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Lipid Peroxidation and Antioxidant Enzyme Activities in the Rat Testis after Cigarette Smoke Inhalation or Administration of Polychlorinated Biphenyls or Polychlorinated Naphthalenes

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ABSTRACT: Lipid peroxidation products and antioxidant enzyme activities were studied in the rat testis following exposures to cigarette smoke, polychlorinated biphenyls (PCBs), or polychlorinated naphthalenes (PCNs). Three hours after a single 1-hour period of smoke inhalation, the levels of fluorescent chromolipids and thiobarbituric acid-reactive species (TBARS) were markedly increased in the testis (+49%, $P < 0.01$, and +43%, $P < 0.05$, respectively). Twelve hours after daily smoking for 1 hour, for 1, 5, or 10 days, such an increase was not found. Activities of the antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), glutathione transferase (GSH-Tr), or hexose monophosphate shunt (HMS) were not affected immediately, 3 hours, or 12 hours after a single smoking session. Twelve hours after smoking for 5 days, the activity of catalase was decreased (−16%, $P < 0.05$). Smoking exposures had no consistent effects on serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), or testosterone concentrations. Single i.p. injections of PCB or PCN mixtures resulted in decreases in testicular SOD activity 1 day after the exposures (−14%, $P < 0.05$, and −51%, $P < 0.01$, respectively). Catalase activity also decreased after both exposures (−30 to −42%, $P < 0.05$, at days 1–7 after PCB exposure, and −37 to −43%, $P < 0.05$, at days 3–7 after PCN exposure). Ninety days after the PCN exposure, activities of GSH-Px and GSH-Tr were decreased in the testis (−20%, $P < 0.05$, and −26%, $P < 0.05$, respectively). The only statistically significant change in lipid peroxidation measurements in the testes of PCB- or PCN-treated rats was a decrease in TBARS by 13% ($P < 0.01$) 1 day after PCN exposure. The main findings of this study were the increase in lipid peroxidation in the rat testis after cigarette smoke inhalation and the impairment of the function of the enzymatic antioxidant defense after exposures to PCBs or PCNs. These results suggest that free radical-dependent mechanisms may play an important role in the testicular toxicity of environmental chemicals.

Key words: Reactive oxygen, oxidative stress, superoxide dismutase, catalase, cigarette smoking, environmental chemicals.

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The incidence of testicular cancer has increased markedly in the developed countries during the last half century (Giwercman and Skakkebaek, 1992). During the same time sperm counts have decreased, and recent data show reduced fertility in 5% of men (Carlsen et al, 1992). In the etiology of testicular cancer and infertility, the exposure of men to a variety of chemicals may be involved (Steinberger, 1981; Feichtinger, 1991). In addition to the classical hypothesis of toxicant action via metabolites binding covalently to DNA or proteins, there is another, less extensively studied, mechanism of toxicity involving production of reactive oxygen species (Halliwell and Gutteridge, 1984, 1988). The accumulating knowledge on the central role of oxygen-derived free radicals in carcinogenesis has emphasized the importance of studies on prooxidant effects of chemicals (Cerutti, 1985). Free radicals can cause the destruction of all cellular structures, but their initiation of lipid peroxidation processes is of special importance. The level of peroxidation products has been used as an index of oxidative stress (Kappus, 1985). The testis tissue and sperm function are particularly vulnerable to the peroxidative injury produced by reactive oxygen species. Defective sperm function of infertile men is associated with increased lipid peroxidation and impaired function of antioxidant enzymes in spermatozoa (Jones et al, 1979; Alvarez et al, 1987; Aitken et al, 1989). Testicular toxicants such as cadmium (Kojima et al, 1990), polycyclic aromatic hydrocarbons (Georgellis et al, 1987), or ethanol (Rosenblum et al, 1985) exert their effects at least in part by free radical-dependent mechanisms. In protection against peroxidative injury, the synergistic action of the antioxidant enzyme system is essential (Fridovich and Freeman, 1986). Prooxidant states often result in impairment of this defense.

Tobacco smoking is a common means of exposure of humans to toxic chemicals. Tobacco smoke contains several thousand compounds, some of which are carcinogens

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(Hoffman and Wynder, 1986). Polycyclic aromatic hydrocarbons in tobacco smoke are toxic to the testis (Ford and Huggins 1963; Mattison, 1982). Complex congener mixtures of polychlorinated biphenyls (PCBs) have been widely used in numerous industrial applications. The toxic properties of these chemicals, e.g., immunotoxicity, hepatotoxicity, teratogenicity, and carcinogenicity, have considerably restricted their use, but several toxic PCB congeners still occur abundantly in our environment (Safe, 1984; McFarland and Clarke, 1989). In addition to other toxic effects, PCBs are noxious to male and female reproductive functions (Safe, 1984; Lione, 1988). PCBs and other chlorinated hydrocarbons are found in the semen of men even if they have not been in contact with such substances (Schlebusch et al, 1989), and higher seminal concentrations of these compounds are associated with reduced fertility (Feichtinger, 1991). Polychlorinated naphthalenes (PCNs) have been used as commercial mixtures from the beginning of this century in several industrial purposes, including the replacement of PCBs, and their occurrence in environmental samples has been demonstrated (Järnberg et al, 1990). In the rat liver, PCBs and PCNs have been observed to increase lipid peroxidation (Kamohara et al, 1984; Dogra et al, 1988; Mäntylä and Ahotupa, 1993).

