**-cingulate cortex; **F**-glial cells in brain parenchyma; **I-L** and **M-P** ependymal cells and cells in subventricular zones of third and lateral ventricles (magnification x 400 and x 1000, respectively). The results are representative findings of 3 mice.

analyzed on frontal sections made approximately at -2.12 mm posterior to the bregma in three groups of mice: 1) intact mice, 2) mice closed in the metabolic chamber, ventilated with fresh air and 3) mice closed in the metabolic chamber, ventilated with gasoline vapor. The data showed that in intact mice MT I+II proteins were expressed predominantly the ependymal and astroglial cells localized in ventricle walls (Figs. 1J,N), and moderately inside the brain parenchyma (Fig. 1F), and in dentate gyrus of hippocampus (Fig. 2N). Stress, provoked by closure of mice in the metabolic chamber upregulated MTs expression in subgranular zone of hippocampus (Fig. 2O), but slightly decreased the number of cells expressing the MTs in brain's parenchyma and in subventricular zones (Figs. 1K,2K). Inhalation of gasoline vapor provoked, however, a dramatic upregulation of MT I/II expression in areas related to olfactory pathways and the major input from the entorhinal cortex. Thus, MT I/II expressing cells were found not only in ventricular subependyma (Fig. 1L,P), but also in cingulate cortex (Fig. 1D,H) and particularly in the subgranular layer of dentate gyrus and the CA3-CA1 fields of the Ammon's horn, where intense cytoplasmic and nuclear MTs-immunoreactivity was seen in the astroglial cells and occasionally on dense network of glial-glial and glial-axonal interactions (Figs. 2D,H,L,P,3A-M). Some MT-expressing cells with ramification were found surrounded by hippocampal granule cells, being occasionally in direct contact with neurons (Fig. 3C). Furthermore, several macrophage-like MTs-expressing cells were found attached to vascular cell walls (Fig. 3H,I), or in the perivascular areas (Fig. 3M), suggesting that some of them might have a hematogenous origin.

