Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 512 (2005) 181–187

www.elsevier.com/locate/ejphar

Effects of thinner exposure on the expression pattern of neural cell adhesion molecules, level of lipid peroxidation in the brain and cognitive function in rats

Giyasettin Baydas[a,*], Faik Ozveren[b], Mehmet Tuzcu[c], Abdullah Yasar[a]

[a]*Department of Physiology, College of Medicine, Firat University, Elazig 23119, Turkey*
[b]*Department of Neurosurgery, College of Medicine, Firat University, Elazig 23119, Turkey*
[c]*Department of Biology, Faculty of Science, Firat University, Elazig, Turkey*

Received 24 February 2005; accepted 24 February 2005
Available online 7 April 2005

Abstract

Thinner containing 60–70% toluene is a neurotoxic mixture, which is widely used as an aromatic industrial solvent. This product has been shown to cause functional and structural changes in the central nervous system. Thinner generates reactive oxygen species and the toxic effects relating to these reactants. We have investigated the effect of exposure to high concentrations (3000 ppm) of thinner for 45 days (1 h/day) on cognitive function and the levels of neural cell adhesion molecules (NCAM) and lipid peroxidation products in the hippocampus, cortex and cerebellum of rats. Thinner exposure caused a significant increase in lipid peroxidation products (malondialdehyde and 4-hydroxyalkenals) in all brain regions. Levels of NCAM 140 and NCAM 180 kDa were significantly decreased in the hippocampus and cortex of the thinner-exposed group. Furthermore, thinner-exposed rats showed cognitive deficits in passive avoidance and Morris water maze tasks.

These cognitive deficits may be due to both elevated oxidative stress and changes in synaptic plasticity. Almost all studied parameters were reversed in animals which were allowed to recover from thinner inhalation, suggesting that the effects of exposure to thinner, at least for 1 h/day for 45 days, are reversible.

Keywords: Thinner; Learning; Memory; NCAM; (Rat)

1. Introduction

Thinner contains toluene, benzene, acetone, methanol and hexane, and is widely used in textiles, paints and solvent-based cleaning fluids. The main agent is the neurotoxin toluene, which accounts for approximately 60–70% of the content of most thinners. Thinner is an abused substance and well known as a neurotoxic agent (Escobar and Aruffo, 1980). The chronic abuse of solvents results in structural and functional impairment of a variety of organs. Thinner fume inhalation is an important cause of encephalopathy and may lead to irreversible brain damage. Particularly, toluene abuse has been shown to cause permanent changes in brain structures which correlate with neural dysfunction (Baelum, 1991; Ladefoged et al., 1991; Hass et al., 1999). Furthermore, Mattia et al. (1993) demonstrated that intraperitoneal injection of toluene caused a significant elevation in the rate of reactive oxygen species generation and a reduction in levels of reduced glutathione (GSH) in the brain. Reactive oxygen species, in turn, damage lipids, proteins and nucleic acid. This leads to neurodegenerative disorders, which mediate behavioral changes.

Acute and chronic effects of toluene on neurons have been well documented (Burmistrov et al., 2001). Toluene can cause central nervous system (CNS) depression, loss of memory and progressive brain and nerve damage

* Corresponding author. Tel.: +90 424 237 00 00; fax: +90 424 237 91 38.
E-mail address: baydas@hotmail.com (G. Baydas).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.02.038

(Benignus, 1981). Exposure to toluene has been shown to decrease spatial learning, as measured with the Morris water maze (Von Euler et al., 1993). Likewise, effects on cognitive function in offspring were observed after prenatal exposure to the structural analogue, xylene (Hass et al., 1995).

Neural cell adhesion molecules (NCAM) are likely candidate molecules which participate in synaptogenesis in neuronal plasticity. Furthermore, NCAM are involved in learning and memory (Schachner, 1997). These molecules are also involved in cellular migration, axonal growth and regeneration of peripheral axons (Davis et al., 1996; Doherty and Walsh, 1994). Any insult, such as thinner and reactive oxygen species, to the CNS may alter the expression pattern of NCAM, thereby impairing learning and memory processes. There are limited data to explain the neurotoxic effects of solvents such as thinner and toluene on behavioral and functional structures of the CNS.