In the present study, we exposed rats to cigarette smoke for 1 hour for 1, 5, or 10 days. Blood carboxyhemoglobin (COHb) determination was used to compare the extent of exposure to that of human smokers. Other rats were exposed to i.p. injections of PCB or PCN mixtures and followed-up for 90 days. In both exposure regimens, the level of lipid peroxidation in the testis was estimated by the formation of fluorescent chromolipids and thiobarbituric acid reactive species (TBARS). Testicular activities of the antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), glutathione transferase (GSH-Tr), and hexose monophosphate shunt (HMS; in smoking experiments) were measured. Also, the effects of smoking exposures on serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were studied.

Materials and Methods

Chemicals

1,1,3,3-tetraethoxypropane, butylated hydroxytoluene, cumene hydroperoxide, epinephrine, glucose-6-phosphate, hydroxen peroxide (H_2O_2), linoleic acid (cis-9,cis-12-octadecadienoic acid, sodium salt), lucigenin (bis-*N*-methylacridinium nitrate), reduced glutathione (GSH), reduced nicotinamide adenine dinucleotide phosphate (NADPH), xanthine, and the enzymes catalase (bovine liver), GSH reductase (baker's yeast), SOD (Cu/Zn-form, bovine erythrocytes), and xanthine oxidase (buttermilk) were all purchased from Sigma Chemical Co. (St. Louis, Missouri). The cigarettes (Marlboro®) were made by Amer-Yhtymä Oy (Tuusula, Finland), under authority of Philip Morris Products Inc. (Richmond, Virginia). The PCB mixture Clophen A 50 (54% chlorine, w/w) was purchased from Bayer AG (Leverkusen, Germany), and the PCN mixture Halowax 1014 (62% chlorine, w/w) was purchased from Ultra-Scientific (Hope, Rhode Island). Halowax 1014 is a mixture of hexa-, penta- (40% each, w/w), and tetrachloronaphthalenes (20%, w/w).

Animals and Treatments

Adult male Sprague-Dawley rats were produced in our own fully accredited vivarium. They were fed with standard laboratory rat chow and water *ad libitum* and kept at 20–22°C with a light/dark cycle of 14 hours light and 10 hours darkness. The appropriate permission for the experiment according to Finnish law was applied for and granted. Cigarette smoke inhalations were carried out by exposing a group of four rats to the smoke of 10 commercial filter cigarettes (containing 0.9 mg nicotine and 14 mg tar per cigarette, as quoted by the National Board of Health of Finland) during 1 hour in an inhalation chamber as described by Uotila and Marniemi (1976). The corresponding control group of four rats was exposed to air flow in an identical chamber in which the cigarette was replaced by a filter. At the end of the daily exposures for 1, 5, or 10 days, the animals were killed by decapitation, under light carbon dioxide anesthesia, 12 hours after the last smoking or sham session. Other groups of four to eight rats were killed either immediately or 3 hours after a single 1-hour smoke or sham exposure. The number of exposed and control groups in the smoking regimen was five, and the total number of animals was 48. PCBs (Clophen A 50) or PCNs (Halowax 1014) were administered by single i.p. injections (100 mg/kg or 20 mg/kg body weight in corn oil, respectively). Control animals were injected with the vehicle only. Groups of four rats were sacrificed 1, 3, 7, 14, or 90 days after the injection. The total number of rats in this exposure regimen was 60.

Tissue Preparation

After decapitation, trunk blood was collected into test tubes and serum prepared by centrifugation. Different blood samples for COHb determinations were obtained by cardiac puncture into heparinized syringes from the rats killed immediately after the smoking or sham sessions. The testes were dissected out, rinsed in 0.15 mol/L KCl, and weighed. A 33% (w/v) testis homogenate was prepared, using an Ultra-Turrax homogenizer (Janke & Kunkel KG, Staufen, Germany), in 0.15 mol/L KCl (0°C). Part of the homogenate was centrifuged (10,000 × *g* for 10 minutes at 4°C), and the supernatant was decanted and saved.