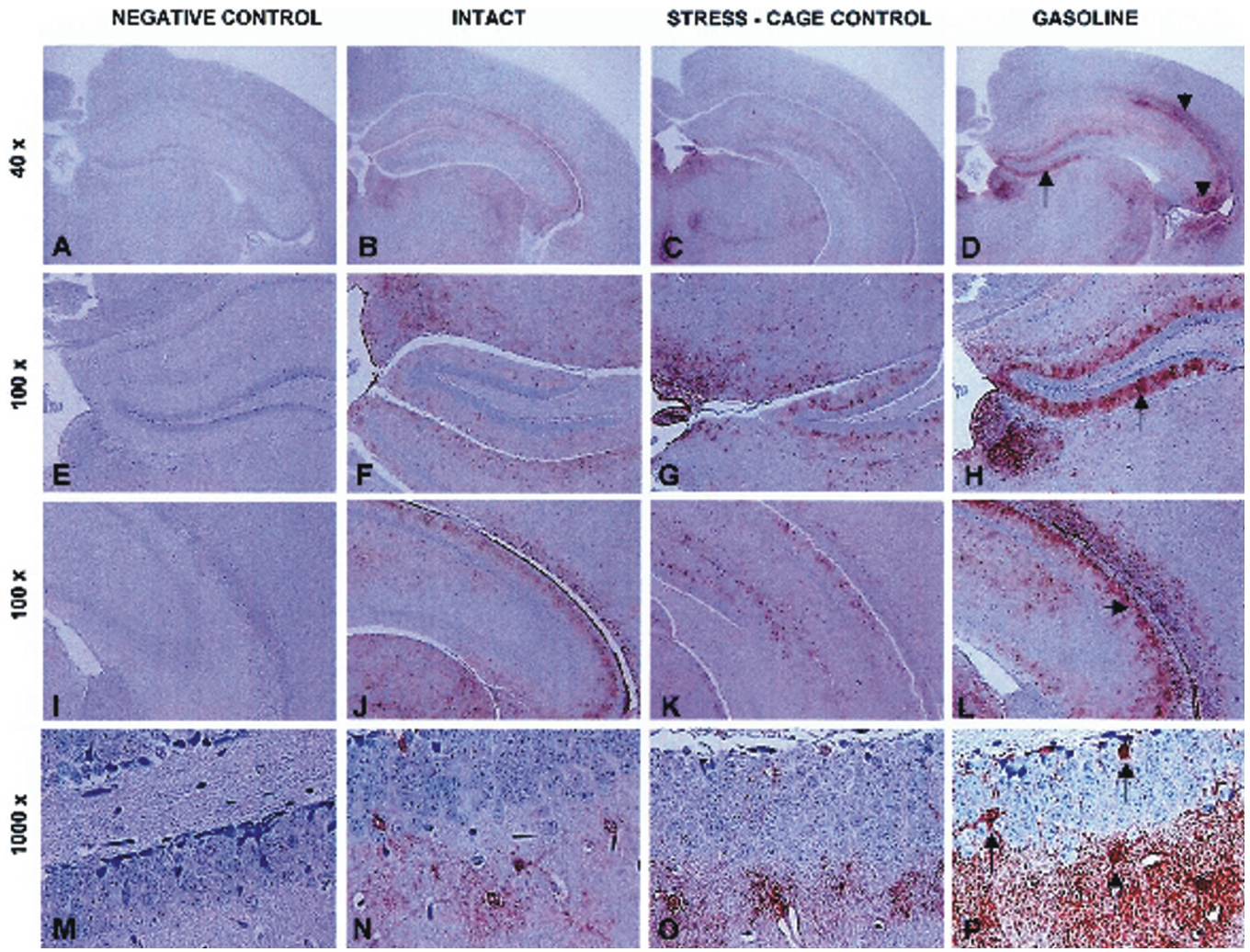

Fig. 2. Expression of MT I+II proteins in the hippocampal zone. **D, H, L** and **P** gasoline-induced upregulation of MTs in the subgranular layer of the dentate gyrus and the CA3-CA1 fields of the Ammon's horn. P-arrows indicate some MT-positive cells in the granular layer in contact with neurons (magnification x 40, x 100 or x 1000, respectively). Results are representative findings of 3 mice.

Simultaneously, we found that the inhalation of gasoline vapor upregulated also the MTs expression in the alveolar epithelium, affecting pneumocytes type I (Fig. 4A) and type II (Fig. 4B), as well as the MTs expression in the kidney. In the latter, we found several cells showing cytoplasmic and nuclear MT-staining in proximal and distal tubules, and in parietal wall of Bowman's capsule (Fig. 5D). The upregulation of MT I/II expression after inhalation of gasoline vapor was confirmed by enumeration of MT-positive cells in the slides of brain, lungs and the kidney, showing that the relative amount of these cells was significantly increased in all the tissues which were directly or indirectly exposed to this toxic agent (Fig. 6; for all organs $p<0.001$ in relation to both control groups), having the greatest effect on the hippocampus (Fig. 6A), and particularly on it's subgranular zone (Fig. 6B).

Discussion

The data show that subacute (10 days) exposure of mice to gasoline vapor induce marked changes not only in the lungs and kidney, but also in the brain, supporting the findings that severe exposure to gasoline in the oil refining and petrochemical industry, gas station employees, traffic-exposed professionals (taxi and bus drivers), and chronic gasoline or petrol sniffing may result in an encephalopathy, organic psychosis, dementia, and generalized tonic-clonic seizures (Mehlman, 1991; Tenenbein, 1997; Varelas et al., 1999;