The purpose of the present study was to examine the behavioral effects of thinner exposure on tests of learning and memory and to evaluate the possible differential expression pattern of NCAM in the hippocampus, cortex and cerebellum of rats.

2. Materials and methods

A total of 120 adult male Wistar rats (Animal research unit, Firat University, Elazig) weighing 200–250 g were used in this study. The rats were kept in an air-conditioned room (23 ± 2 °C), under a 12-h light/12-h dark cycle. Food and tap water were available ad libitum. Rats were randomly divided into three groups. Two groups (40/group) were exposed to inhalation of 3000 ppm thinner (1 h/day) between 1700 and 1800 h for 45 days. The exposure to thinner was performed in whole-body inhalation chamber with glass walls. The control group ($n=40$) was exposed to fresh air. One of the thinner-exposed groups ($n=40$) was allowed to recover for 45 days. All protocols described were reviewed and approved by the Local Institutional Committee for the Ethical Use of Animals.

2.1. Passive avoidance test

A one-trial step-down type of passive avoidance task was used to evaluate the memory retention deficits in rats, as previously described (Sharma and Gupta, 2001). Training was carried out 24 h after the last exposure to thinner. The apparatus consisted of a two-compartment dark/light shuttle box. The floor of the dark compartment consisted of a stainless steel shock grid floor. Electric shocks were delivered to the grid floor with a stimulator. During the training session, each rat was gently placed in the light chamber and, as the rat turned into the dark chamber, a foot shock was delivered for 5 s. The retention test was carried out 24 h after training, in a similar manner; latency time was measured by placing the animals in the light chamber and recording their latency to enter the dark compartment. Animals had free access to both compartments for 10 min. Short latencies indicate poor retention.

2.2. Morris water maze learning performance

The Morris water maze (Morris et al., 1982) was selected as a test of spatial learning and memory. A circular water tank (120 cm diameter, 50 cm height) was filled to a depth of 30 cm with water at 25 ± 1 °C and the tank was divided into four equal quadrants, labeled N–S–E–W. The water was made opaque by the addition of semi-skimmed milk. An escape platform (10 cm diameter) was placed in one of the four maze quadrants (the target quadrant) and submerged 1.5 cm below the water surface. The platform remained in the same quadrant during the entire experiment. The rats were required to find the platform using only distal spatial cues available in the testing room. Cues were constant throughout testing. The rats were trained for four trials per day for 5 consecutive days to locate and escape onto the platform. A different starting position was used on each trial (not containing the platform). A trial began by placing the rat into the water facing the wall of the pool at one of the starting points. The rats were allowed to swim freely to find the hidden platform within 60 s and after reaching platform they were allowed to stay on the platform for 30 s before being returned to the cage, which was warmed with a heating pad, to await the next trial. There was a 30-s intertrial interval. If a rat failed to locate the escape platform within this time, it was gently assisted onto the platform and allowed to remain there for the same amount of time. The time to reach the platform (latency in seconds) was recorded and analyzed by software program.

2.3. Probe trial

A probe trial was performed in which memory consolidation was assessed. The time spent in the target quadrant indicates the degree of memory consolidation that has taken place after learning. On the day after the last acquisition trial, animals were tested in a probe test. In the probe trial, the rats were placed into the pool as in the training trial, except that the hidden platform was removed from the pool. For these trials, percentage time in the target quadrant, target site crossings and average distance from the platform site were recorded. The time spent in the target quadrant was used as a measure of spatial memory.

One group of rats was allowed to recover for a period of 45 days after exposure to thinner. After that time, thinner-exposed rats were tested for 5 days with four consecutive trials per day with a 30-s intertrial interval.

To test the possible deficits in sensorimotor processes and vision impairment, rats were tested in the water maze with a visible platform at a new location on the final day of the training (Kamal et al., 2000). For the visual test, the black target platform was placed inside the pool 1 cm above

the water line. Latency times to reach the platform were recorded for each trial.