Lipid Peroxidation Measurements

Fluorescent chromolipids were assayed in testis homogenates essentially as described by Esterbauer et al (1986). The samples were eluted with chloroform–methanol (2:1; v/v) and centrifuged at 2,000 × *g* for 10 minutes. The organic phase was evaporated under N_2 to dryness and redissolved in chloroform–methanol (10:1), and its fluorescence was measured at 360 nm (excitation)/430 nm (emission). An unpromoted thiobarbituric acid test was performed using 100 μl of homogenate, which was added to 400

μl of phosphate buffer, pH 7.4. Standard samples contained 1,1,3,3-tetraethoxypropane instead of homogenate. Thereafter, 500 μl of 30% trichloroacetic acid and 50 μl of 2% (w/v) butylated hydroxytoluene in ethanol were added to the reaction mixture. To start the reaction, 500 μl of thiobarbituric acid (375 mg/50 ml H_2O) was added, and the tubes were heated in a boiling water bath for 15 minutes. Then the tubes were cooled, and after centrifugation (2,000 × *g* for 10 min) the absorbance was measured at 535 nm (Bird and Draper, 1984).

Enzyme Assays

In the cigarette smoke experiment, the activity of SOD (Enzyme Commission [EC] no. 1.15.1.1) was measured by inhibition of xanthine–xanthine oxidase-dependent chemiluminescence enhanced by lucigenin and linoleate (Laihia et al, 1993). The reaction mixture was made by adding 55 μl of the sample dilution (1:2,000 in assay medium), 20 μl of xanthine oxidase (420 U/L), 20 μl of 0.1 mmol/L lucigenin, and 20 μl of 200 mmol/L linoleic acid to 385 μl of assay medium—50 mmol/L potassium phosphate buffer, pH 10.0. The superoxide-producing reaction was initiated by automated dispensing of 60 μl of 1.45 mmol/L xanthine. Chemiluminescence was measured for 7 minutes at 35°C. The inhibition of chemiluminescence was calculated as a percent of the value of buffer as a sample. All measurements were performed in duplicate cuvettes with a Bio-Orbit 1251 Luminometer (Bio-Orbit Ltd., Turku, Finland). In the PCB and PCN experiments, SOD was assayed spectrophotometrically by inhibition of epinephrine autoxidation (Misra and Fridovich, 1972). Samples were added to reaction mixture made in sodium carbonate buffer, pH 10.2, and the absorbance change was recorded at 480 nm. The activity of catalase (EC 1.11.1.6) was determined by measuring the rate of disappearance of 15 mmol/L H_2O_2 at 240 nm (Beers and Sizer, 1952). The results of SOD and catalase assays were expressed as U of enzyme activity per g tissue with reference to standard samples of purified enzyme preparation. GSH-Px (EC 1.11.1.9) was assayed by measuring the oxidation of $NADPH^+$ at 340 nm, with cumene hydroperoxide as the substrate (Paglia and Valentine, 1967). In the assay of GSH-Tr (EC 2.5.1.18), 1-chloro-2,4-dinitrobenzene was used as the substrate, and the absorbance change was recorded at 340 nm (Habig et al, 1974). The activity of HMS was assayed using glucose-6-phosphate as the substrate and 340 nm as the recording wavelength (Glock and McLean, 1953). Because the total NADPH production was measured, the HMS activity represents the sum of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities. The results of GSH-Px, GSH-Tr, and HMS assays are expressed as nmol product per minute per g tissue. SOD and catalase activities were determined in tissue homogenates, whereas the centrifuged supernatant fluid was used in GSH-Px, GSH-Tr, and HMS assays.

Hormone Measurements

Serum FSH was measured by a double-antibody radioimmunoassay (RIA), using reagents supplied by the National Institutes of Health (Bethesda, Maryland). Iodinations with Na[^{125}I]iodide of the purified hormone preparations were performed using the chloramine-T method (Hunter and Greenwood, 1962). Testosterone was measured from diethylether extracts of the sera by RIA as described previously (Huhtaniemi et al, 1985). A new supersensitive immunofluorometric assay was used for serum LH measurements (Haavisto et al, 1993). The method is based on two monoclonal antibodies, one to human and the other to bovine LH, and on signal detection by time-resolved fluorescence (Delfia®; Wallac Oy, Turku, Finland).

COHb Determinations

COHb concentrations in fresh blood samples were determined using an IL-282 Co-Oximeter (Instrumentation Laboratories, Lexington, Massachusetts).

Statistical Analysis

Two-tailed Student's *t*-test was used to compare the exposure groups with the corresponding control groups. A P value ≤ 0.05 was considered statistically significant.

Results

Effects of Cigarette Smoke Inhalation

Lipid Peroxidation—Immediately after smoke inhalation for 1 hour, no effect was observed in the level of fluorescent chromolipids in the testis (Fig. 1a). Three hours after smoking, the amount of fluorescent products was increased above the level in the testes of sham-exposed rats (+49%, $P < 0.01$). In parallel, testicular TBARS (Fig. 1b) were not significantly different from the control level immediately after smoking, but the elevation in this parameter at 3 hours reached statistical significance (+43%, $P < 0.05$). Twelve hours after cigarette smoke inhalation for 1, 5, or 10 days, no statistically significant changes were observed in fluorescent chromolipids or TBARS in the testes of smoke-exposed rats compared to the sham-exposed group of animals.