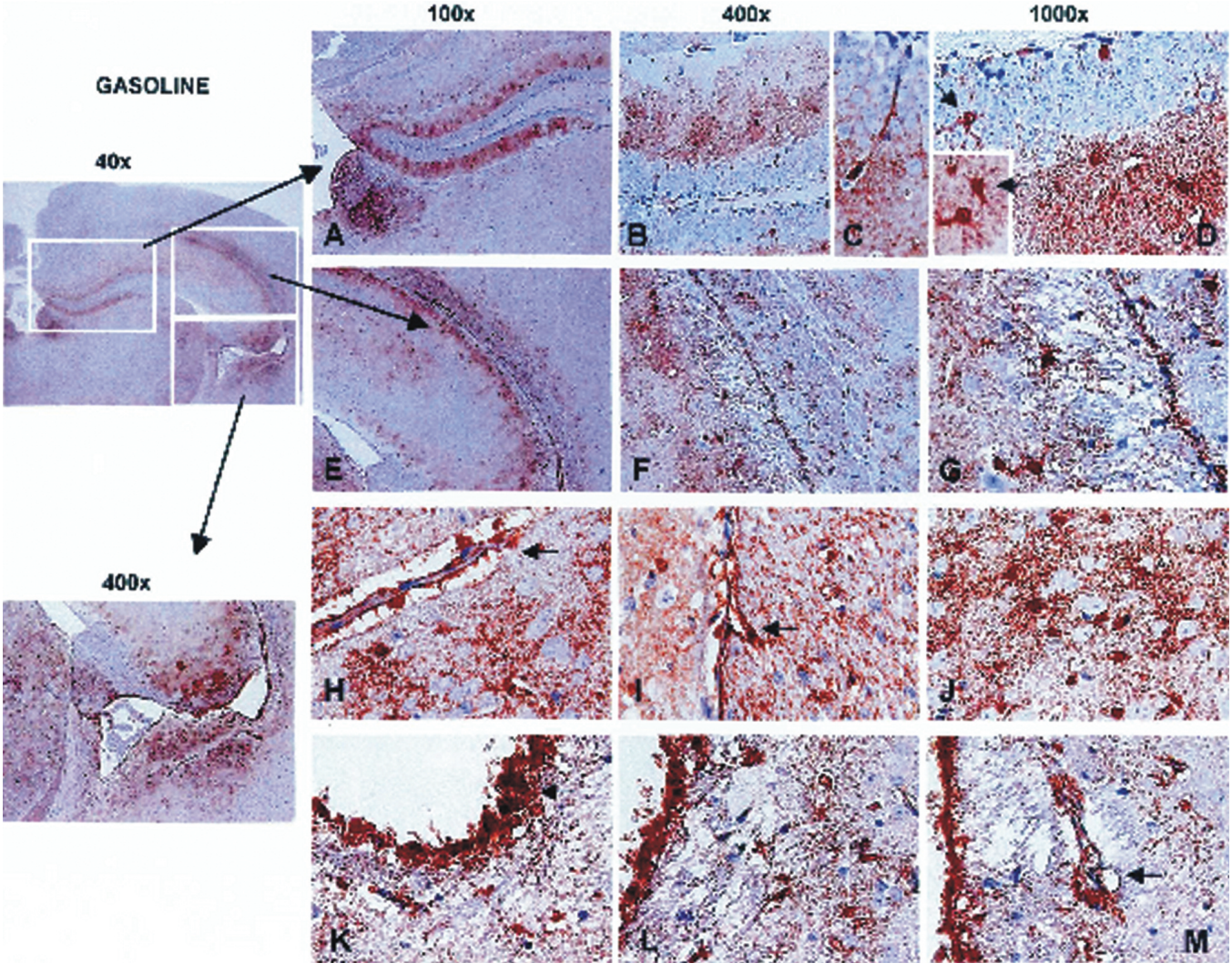

Fig. 3. High gasoline-induced expression of MT I+II proteins in the subgranular layer of dentate gyrus (**A-D**), CA3-CA1 field (**E-G**), in the subventricular zone of lateral ventricles (**H-J**), and around the lateral ventricles (**K-M**). C-MT positive cells in direct contact with neurons. **H, I, M**-MTs-expressing cells attached to vascular cell walls and in perivascular areas (magnification x 40, x 100, x 400 and x 1000, respectively). The results are representative findings of 3 mice.