2.4. Western blot of NCAM

All rats were killed by decapitation one day after the Morris water maze learning test. The brain tissues were removed and the hippocampus, cerebral cortex and cerebellum were dissected. Samples were used fresh or kept at −80 °C. Fresh or frozen tissue samples were homogenized 1:10 (w/v) in buffer [10 mM Tris–HCl, pH 7.4, 0.1 mM NaCl, 0.1 mM phenylmethylsulphonyl fluoride (PMSF), 5 μM soybean (soluble powder; Sigma, St. Louis, MO, USA) as trypsin inhibitor]. Homogenates were centrifuged at 60,000×*g* for 60 min. Pellets were washed and resuspended in homogenization buffer and recentrifuged at 60,000×*g* for 60 min. The resulting pellets were washed and resuspended in buffer (25 mM Tris–HCl, pH 7.4, 0.1 mM PMSF and 2% Triton X-100).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer containing 2% β-mercaptoethanol was added to the supernatants. Equal amounts of protein were applied in each lane, separated on SDS-PAGE using 7.5–17.5 gradient gel as described previously (Laemmli, 1970; Baydas et al., 2002). Separated proteins were transferred to nitrocellulose filters (Schleich and Schuell Inc. USA) electrophoretically using an electroblotter. Non-specific binding was prevented by incubation with 1% bovine serum albumin in 100 mM NaCl, 20 mM Na_2PO_4 and 20 mM NaH_2PO_4 at pH 7.2. Primary antibody (polyclonal rabbit anti-rat NCAM antibody) was diluted (1:2000) in the same buffer containing 0.05% Tween-20. The nitrocellulose membrane was incubated overnight at 4 °C with NCAM antibody. The blots were washed and incubated for 1 h with a secondary antibody, a goat anti-rabbit Ig peroxidase conjugated (Sigma, UK). Specific binding was detected using diaminobenzidine and H_2O_2 as substrates. The relative amount of immunoreactive NCAM isoforms on Western blots was quantified in arbitrary units by scanning blots using a computerized software program (LabWorks 4.0; UVP, Inc. Cambridge, UK).

2.5. Protein, lipid peroxidation and glutathione assays

Total protein levels were measured according to Lowry et al. (1951). Tissue lipid peroxidation (malondialdehyde+4-hydroxyalkenals) was determined using a lipid peroxidation-586 kit (Oxis International, Inc., Corvallis, OR, USA); the method is based on the reaction of *N*-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals at 45 °C. GSH levels were determined according to the method of Ellman (1959).

2.5.1. Statistical analysis

The spatial learning data were analyzed by two-way analysis of variance (ANOVA) for repeated measures, followed by Fisher's post hoc test. Biochemical data and probe test data were analyzed by one-way ANOVA followed by Dunnett's *t*-test. The relationship between lipid peroxidation and behavioral impairment was assessed with Pearson correlation coefficients.

3. Results

3.1. Lipid peroxidation and GSH levels

After thinner fume inhalation, the levels of lipid peroxidation products, i.e. malondialdehyde and 4-hydroxyalkenals, in the hippocampus, cortex and cerebellum were significantly increased compared to those of control (Table 1). The rats allowed to recover after thinner inhalation did not show any significant rise in lipid peroxidation product level as compared to the control group. There was no significant effect of thinner inhalation on the levels of the antioxidant glutathione in any brain region.

3.2. Passive avoidance test

The mean initial latency in the passive avoidance test was not different in thinner-exposed rats and control rats ($P>0.05$; Fig. 1A). The retention of the passive avoidance response was different from that of the control group: the mean retention latency in the thinner-exposed group was significantly shorter ($P<0.01$; Fig. 1B) than that of the control rats. A decrease in retention latency indicates impairment in memory retention of the passive-avoidance task in the thinner-exposed rats. However, thinner-exposed rats which were allowed to recover for 45 days performed the retention test, 24 h after the training session, as well as the control rats.

Table 1
The levels of lipid peroxidation products (as malondialdehyde+4-hydroxialkenals) and GSH in different brain regions (mean ± S.D.)

	Control	Thinner	Recovery
Hippocampus			
Lipid peroxidation products (nmol/mg protein)	1.40 ± 0.2	2.45 ± 0.3[a]	1.50 ± 0.1[b]
GSH (μg/g tissue)	450 ± 42	430 ± 40	445 ± 41
Cortex			
Lipid peroxidation products (nmol/mg protein)	2.20 ± 0.3	3.25 ± 0.3[a]	2.45 ± 0.3[c]
GSH (μg/g tissue)	440 ± 40	435 ± 42	442 ± 40
Cerebellum			
Lipid peroxidation products (nmol/mg protein)	1.50 ± 0.2	3.05 ± 0.3[d]	1.60 ± 0.2[b]
GSH (μg/g tissue)	460 ± 45	440 ± 42	455 ± 45

[a] $P<0.01$ vs. control values.
[b] $P<0.001$ vs. thinner group.
[c] $P<0.01$ vs. thinner group.
[d] $P<0.001$ vs. control values.