Antioxidant Enzyme Activities—No significant differences were observed in testicular activities of antioxidant enzymes between the cigarette smoke-exposed and sham-exposed rats 0 or 3 hours after a single exposure. Catalase activity (Fig. 2a) in the smoke-exposed rats was below the level of control rats after exposure for 1, 5, and 10 days, and GSH-Px activity was below the control level after 5 and 10 days of smoke exposure (Fig. 2b). However, only the decrease in catalase after smoking for 5 days was statistically significant (−16%, $P < 0.05$). Cigarette smoke inhalation for 1, 5, or 10 days did not affect testicular activities of SOD, GSH-Tr, or HMS.

Blood COHb and Testis Weights—No COHb was detected in the blood of sham-exposed rats, whereas an increased level of blood COHb was found immediately after 1 hour of cigarette smoke inhalation (0 ± 0.03, n = 4, vs. 9.0 ± 1.1, n = 4; mean % ± standard error [SE] in the sham-exposed vs. smoke-exposed rats, respectively). Testis weights were not affected by smoke exposure.

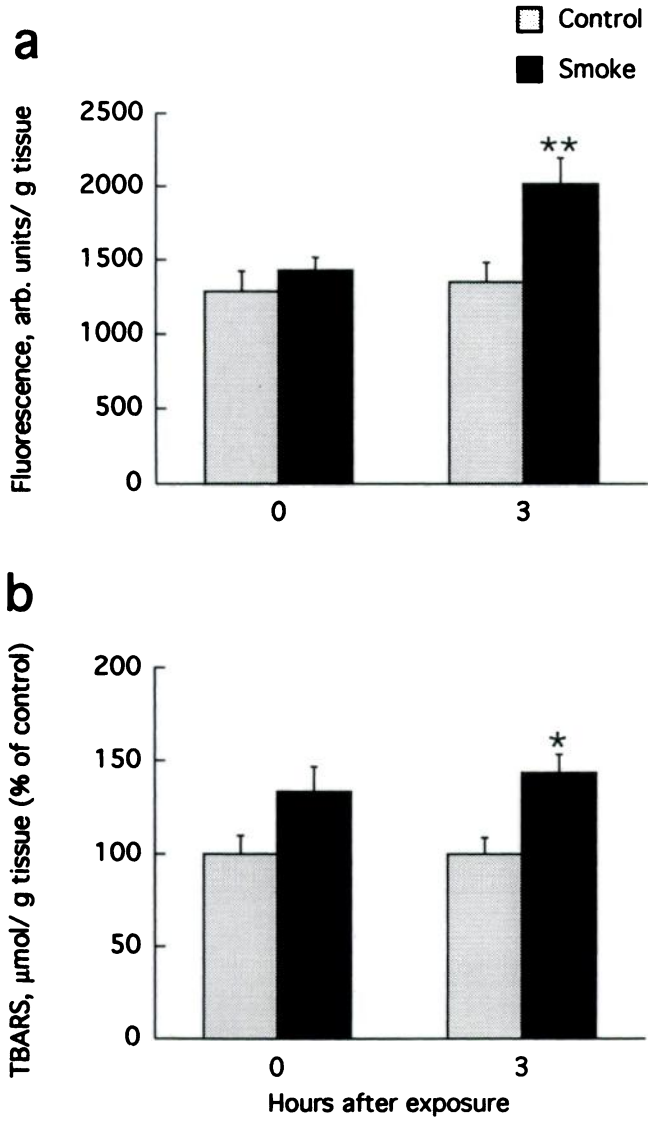

FIG. 1. Fluorescent chromolipids (a) and thiobarbituric acid-reactive species (TBARS) (b) in the rat testis immediately or 3 hours after single 1-hour cigarette smoke or sham exposure. Mean ± SEM; $n = 4$, except $n = 7$–8 for fluorescence at 3 hours after the exposure. The asterisks indicate a significant difference from the control: *, $P < 0.05$; **, $P < 0.01$.