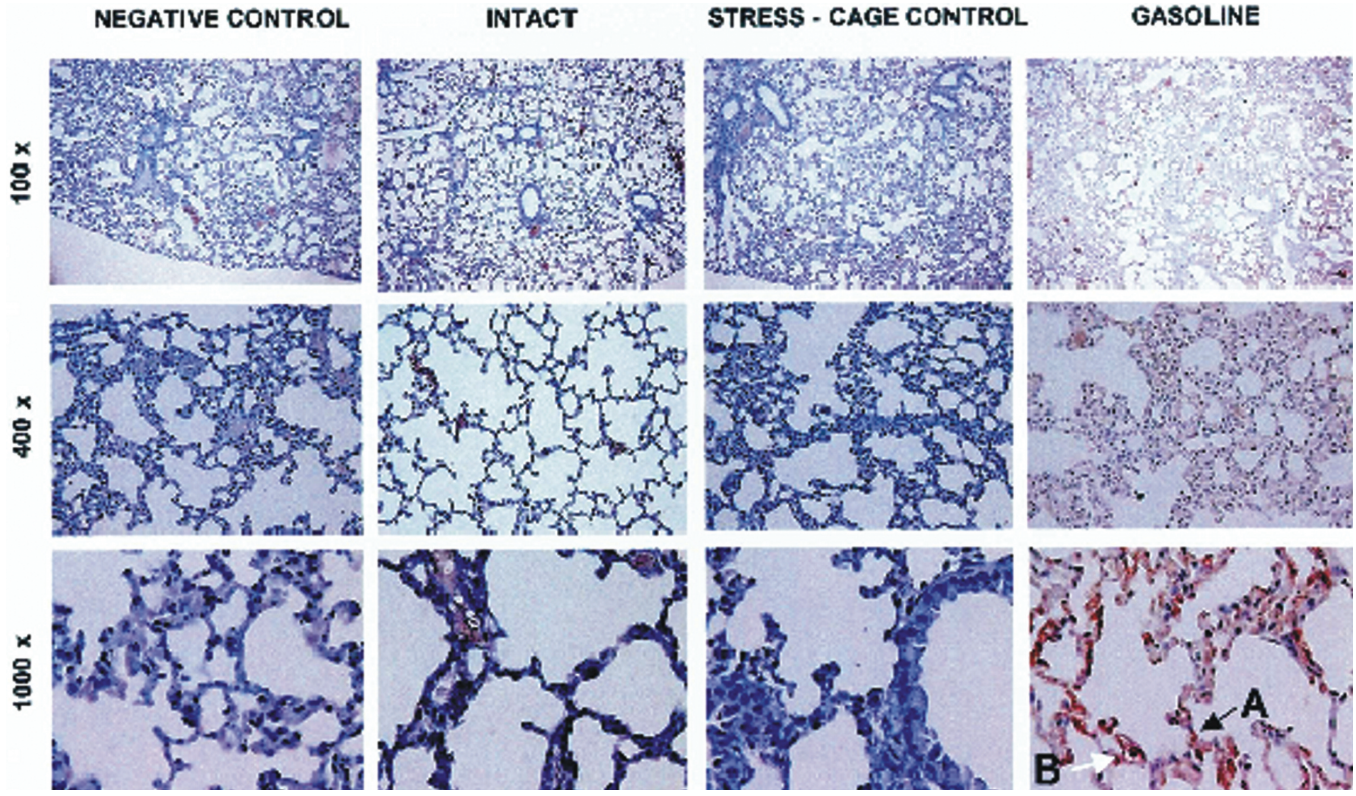

Fig. 4. Expression of MT I+II proteins in the alveolar epithelium. Gasoline-induced upregulation of MTs in pneumocytes type I (A) and type II (B) (magnification x 100, x 400 and x 1000, respectively). The results are representative findings of 3 mice.

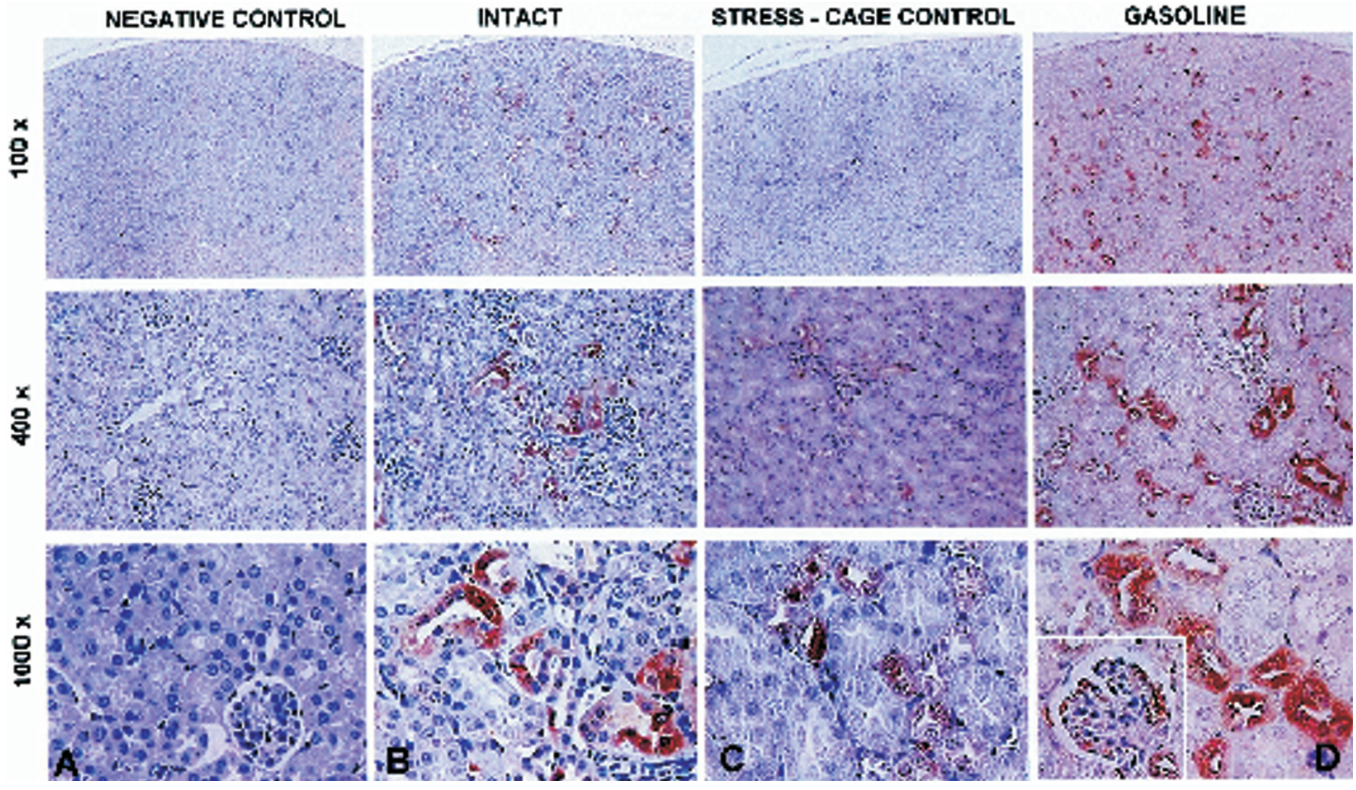

Fig. 5. Expression of MT I+II proteins in the kidney. D-MTs expressing cells in the proximal and distal tubules, and in parietal wall of Bowman's capsule (magnification x 100, x 400 and x 1000, respectively). The results are representative findings of 3 mice.