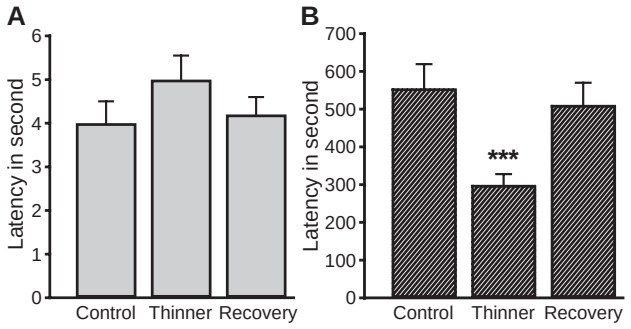

Fig. 1. Effects of thinner inhalation on the initial latency (A) and retention latency (B) in the passive avoidance test. Retention latency time was recorded to a maximum of 600 s (***$P<0.001$ vs. control).

3.3. Morris water maze test

Distances traveled to find the hidden platform during the acquisition phase of the experiment are shown in Fig. 2. The mean escape latency for the trained rats decreased from 56 to 8 s over the course of the 20 learning trials. The mean latencies in thinner-exposed and control rats were similar in the first trial, which suggests that their motor performance (ability to swim) was unaffected by thinner inhalation, whereas the thinner-exposed group tended to take longer than the controls to find the platform in the following trials (Fig. 2) The performance was impaired in the thinner-exposed rats compared to the controls and the latencies were higher in the thinner-exposed group ($P<0.05$). The performance of thinner-exposed and control rats in the trial with the visible platform was not different (latencies: 7.3 ± 0.9 and 8.1 ± 1.1 s in control and thinner-exposed rats, respectively). The overall escape latency for the thinner-exposed rats allowed to recover for 45 days was significantly different from that of the thinner-exposed group but was not significantly different from that of the controls (Fig. 2). Thus, allowing animals to recover largely prevents the impaired performance in the water maze.

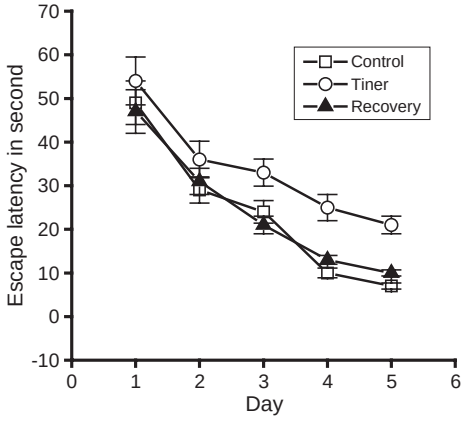

Fig. 2. Effects of chronic thinner inhalation on the acquisition of spatial learning in the Morris water maze. Latency of rats to find the hidden platform on 5 consecutive days. Learning performance was impaired in thinner-exposed rats compared to the controls and the latencies were higher in the thinner-exposed group ($P<0.05$).

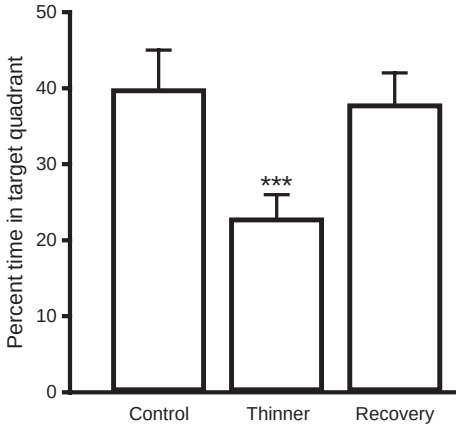

Fig. 3. Effects of chronic thinner inhalation on the mean percentage of time spent in the target quadrant in which the platform had previously been located during acquisition (***$P<0.001$ vs. control).