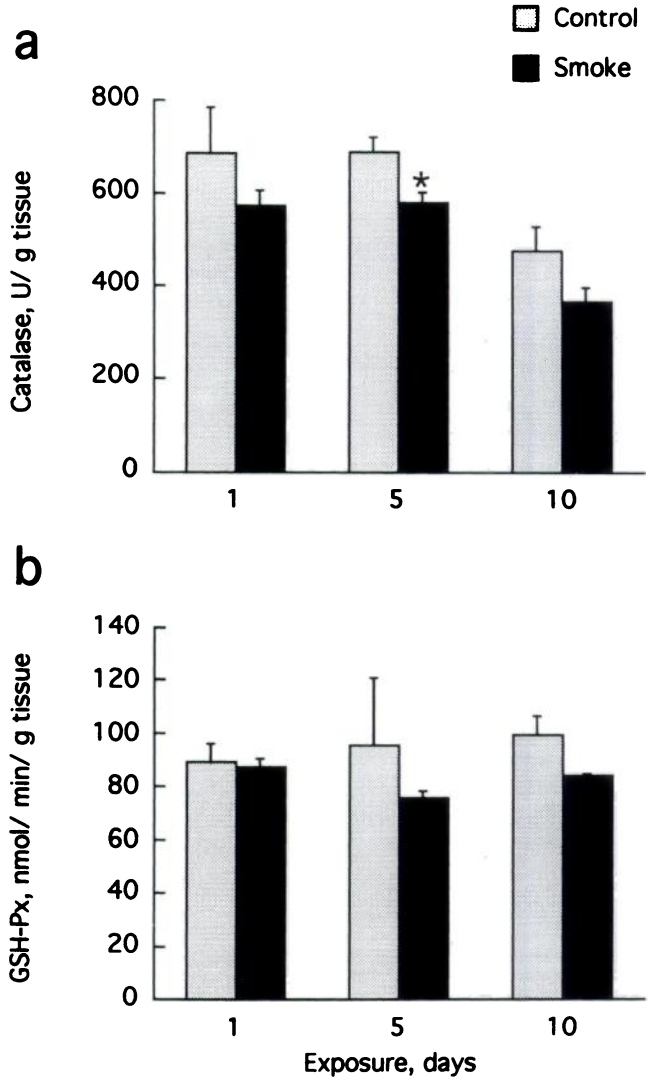

FIG. 2. Activities of catalase (a) and glutathione peroxidase (GSH-Px) (b) in the rat testis 12 hours after daily cigarette smoke or sham exposures for 1, 5, or 10 days. Mean ± SEM; $n = 4$. The asterisk (*) indicates a significant difference from the control, $P < 0.05$.

Pituitary–Testicular Hormones—FSH, LH, and testosterone were measured in serum of rats killed 3 hours after a single smoke or sham exposure or 12 hours after exposure for 1, 5, or 10 days. Cigarette smoke inhalation had no effect on serum concentrations of these hormones.

Effects of PCBs

Lipid Peroxidation—Fluorescent chromolipids were determined 7 and 90 days after the single doses of PCBs and PCNs. No differences were found between the levels in exposed and control rats. Testicular TBARS were reduced below control level by day 1 after the administration of PCNs (−13%, $P < 0.01$; Fig. 3). Thereafter, the level of TBARS in the testes of PCB- or PCN-exposed rats did not differ significantly from that of the control rats during the 90-day follow-up.

Antioxidant Enzyme Activities—Testicular SOD activity was reduced below control by day 1 after administration of PCBs (−14%, $P < 0.05$) and PCNs (−51%, $P < 0.01$; Fig. 4a). It recovered to control level by day 3. At 90 days after the dosing, testicular SOD activity was increased in PCB-treated rats (+49%, $P = 0.05$). Also, in the PCN-treated group, this activity was higher on day 90 than in the controls, but the increase was statistically not significant. Catalase activity in the testis was markedly reduced after exposures with PCB and PCN (Fig. 4b). It was decreased by day 1 after the PCB dose (−30%, $P <$

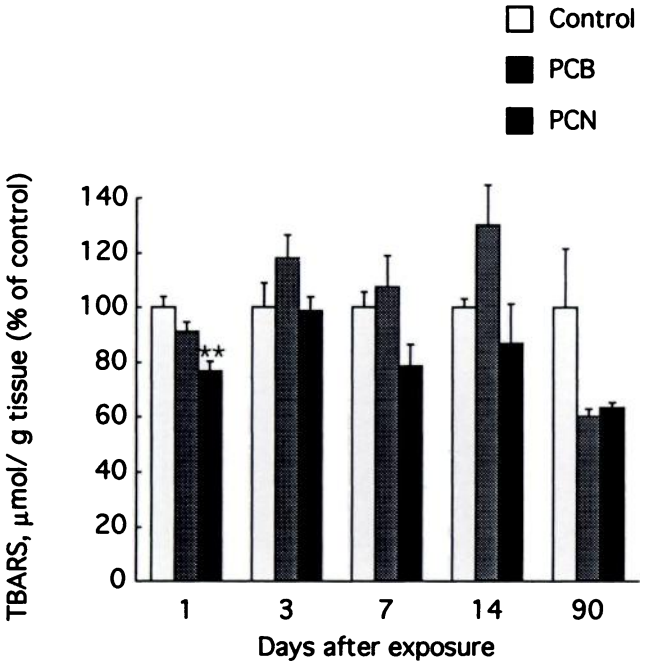

FIG. 3. TBARS in the rat testis after i.p. injection of PCB or PCN mixture. Mean ± SEM; $n = 4$, except $n = 3$ for PCN-treated rats 14 days after exposure. The asterisks (**) indicate a significant difference from the control, $P < 0.01$.