Takamiya et al., 2003; Cairney et al., 2005), which were occasionally seen also in our experimental group of mice (not shown). Additionally, our data emphasize that gasoline-induced injury in all damaged tissues elicits a marked overexpression of MT I/II, confirming that these isoforms might have a significant protective role (Choudhuri et al., 1995; Nakajima and Suzuki, 1995; Aschner et al., 1997; Moffatt and Denizeau, 1997; Hidalgo and Carrasco, 1998; Hidalgo et al., 2001; Giralt et al., 2002; West et al., 2004; Dittmann et al., 2005; Mocchegiani et al., 2005; Penkowa, 2006).

As presented on Figs 1-3, within the brain, it is likely that the MT-I/-II production was up-regulated primarily in resident astroglial cells, which may act as soldiers in the innate immune recognition and in clearance of pathogens and toxic cell debris, but the findings of numerous MTs positive macrophage-like cells, lining the perivascular space, might indicate that some of these cells were migratory cells derived from the bone marrow. In any case, we hypothesize that the induction of MTs in the brain was mediated by some factors diffusing from the injury sites, since the changes occurred mostly in hippocampus which receives a strong olfactory input from the olfactory bulb and the entorhinal cortex, as well as in the cells that represent the blood-brain barrier (BBB), such as endothelial cells, pericytes and astrocyte foot processes, suggesting that toxic gasoline components were present in the blood and the liquor and/or transferred along the olfactory pathway to the CNS. Both possibilities are implied by data showing that intranasally administered substances may be distributed from the nasal cavity into the brain by absorption into systemic circulation followed by filtration through the BBB or choroid plexus, as well as by absorption from the nasal cavity into olfactory nerves, followed by neuronal distribution directly into the brain (Aschner, 2000; Yokel, 2002). The processes depend on diffusion, as well as on carrier- and receptor-mediated transport, which all might be relevant in our experimental model, since gasoline commonly contains appreciable amounts of polar fuel components, such as benzene, toluene, ethylbenzene, aniline and phenol, as well as several trace metals, like Pb, Cu, Zn, Mn which may either diffuse through the BBB, or may be transported by cell membrane carriers into and out of the brain (Aschner, 2000; Yokel, 2002). Supporting these possibilities it was reported that nasally applied drugs and some inhaled chemicals reach areas of the CNS using extracellular pathways, that are associated with peripheral components of the olfactory and trigeminal systems, as well as an intracellular pathway, that enables the adsorptive endocytosis of agents into olfactory sensory neurons and subsequent anterograde axoplasmic transport within olfactory sensory neurons to olfactory bulbs, rostral brain and hippocampal formation (Amaral and Witter, 1989; Vanderwolf, 2001; Suzuki and Amaral, 2003; Thorne et al., 2004).

In agreement with current dogma about the neuroprotective effects MTs, which is well documented by excellent reviews (Choudhuri et al., 1995; Nakajima and Suzuki, 1995; Aschner et al., 1997; Hidalgo and Carrasco, 1998; Hidalgo et al., 2001; Giralt et al., 2002; West et al., 2004; Penkowa, 2006), we therefore speculate that toxic gasoline components, present in the brain induced chemical or physical injury of axons, or directly stimulated protective astrocytes or microglia,

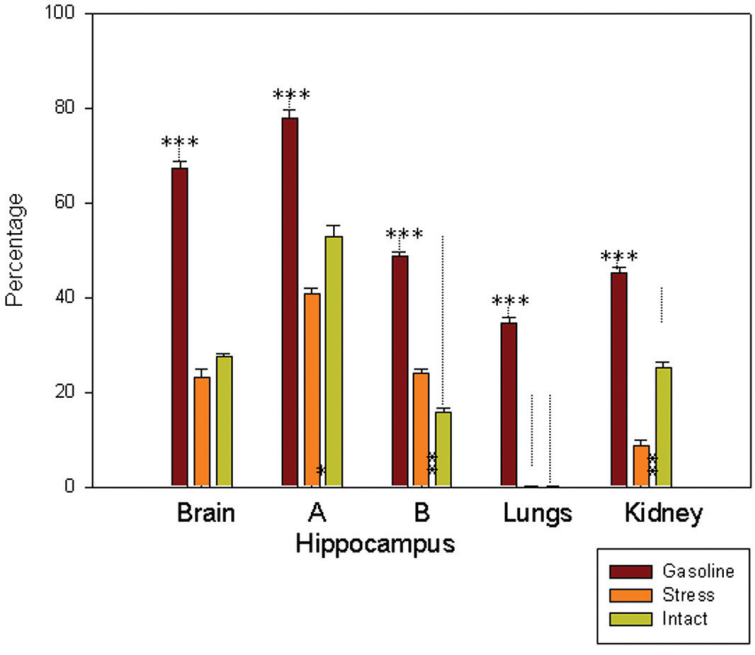

Fig. 6. Relative number of cells that express MT I+II, found in the sections of brain, hippocampus (A-whole hippocampus; B-in subgranular zone of the dentate gyrus), lungs and kidney stained by anti-MT I+II antibody. Total number of cells and cells stained by anti-MT I+II antibody were counted in 5 randomly chosen fields under a light microscope (magnification 400x) in 3 mice per experimental group. The data are expressed as percentage of MT I/II positive cells (mean ± SEM), calculated by dividing the MT-expressing cells by the number of total cells. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