3.4. Probe trial

Data from the probe trial of the Morris water maze study, which measures how well the animals had learned and consolidated the platform location during the 5 days of training, indicated significant differences between the groups ($P<0.001$; Fig. 3). Thinner-exposed rats performed significantly poorer in comparison to the control group, spending 43% less time in the target quadrant than the control group. The rats allowed to recover showed no significant difference from the control rats in the probe test.

3.5. The levels of NCAM isoforms

NCAM levels were measured in different parts of the brains of the thinner-exposed and control rats. The antibody against NCAM allowed the detection of bands at 120, 140 and 180 kDa in rat brain (Fig. 4). In the hippocampus, thinner inhalation significantly decreased NCAM isoforms compared to the control values. The greatest decrease was found for the NCAM 180 isoform. Similarly, there was a trend for the expression of these three isoforms to be reduced in the cortex of thinner-exposed rats as compared to controls. However, levels of NCAM 120 and 140 kDa were significantly increased, whereas that of NCAM 180 kDa was not changed in the cerebellum of rats after thinner inhalation (Fig. 5).

In the rats allowed to recover the levels of NCAM 120 and 140 were increased in the cortex but reduced in the cerebellum compared to the levels in thinner-exposed group. Levels of NCAM 180 were significantly increased in all brain regions in rats allowed to recover for 45 days (Figs. 4 and 5).

4. Discussion

One of the important pathways of thinner neurotoxicity is thought to involve the generation of reactive oxygen

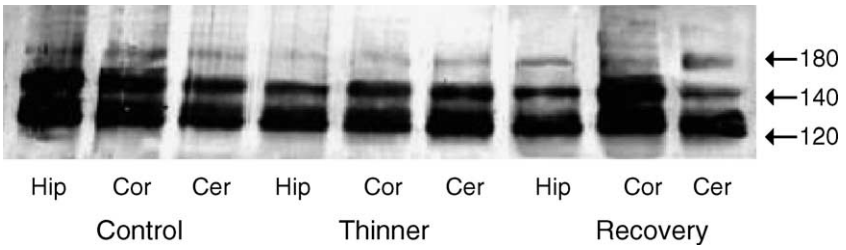

Fig. 4. Western blot analysis of NCAM polypeptide in the hippocampus, cortex and cerebellum of control and thinner-exposed rats. The antibody against NCAM allowed the detection of bands at 120, 140 and 180 kDa in rat brain. Hip: hippocampus, Cor: cortex, Cer: cerebellum.

species, which induce oxidative damage to lipids, proteins and nucleic acids. Exposure to toluene, both in vivo and in vitro, leads to reactive oxygen species formation in many tissues including brain tissue (Mattia et al., 1993). Halifeoglu et al. (2000) reported that thinner elevates the levels of malondialdehyde in serum of people working with paint thinner. In our previous study (Baydas et al., 2003), we also indicated that toluene-containing thinner increases lipid peroxidation in different brain regions and that these increases are inhibited by the antioxidant melatonin.

We demonstrate here that thinner-exposed rats had significantly elevated levels of lipid breakdown products in several brain regions. The generation of reactive oxygen species, which cause neurodegeneration, may underlie the cognitive deficits found in thinner-exposed rats. In the present study, thinner exposure-related effects on cognitive functions were observed in both the Morris water maze and passive avoidance tests in rats. We found that exposure to a high level of thinner caused a spatial learning deficit. These findings are in agreement with the results of other investigators who showed that toluene exposure stimulates reactive oxygen species formation (Mattia et al., 1993; Burmistrov et al., 2001; Halifeoglu et al., 2000) and that memory is impaired by oxidative stress (Bickford et al., 2000; Forster et al., 1996). The correlation analyses revealed a region-specific association between lipid peroxidation products and impairment scores. The retention latency in the passive avoidance test was correlated with increased levels of lipid peroxidation products in the cortex and hippocampus ($r=-0.52$, $P<0.05$; $r=-0.58$, $P<0.01$, respectively). Furthermore, the increase in lipid breakdown products within the cortex and hippocampus was also correlated with the time spent in the target quadrant ($r=-0.60$, $P<0.01$; $r=-0.65$, $P<0.001$) suggesting that increase in lipid peroxidation in the hippocampus and cortex decreases learning and memory performance. These find-