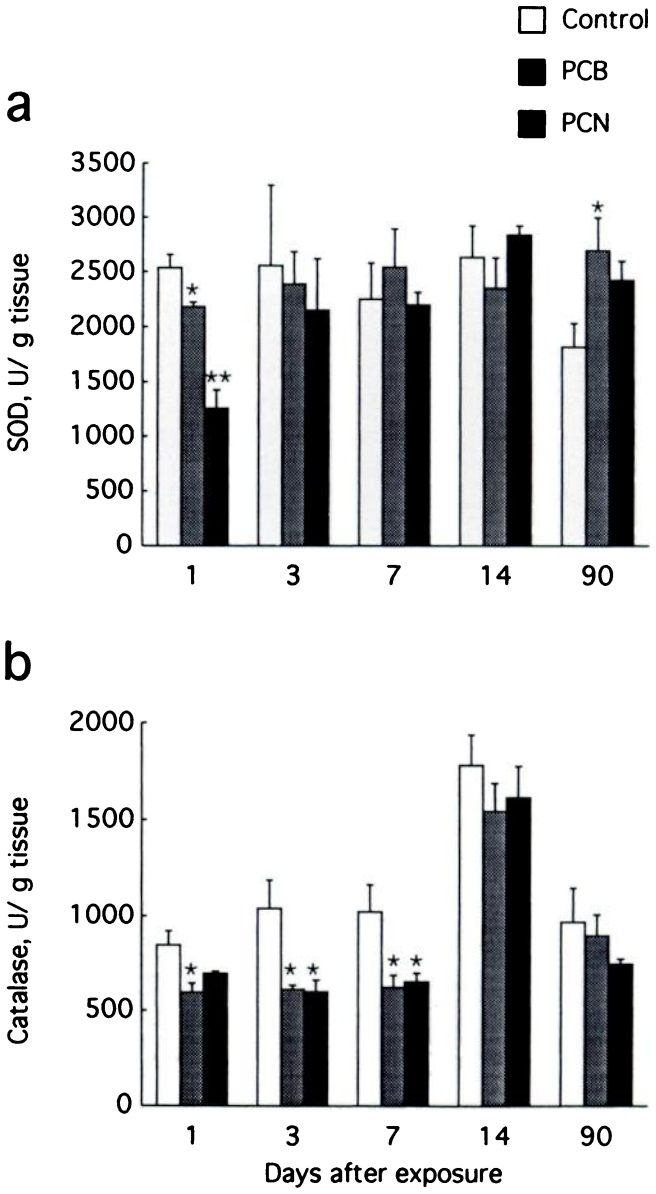

FIG. 4. Activities of superoxide dismutase (SOD) (a) and catalase (b) in the rat testis after i.p. injection of PCB or PCN mixture. Mean ± SEM; $n = 4$, except $n = 3$ for PCN-treated rats 14 days after exposure. The asterisks indicate a significant difference from the control: *, $P \leq 0.05$; **, $P < 0.01$.

0.05), and it remained reduced (−42%, $P < 0.05$, and −39%, $P < 0.05$, at days 3 and 7, respectively) until reaching control level by day 14. After PCN administration, catalase activity was decreased on days 3 and 7 (−43%, $P < 0.05$, and −37%, $P < 0.05$, respectively). Thereafter, testicular catalase was at the control level to the end of the 90-day follow-up. Administration of PCBs or PCNs did not alter GSH-Px activity in the testis at days 1–14 after the doses (Fig. 5a). By day 90 after PCN administration, GSH-Px activity was decreased (−20%, $P < 0.05$). GSH-Tr activity followed a similar pattern (Fig. 5b); there was no significant difference between exposed and control animals until a decrease occurred in the testes of PCN-exposed rats on day 90 (−26%, $P < 0.05$). At the same time, testicular GSH-Tr activity in PCB-exposed rats was also below the level of the control group, but the difference was statistically not significant.

Testis Weights—Administration of PCB or PCN mixtures did not alter testis weights when compared to respective control groups during the 90-day follow-up.

Discussion

In the rat testis, indicators of lipid peroxidation were increased at 3 hours after cigarette smoke inhalation. Lipid peroxidation was measured by end-products in the network of chemical reactions, and results of thiobarbituric acid tests are known to be nonspecific (Janero, 1990). Nevertheless, the marked parallel increases in TBARS and fluorescent chromolipids presumably reflect increased peroxidation of testicular lipids. An increase in these parameters in testis homogenate might also reflect infiltration of inflammatory cells or an increase in oxidized plasma lipoproteins (Harats et al, 1990). However, these sources of peroxidation products seem to play only a minor role in tissue homogenates, because we found no increase in lipid peroxidation in liver homogenate after similar smoke exposures (unpublished results). The in-