which upregulated their MTs I/II production, responding to rescue signals generated by injured neurons or to other types of mediators released from damaged cells such as cytokines, heavy metals, free zinc, metalloproteases, growth factors and neurotransmitters derived from several cooperating and anatomically overlapping extracellular signaling pathways (Chung et al., 2004; Hagg, 2005; Hauwel et al., 2005; Ladeby et al., 2005; Streit, 2005; Penkowa, 2006). Interestingly, the areas containing the MTs expressing cells in our experiments (ventricular subependyma and the dentate gyrus subgranular zone of hippocampus), correspond with areas described as specialized local niches, which in adult brain contain neuronal and glial progenitor cells able to differentiate upon injury into glial-like cells with inherent "protective " or "regulatory " activities (Hauwel et al., 2005; Hagg, 2005). Moreover, as multipotential progenitors, particularly these cells can effectively generate myelinating oligodendrocytes and promote the clearance of pathogen and toxic cell debris, after switching to a "chaperone" phenotype to nurture tissue repair and rescue the dying neurons (Goldman and Sim, 2005; Hagg, 2005; Hauwel et al., 2005). Our data showing the near contact of MTs expressing cells and neurons (Fig. 3) probably support this possibility.

The mechanisms of these neuroprotective and reparatory effects of MTs in the mammalian CNS and other tissues, are still unclear, but include the zinc-binding property of the protein (seven zinc ions per protein molecule), sequestering of the zinc in the cell nucleus and regulation of cellular cycle and proliferation, protective effects against heavy metals and DNA damaging effects of nitric oxide, their ability to scavenge free radicals or directly reduce the inflammatory response, decreasing the level of nuclear transcription factor kappa B and proapoptotic molecules or modulating the functions of lymphocytes (Schwarz et al., 1997; Abdel-Mageed and Agrawal 1997; Coyle et al., 2002; Sato and Kondoh, 2002; Penkowa, 2006).

In stressful conditions and after exposure to heavy metals Zn can be readily displaced from its binding sites in MTs by other metals including Cd, Pb, Bi, Ni, Ag, Hg, and Mn and transferred onto the oxidized form of glutathione, having an important role in the glutathione redox cycle and other neuron functions (Gilbert, 2000; Miles et al., 2000), but the transcription of MT could be upregulated by glucocorticoid responsive elements, by the antioxidant (or electrophile) response element, as well as by the elements activated by STAT (signal transducers and activators of transcription) proteins through cytokine signaling, indicating that MTs are multifunctional proteins which are critically involved in the control of inflammation, acute phase response and cell cycle progression and apoptosis participating in a variety of cellular functions (Aschner et al., 1997; Moffatt and Denizeau, 1997; Hidalgo and Carrasco, 1998; Miles et al., 2000; Hidalgo et al., 2001; Coyle et al., 2002; Giralt et al., 2002; Sato and Kondoh, 2002; Theocharis et al., 2004; West et al., 2004; Penkowa, 2006).

This conclusion is supported also by our findings that gasoline-vapor upregulated the MT-expression in alveolar epithelium (Fig. 4) and in the kidney (Fig. 5), where cells were directly exposed to inhaled gasoline vapor or to gasoline metabolites, present in the glomerular filtrate, indicating that endogenous MTs may be protective molecules acting also against airway inflammation and toxic injuries in the tubules, where they might be induced by several proinflammatory cytokines, such as IL-1, IL-6, TNF-α, and interferons (Nordberg and Nordberg, 2000; Takano et al., 2004; Wesselkamper et al., 2006).

In summary, although the mechanisms are still unclear, the present study demonstrates that repeated exposure of mice to gasoline vapor causes high MT-I/II upregulation in tissues which were directly or indirectly exposed to toxic components, resulting in prominent upregulation of MTs expressing cells in CNS (ependymal cells, astroglial cells in subventricular zone and brain parenchyma, subgranular and CA1-CA3 zone of the dentate gyrus, perivascular macrophage-like cells), in the lungs (pneumocytes type I and type II) and in the kidney (parietal wall of Bowman capsule, proximal and distal tubules), pointing to their protective and growth-regulatory effects in the brain and on other places of injuries.

Acknowledgements. This work was supported by a grant from the Croatian Ministry of Science (Projects nos. 0062018 and 0062081).