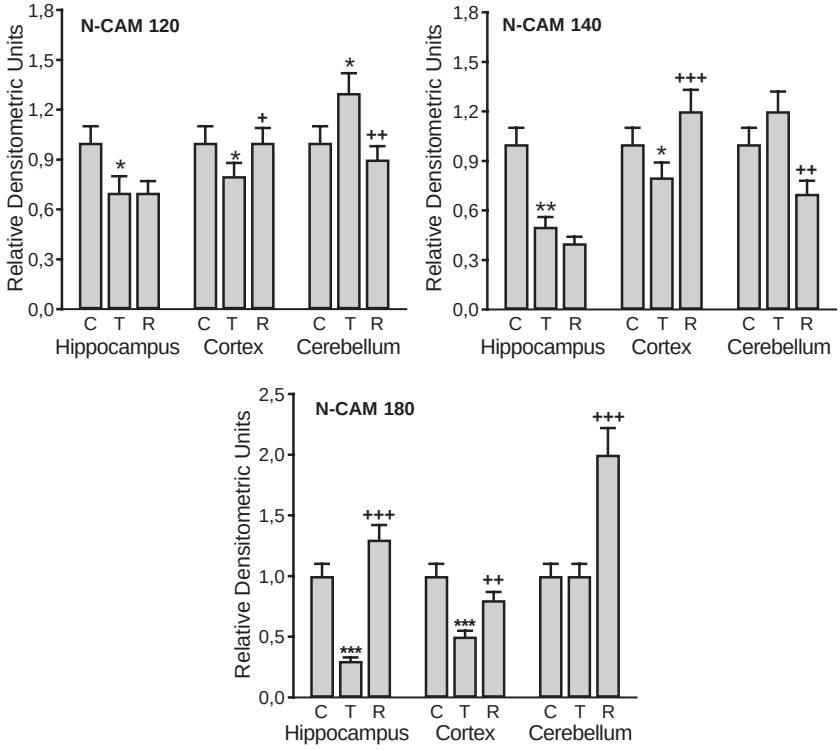

Fig. 5. Relative densitometric analysis of NCAM 120, 140 and 180 kDa from control and thinner-exposed rats (C: control, T: thinner, R: recovery). Thinner-exposure significantly changed the expression pattern of NCAM. Allowing animals to recover largely prevents the impaired pattern of NCAM expression. Values are means ± S.D.; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. control; $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$ vs. thinner group.

ings further indicate that the diminished cognitive performance of the thinner-exposed rats involves oxidative molecular damage in different regions of the brain. We also found that, if the rats were allowed to recover for 45 days after thinner exposure, the levels of lipid peroxidation products returned to normal and that cognitive performance improved considerably. Thus, the effects of thinner inhalation, at least for 1 h/day for 45 days, on oxidative stress and cognitive functions appear to be reversible. This in turn suggests that the impaired cognitive function of the rats exposed to thinner depends of neurochemical changes, such as elevated levels of lipid peroxidation products, rather than on persistent structural changes.

The second important observation of the current study is the changes in the expression of NCAM in different brain regions of thinner-exposed rats. To our best knowledge, this is the first report showing changes in NCAM in the hippocampus and cortex of thinner-exposed rats. NCAM contributes to the structural organization of the nervous system during brain development and also participates in synaptic modification in the mature brain. Furthermore, it has been indicated that NCAM may contribute to neural regeneration. The expression of NCAM was impaired in the thinner-exposed rats compared with the controls. Several recent studies indicate a role for NCAM in learning and establishment of long-term memory (Fields and Itoh, 1996; Murase and Schuman, 1999).

In the present study, we found that chronic exposure to thinner induced a significant reduction in the expression of NCAM 140 and NCAM 180 in both the hippocampus and the cortex. Ethanol exposure has been reported to cause a significant reduction in the levels of NCAM 140 and NCAM 180 at the end of the postnatal period, when synapse formation is completed (Minana et al., 2000).