crease in lipid peroxidation was of short duration; 12 hours after smoking for 1, 5, or 10 days no effect was observed. The vast number of compounds in tobacco smoke includes many potential prooxidants. Cigarette tar contains redox cycling species, capable of reducing molecular oxygen to produce superoxide anion (Church and Pryor, 1985). Polycyclic aromatic hydrocarbons benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene are largely metabolized via free radical-dependent pathways in the rat testis (Georgellis et al, 1987). Nicotine (Mattison, 1982), cadmium (Hoffmann and Wynder, 1986), and radioactive particles (Ravenkolt, 1982) are other constituents of tobacco smoke suggested to adversely affect the testis. Increased lipid peroxidation may be involved in the action of these toxicants as well. It remains unknown whether chronic exposure to cigarette smoke leads to oxidative damage in the testis. Long-term smoke exposure has resulted in decreased or unchanged lipid peroxidation in rat lungs (Chow, 1993). This finding may be explained by adaptation of the antioxidant defense system. Increased levels of vitamin E and activities of antioxidant enzymes have been found in the lungs of chronically smoke-exposed rats (Chow, 1993).

Testicular catalase and GSH-Px activities were somewhat lower at 12 hours after 5 and 10 days of smoke-exposure than after sham sessions, but this finding was statistically significant only in the case of catalase after exposure for 5 days. Previously, Gupta et al (1988) observed increased SOD activities in the rat liver after exposure to cigarette smoke for 35 days, whereas pulmonary SOD was not affected. In their study, pulmonary and hepatic activities of catalase, GSH-Px, and GSH reductase were not affected by inhalation of cigarette smoke. Unpublished data from our own laboratory show induction of some of the antioxidant enzymes in rat lung and liver after smoking exposure. The present results suggest that testicular catalase and GSH-Px are susceptible to inactivation by tobacco smoke constituents, but more experimental material is needed to strengthen this finding. The results of pituitary–testicular hormone measurements showed no clear effects by smoke inhalation. In human smokers, serum testosterone levels are lower than in nonsmokers (Mattison, 1982).

Blood COHb levels (mean 9.0%) of the rats studied were near the levels of human smokers (1.3–9.0%; Rickert and Robinson, 1981). In the rat, cigarette smoke inhalation results in higher blood COHb relative to total particulate matter intake than in humans, because the exchange of inhaled gases with pulmonary capillary blood is fast, due to rapid respiratory and circulatory rates (Loscutoff et al, 1982). The findings of the present study suggest that tobacco smoking may result in oxidative stress in the human testis as well. In addition, if the antioxidant defense system is impaired, smokers would possibly be more susceptible than nonsmokers to other free radical-producing testicular toxicants. The effects of cigarette smoking on sperm quality have been studied extensively, but the results have been contradictory. Stillman et al (1986) concluded in their review that literature as a whole reflects impairment of sperm density, motility, and morphology in human smokers. Thereafter, corresponding findings were made in several studies (Rantala and Koskimies, 1986; Saaranen et al, 1987; Marshburn et al, 1989), although some of the recent epidemiological studies have shown no effects of smoking on sperm function (Vogt et