References

Abdel-Mageed A. and Agrawal K.C. (1997). Antisense down-regulation of metallothionein induces growth arrest and apoptosis in human breast carcinoma cells. Cancer Gene Ther. 4, 199-207.

Amaral D.G. and Witter M.P. (1989). The three-dimensional organization of the hippocampal formation, a review of anatomical data. Neuroscience 31, 571-591.

Aschner M. (2000). Manganese: brain transport and emerging research needs. Environ. Health Perspect. 108, 429-432.

Aschner M., Cherian M.G., Klaassen C.D., Palmiter R.D., Erickson J.C. and Bush A.I. (1997). Metallothioneins in brain--the role in physiology and pathology. Toxicol. Appl. Pharmacol. 142, 229-242.

Cairney S., Maruff P., Burns C.B., Currie J. and Currie B.J. (2005). Neurological and cognitive recovery following abstinence from petrol sniffing. Neuropsychopharmacology 30, 1019-1027.

Chang L.W. (1990). The neurotoxicology and pathology of organomercury, organolead, and organotin. J. Toxicol. Sci. 15, 125-151.

Choudhuri S., Kramer K.K., Berman N.E., Dalton T.P., Andrews G.K. and Klaassen C.D. (1995). Constitutive expression of metallothionein genes in mouse brain. Toxicol. Appl. Pharmacol. 131, 144-154.

Chung R.S., Adlard P.A., Dittmann J., Vickers J.C., Chuah M.I. and West A.K. (2004). Neuron-glia communication, metallothionein expression is specifically up-regulated by astrocytes in response to neuronal injury. J. Neurochem. 88, 454-461.

Coyle P., Philcox J.C., Carey L.C. and Rofe A.M. (2002). Metallothionein, the multipurpose protein. Cell. Mol. Life Sci. 59,

627-647.

Dittmann J., Fung S.J., Vickers J.C., Chuah M.I., Chung R.S. and West A.K. (2005). Metallothionein biology in the ageing and neurodegenerative brain. Neurotox. Res. 7, 87-93.

Flanagan R.J. and Ives R.J. (1994). Volatile substance abuse. Bull. Narc. 46, 49-78.

Gilbert D.L. (2000). Fifty years of radical ideas. Ann. NY Acad. Sci. 899, 1-14.

Giralt M., Penkowa M., Lago N., Molinero A and Hidalgo J. (2002). Metallothionein-1+2 protect the CNS after a focal brain injury. Exp. Neurol. 173, 114-12.

Goldman S.A. and Sim F. (2005). Neural progenitor cells of the adult brain. Novartis Found. Symp. 265, 66-80; discussion 82-97.

Goodheart R.S. and Dunne J.W. (1994). Petrol sniffer's encephalopathy. A study of 25 patients. Med. J. Aust. 160, 178-181.

Hagg T. (2005). Molecular regulation of adult CNS neurogenesis, an integrated view. Trends Neurosci. 28, 589-595.

Hastings L. and Evans J.E. (1991). Olfactory primary neurons as a route of entry for toxic agents into the CNS. Neurotoxicology 12, 707-714.

Hauwel M., Furon E., Canova C., Griffiths M., Neal J. and Gasque P. (2005). Innate (inherent) control of brain infection, brain inflammation and brain repair: the role of microglia, astrocytes, "protective" glial stem cells and stromal ependymal cells. Brain. Res. Brain. Res. Rev. 48, 220-233.

Hidalgo J. and Carrasco J. (1998). Regulation of the synthesis of brain metallothioneins. Neurotoxicology 19, 661-666.

Hidalgo J., Aschner M., Zatta P. and Vasak M. (2001). Roles of the metallothionein family of proteins in the central nervous system. Brain. Res. Bull. 55, 133-145.

Hong J.Y., Wang Y.Y., Bondoc F.Y., Yang C.S., Lee M. and Huang W.Q. (1997). Rat olfactory mucosa displays a high activity in metabolizing methyl tert-butyl ether and other gasoline ethers. Fundam. Appl. Toxicol. 40, 205-210. Erratum in: Toxicol. Sci. (1998). 43, 78.

Ladeby R., Wirenfeldt M., Garcia-Ovejero D., Fenger C., Dissing-Olesen L., Dalmau I. and Finsen B. (2005). Microglial cell population dynamics in the injured adult central nervous system. Brain Res. Brain Res. Rev. 48, 196-206.

Mehlman M.A. (1991). Dangerous and cancer-causing properties of products and chemicals in the oil refining and petrochemical industry, Part I. Carcinogenicity of motor fuels, gasoline. Toxicol. Ind. Health 7, 143-152.

Miles A.T., Hawksworth G.M., Beattie J.H. and Rodilla V. (2000). Induction, regulation, degradation, and biological significance of mammalian metallothioneins. Crit. Rev. Biochem. Mol. Biol. 35, 35-70.