The mechanism by which thinner exposure alters the pattern of NCAM expression is not known. Chronic exposure to stress induces a considerable degree of structural plasticity in the adult brain, especially in the hippocampus, where these changes are accompanied by impairments in cognitive performance (Nacher et al., 2004). It is postulated that chronic stress interferes with the mechanisms involved in the synthesis of NCAM (Venero et al., 2002). Thinner exposure provides a relevant example of chronic stress which generates reactive oxygen species and provides structural and functional changes in the nervous system (Mattia et al., 1993; Burmistrov et al., 2001). In support of this hypothesis, the level of lipid breakdown products was increased significantly in the hippocampus, cortex and cerebellum. A loss of NCAM 180 has been found to be accompanied by cell injury following exposure to the ototoxicant trimethyltin chloride, which involves reactive oxygen species generation, and to be related to cytoskeletal alterations and destabilization of cellular contacts (Dey et al., 1994; Clerici, 1996). We have previously indicated that (Baydas et al., 2002) NCAM is downregulated in the hippocampus of rats under constant light, which lead to the generation of reactive oxygen species (Baydas et al., 2001). Thus, we speculate that the generation of reactive oxygen species induced by thinner exposure alters the pattern of NCAM expression and suggest that modification of NCAM could affect cognitive function in rats. We found here that thinner exposure, like the ototoxicant trimethyltin chloride, reduced the levels of NCAM 140 and NCAM 180 in the cortex and hippocampus. The changes in the expression of NCAM observed in the present study might in part underlie the impaired cognitive performance induced by thinner exposure.

One possible explanation for the learning and memory deficits in the thinner-exposed rats is that synaptic remodelling and plasticity require optimal NCAM concentrations that are permissive for activity-dependent synaptic sprouting (Bailey, 1999). Thus, thinner exposure causes down-regulation of NCAM 140 and NCAM 180 in the hippocampus, and this in turn inhibits the formation of new synapses required for learning and memory.

In summary, the present findings indicate that exposure to thinner causes learning and memory deficits, probably by generating reactive oxygen species formation and down-regulating NCAM 140 and NCAM 180 expression in the hippocampus and cortex of rats. Further studies are needed to assess the above-mentioned hypothesis.

Acknowledgments

This work was supported by the TUBITAK (SBAG-2551) and Firat University Research Foundation.

References

Baelum, J., 1991. Human solvent exposure: factors influencing the pharmacokinetics and acute toxicity. Pharmacol. Toxicol. 1, 1–36.

Bailey, C.H., 1999. Structural changes and the storage of long-term memory in Aplysia. Can. J. Physiol. Pharm. 77, 738–747.

Baydas, G., Ercel, E., Canatan, H., Donder, E., Akyol, A., 2001. Effect of melatonin on oxidative status of rat brain, liver and kidney tissues under constant light exposure. Cell Biochem. Funct. 19, 37–41.

Baydas, G., Nedzvetsky, V.S., Nerush, P.A., Kirichenko, S.V., Demchenko, H.M., Reiter, R.J., 2002. A novel role for melatonin: regulation of the expression of cell adhesion molecules in the hippocampus, cortex and cerebellum. Neurosci. Lett. 326, 109–112.

Baydas, G., Reiter, R.J., Nedzvetskii, V.S., Yasar, A., Tuzcu, M., Ozveren, F., Canatan, H., 2003. Melatonin protects the central nervous system of rats against toluene-containing thinner intoxication by reducing reactive gliosis. Toxicol. Lett. 3, 169–174.

Benignus, V.A., 1981. Neurobehavioral effects of toluene: a review. Neurobehav. Toxicol. Teratol. 3, 407–415.

Bickford, P.C., Gould, T., Briederick, L., Chadman, K., Pollock, A., Young, D., Shukitt-Hale, B., Joseph, J., 2000. Antioxidant-rich diets improve cerebellar physiology and motor learning in aged rats. Brain Res. 2, 211–217.

Burmistrov, S.O., Arutyunyan, A.V., Stepanov, M.G., Oparina, T.I., Prokopenko, V.M., 2001. Effect of chronic inhalation of toluene and dioxane on activity of free radical processes in rat ovaries and brain. Bull. Exp. Biol. Med. 3, 832–836.

Clerici, W.J., 1996. Effects of superoxide dismutase and U74389G on acute trimethyltin-induced cochlear dysfunction. Toxicol. Appl. Pharmacol. 136, 236–242.

Davis, G.W., Schuster, C.M., Goodman, C.S., 1996. Genetic dissection of structural and functional components of synaptic plasticity: III. CREB is necessary for presynaptic functional plasticity. Neuron 17, 669–679.