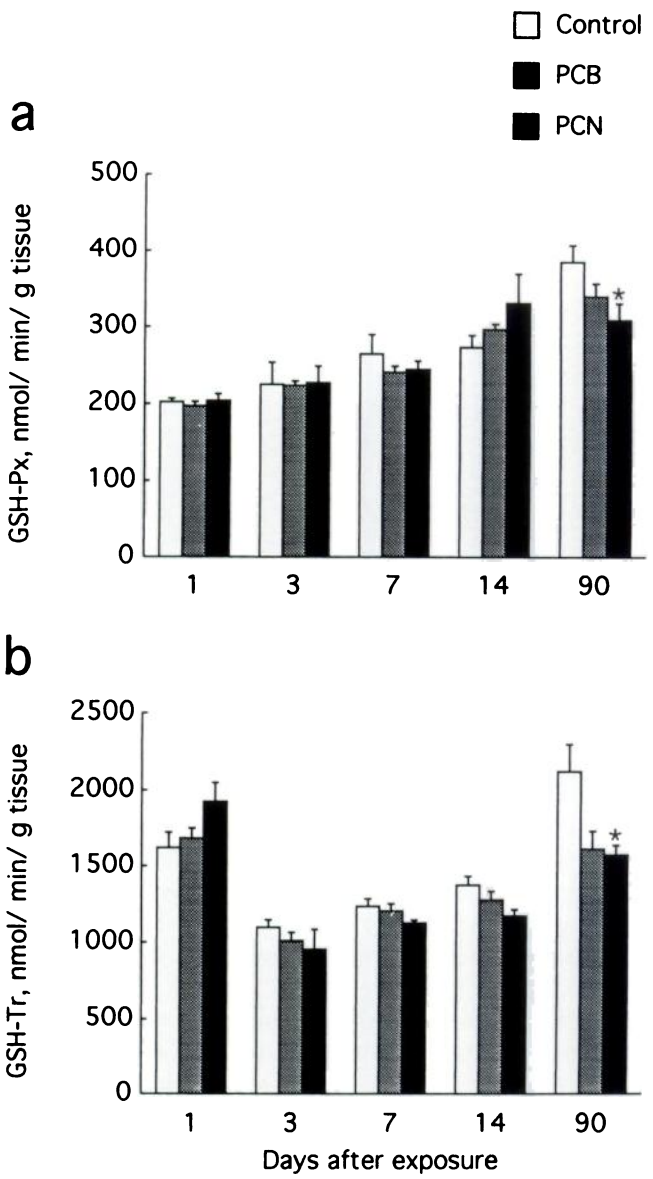

FIG. 5. Activities of GSH-Px (**a**) and glutathione transferase (GSH-Tr) (**b**) in the rat testis after i.p. injection of PCB or PCN mixture. Mean ± SEM; $n = 4$, except $n = 3$ for PCN-treated rats 14 days after exposure. The asterisk (*) indicates a significant difference from the control, $P < 0.05$.

al, 1986; Dikshit et al, 1987; Oldereid et al, 1989; Holzki et al, 1991; Osser et al, 1992). Acrosin activity (Gerhard et al, 1989) and sperm penetration (Close et al, 1990) are impaired among smokers. Paternal smoking did not affect fertility of couples in studies of Tokuhata (1968) and Baird and Wilcox (1985), but Suonio et al (1990) found an increase in the risk of conception delay. Determinations of the antioxidant defense system and free radical metabolism in long-term smoking studies are needed for further evaluation of the oxidative stress in the testis due to tobacco smoking.

In the second experiment of this study, rats were exposed to mixtures of PCB and PCN compounds. The doses used are known to have long-term effects on hepatic drug metabolizing enzyme activities in the rat (Parkki et al, 1977; Ahotupa and Aitio, 1980). SOD activity was decreased markedly 1 day after the exposures. Catalase activity was decreased on days 1–7 and 1–3 after PCB and PCN, respectively. Catalase can be inactivated by superoxide anion radical (Kono and Fridovich, 1982). In the present study, the decrease in SOD activity may have resulted in accumulation of superoxide anions, which subsequently inactivated catalase. If hydrogen peroxide is not removed effectively enough by catalase and glutathione peroxidase, it may in turn inactivate SOD (Bray et al, 1974; Sinet and Garber, 1981). Rapid recovery of SOD activity suggests that no extensive accumulation of hydrogen peroxides occurred. Impairment of enzymatic antioxidant functions has been observed in various tissues after exposure, e.g., to hyperoxia (Ahotupa et al, 1992), *N*-nitrosoamines (Ahotupa et al, 1987), UV-irradiation (Punnonen et al, 1991), γ-irradiation (Kergonou et al, 1981), or cryptorchidism (Ahotupa and Huhtaniemi, 1992). As described above, the antioxidant enzymes are mutually protective, and maintenance of different enzyme activities is needed for the synergistic function of the defensive system. Exposure to PCN had the most prominent acute effect on SOD. It also seems to have caused permanent testicular injury, because after 3 months of recovery the activities of GSH-Px and GSH-Tr were decreased in the PCN-exposed testes. These enzymes detoxify lipid peroxidation products. Presumably, the effects of PCN resulted in a decrease in the production of substrates for GSH-Px and GSH-Tr. However, there were no significant drops in TBARS or fluorescent chromolipids.

Exposures to PCB or PCN did not increase the lipid peroxidation level parallel with inactivation of SOD and catalase. Instead, the formation of TBARS was decreased 1 day after the dose of PCN. These findings seem contradictory to the proposed prooxidative effects of the exposures. They may indicate that nonenzymatic antioxidants are capable of defending testis tissue against extensive oxidative damage, regardless of impaired function of the enzymatic defense system. However, assays of peroxidation products do not always indicate oxidative damage (Kappus, 1985; Janero, 1990). Free radicals may in part exert their effects without increasing peroxidation. Actually, metabolites of certain free radical-producing xenobiotics have been observed to inhibit lipid peroxidation (Pederson and Aust, 1974; Kappus, 1985). In addition, due to the complexity of lipid peroxidation chemistry, most methods used for determining its extent offer only an indirect and narrow view into this chemical process (Smith and Anderson, 1987; Janero 1990). On the other hand, the most toxic coplanar polyhalogenated aromatic hydrocarbon, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), has been shown to induce a long-lasting (at least 12 days) lipid peroxidation in rat testis. This was associated with a small decrease in SOD and GSH-Px activities, but catalase activity remained unchanged (Al-Bayati et al, 1988).

Taken together, exposures to cigarette smoke and PCBs or PCNs resulted in free radical-mediated toxicity in the rat testis. The smoking experiment is comparable to the exposure of human smokers. The clear increase in lipid peroxidation after a single smoking session, however transient in duration, suggests that smoking may result in potentially destructive oxidative stress in the testis. In the exposure to PCBs or PCNs, the doses used were much higher than those commonly due to environmental exposure. Administration of these compounds was found to result in marked impairment in the enzymatic antioxidant defense of the testis. Increased production of free radicals or decreased function of the defense systems may play a role in testicular toxicity of different kinds of chemicals.

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