Mocchegiani E., Bertoni-Freddari C., Marcellini F. and Malavolta M. (2005). Brain, aging and neurodegeneration: role of zinc ion availability. Prog. Neurobiol. 75, 367-390.

Moffatt P. and Denizeau F. (1997). Metallothionein in physiological and physiopathological processes. Drug. Metab. Rev. 29, 261-307.

Nakajima K. and Suzuki K. (1995). Immunochemical detection of metallothionein in brain. Neurochem. Int. 27, 73-87.

Nordberg M. and Nordberg G.F. (2000). Toxicological aspects of metallothionein. Cell. Mol. Biol. (Noisy-le-grand) 46, 451-463.

Penkowa M. (2006). Metallothioneins are multipurpose neuroprotectants during brain pathology. FEBS J. 273, 1857-1870.

Persson E., Henriksson J., Tallkvist J., Rouleau C. and Tjalve H. (2003).Transport and subcellular distribution of intranasally administered zinc in the olfactory system of rats and pikes.Toxicology 191, 97-108.

Sato M. and Kondoh M. (2002). Recent studies on metallothionein, protection against toxicity of heavy metals and oxygen free radicals. Tohoku J. Exp. Med. 196, 9-22.

Schwarz M.A., Lazo J.S., Yalowich J.C., Allen W.P., Whitmore M., Bergonia H.A., Tzeng E., Billiar T.R., Robbins P.D., Lancaster J.R. Jr and Pitt B.R. (1995). Metallothionein protects against the cytotoxic and DNA-damaging effects of nitric oxide. Proc. Natl. Acad. Sci. USA 92, 4452-4456.

Shimada A., Nagayama Y., Morita T., Yoshida M., Suzuki J.S., Satoh M. and Tohyama C. (2005). Localization and role of metallothioneins in the olfactory pathway after exposure to mercury vapor. Exp. Toxicol. Pathol. 57, 117-125.

Streit W.J. (2005). Microglia and neuroprotection, implications for Alzheimer's disease. Brain Res. Brain Res. Rev. 48, 234-239.

Suzuki W.A. and Amaral D.G. (2003). Perirhinal and parahippocampal cortices of the macaque monkey: cytoarchitectonic and chemoarchitectonic organization. J. Comp. Neurol. 463, 67-91.

Takamiya M., Niitsu H., Saigusa K., Kanetake J. and Aoki Y. (2003). A case of acute gasoline intoxication at the scene of washing a petrol tank. Leg. Med. (Tokyo) 5, 165-169.

Takano H., Inoue K., Yanagisawa R., Sato M., Shimada A., Morita T., Sawada M., Nakamura K., Sanbongi C. and Yoshikawa T. (2004). Protective role of metallothionein in acute lung injury induced by bacterial endotoxin. Thorax 59, 1057-1062.

Tallkvist J., Persson E., Henriksson J. and Tjalve H. (2002). Cadmium-metallothionein interactions in the olfactory pathways of rats and pikes. Toxicol. Sci. 67, 108-113.

Tenenbein M. (1997). Leaded gasoline abuse, the role of tetraethyl lead. Hum. Exp. Toxicol. 16, 217-22.

Theocharis S.E., Margeli A.P., Klijanienko J.T. and Kouraklis G.P. (2004). Metallothionein expression in human neoplasia. Histopathology 45, 103-118.

Thorne R.G., Pronk G.J., Padmanabhan V. and Frey W.H. 2nd (2004). Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. Neuroscience 127, 481-496.

Vanderwolf C.H. (2001). The hippocampus as an olfacto-motor mechanism: were the classical anatomists right after all? Behav. Brain. Res. 127, 25-47.

Varelas P.N., Syrigou A.I., Kotoulas G., Kapaki E.N., Athanasopoulou C., Spanaki M.V. and Papageorgiou C.T. (1999). Cortical atrophy detected by computed tomography in gasoline station attendants. Sci. Total. Environ. 239, 143-149.

Wesselkamper S.C., McDowell S.A., Medvedovic M., Dalton T.P., Deshmukh H.S., Sartor M.A., Case L.M., Henning L.N., Borchers M.T., Tomlinson C.R., Prows D.R. and Leikauf G.D. (2006). The role of metallothionein in the pathogenesis of acute lung injury. Am. J. Respir. Cell. Mol. Biol. 34, 73-82.

West A.K., Chuah M.I., Vickers J.C. and Chung R.S. (2004). Protective role of metallothioneins in the injured mammalian brain. Rev. Neurosci. 15, 157-166.

Yasutake A., Sawada M., Shimada A., Satoh M. and Tohyama C. (2004). Mercury accumulation and its distribution to metallothionein in mouse brain after sub-chronic pulse exposure to mercury vapor. Arch. Toxicol. 78, 489-495.

Yokel R.A. (2002) Brain uptake, retention, and efflux of aluminum and manganese. Environ. Health Perspect. 110, 699-704.

Accepted November 27, 2006