Dey, P.M., Graff, R.D., Lagunowich, L.A., Reuhl, K.R., 1994. Selective loss of the 180-kDa form of the neural cell adhesion molecule in hippocampus and cerebellum of the adult mouse following trimethyltin administration. Toxicol. Appl. Pharmacol. 126, 69–74.

Doherty, P., Walsh, F.S., 1994. Signal transduction events underlying neurite outgrowth stimulated by cell adhesion molecules. Curr. Opin. Neurobiol. 4, 49–55.

Ellman, G.L., 1959. Tissue sulphydryl groups. Arch. Biochem. Biophys. 82, 70–77.

Escobar, A., Aruffo, C., 1980. Chronic thinner intoxication: clinico-pathologic report of a human case. J. Neurol. Neurosurg. Psychiatry 43, 986–994.

Fields, R.D., Itoh, K., 1996. Neural cell adhesion molecules in activity-dependent development and synaptic plasticity. Trends Neurosci. 19, 473–480.

Forster, M.J., Dubey, A., Dawson, K.M., Stutts, W.A., Lal, H., Sohal, R.S., 1996. Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. Proc. Natl. Acad. Sci. U. S. A. 14, 4765–4769.

Halifeoglu, I., Canatan, H., Ustundag, B., Ilhan, N., Inanc, F., 2000. Effect of thinner inhalation on lipid peroxidation and some antioxidant enzymes of people working with paint thinner. Cell Biochem. Funct. 18, 263–267.

Hass, U., Lund, S.P., Simonsen, L., Fries, A.S., 1995. Effects of prenatal exposure to xylene on postnatal development and behavior in rats. Neurotoxicol. Teratol. 17, 341–349.

Hass, U., Lund, S.P., Hougaard, K.S., Simonsen, L., 1999. Developmental neurotoxicity after toluene inhalation exposure in rats. Neurotoxicol. Teratol. 4, 349–357.

Kamal, A., Biessels, G.J., Duis, S.E., Gispen, W.H., 2000. Learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: interaction of diabetes and ageing. Diabetologia 43, 500–506.

Ladefoged, O., Strange, P., Moller, A., Lam, H.R., Ostergaard, G., Larsen, J.J., Arlien-Soborg, P., 1991. Irreversible effects in rats of toluene (inhalation) exposure for six months. Pharmacol. Toxicol. 5, 384–390.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Mattia, C.J., Ali, S.F., Bondy, S.C., 1993. Toluene-induced oxidative stress in several brain regions and other organs. Mol. Chem. Neuropathol. 3, 313–328.

Minana, R., Climent, E., Barettino, D., Segui, J.M., Renau-Piqueras, J., Guerri, C., 2000. Alcohol exposure alters the expression pattern of neural cell adhesion molecules during brain development. J. Neurochem. 75, 954–964.

Morris, R.G., Garrud, P., Rawlins, J.N., O'Keefe, J., 1982. Place navigation impaired in rats with hippocampal lesions. Nature 297, 681–683.

Murase, S., Schuman, E.M., 1999. The role of cell adhesion molecules in synaptic plasticity and memory. Curr. Opin. Cell Biol. 11, 549–553.

Nacher, J., Pham, K., Gil-Fernandez, V., McEwen, B.S., 2004. Chronic restraint stress and chronic corticosterone treatment modulate differentially the expression of molecules related to structural plasticity in the adult rat piriform cortex. Neuroscience 126, 503–509.

Schachner, M., 1997. Neural recognition molecules and synaptic plasticity. Curr. Opin. Cell Biol. 9, 627–634.

Sharma, M., Gupta, Y.-K., 2001. Intracerebroventricular injection of streptozotocin in rats produces both oxidative stress in the brain and cognitive impairment. Life Sci. 68, 1021–1029.

Venero, C., Tilling, T., Hermans-Borgmeyer, I., Schmidt, R., Schachner, M., Sandi, C., 2002. Chronic stress induces opposite changes in the mRNA expression of the cell adhesion molecules NCAM and L1. Neuroscience 115, 1211–1219.

von Euler, G., Ogren, S.O., Li, X.M., Fuxe, K., Gustafsson, J.A., 1993. Persistent effects of subchronic toluene exposure on spatial learning and memory, dopamine-mediated locomotor activity and dopamine D2 agonist binding in the rat. Toxicology 19, 223–232.