BIOPHYSICS AND BIOCHEMISTRY

Effect of Chronic Inhalation of Toluene and Dioxane on Activity of Free Radical Processes in Rat Ovaries and Brain

S. O. Burmistrov, A. V. Arutyunyan, M. G. Stepanov, T. I. Oparina, and V. M. Prokopenko

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The effects of toluene and dioxane inhalations on the intensity of free radical oxidation in rat ovaries and brain cortex were studied. Both toxins in a dose 10-fold surpassimg the maximum permissible concentration increased activity of glutathione peroxidase in brain tissue; moreover, toluene increased chemiluminescence intensity, which attested to activation of free radical processes. In ovarian tissue toluene increased activities of glutathione peroxidase and catalase and the intensity of lipid peroxidation. These changes were associated with the appearance of normally absent circadian rhythm.

Key Words: *toluene; dioxane; free radicals; ovaries; brain; circadian rhythms*

Disturbances in the nervous and reproductive systems are the most unfavorable outcomes caused by xenobiotics. Numerous studies demonstrated adverse effects of environmental pollution on the female reproductive system resulting in deterioration of child health [1,8]. Activation of free radical processes, accumulation of lipid peroxidation (LPO) products and modulation of protein structure are universal mechanisms impairing functions of organs, tissues, and cells [13]. This mechanism underlies the effect of many toxic substances, including organic solvents acetone, ethanol, and toluene entering the organism via inhalation (which is the most typical of large cities) [4,7]. We previously showed that toluene and dioxane modulate daily rhythms of biogenic amines and free radical processes in the hypothalamus (brain structure responsible for regulation of the reproduction cycle) [2].

Laboratory of Perinatal Biochemistry, D. O. Ott Institute of Obstetrics and Gynecology, Russian Academy of Medical Sciences, St. Petersburg

Here we investigated toxic effects of toluene and dioxane inhalation on protein peroxidation (PPO) and activity of catalase and glutathione peroxidase (GPx) in rat brain cortex and ovaries and on circadian rhythms of these parameters.

MATERIALS AND METHODS

Experiments were carried out on female rats (180-200 g). The animals were put into 400-liter inhalation chambers (air flow rate 30 liters/min) and exposed to toxic vapors for 4 h a day, 5 days a week, during 1 month. The concentrations of xenobiotic were maximum permissible concentration (MPC) for working zone of industrial plants and 10-fold MPC, *i.e.* 50 and 500 mg/m^3 for toluene and 10 and 100 mg/m^3 for dioxane, respectively. The rats were sacrificed at 11:00 or 17:00. The ovaries and brain cortex were isolated and frozen at -20°C. For biochemical analysis, the tissues were homogenized in 0.15 M K,Na-phosphate buffer (pH 7.8) and centrifuged for 20 min as 23,000*g*. Catalase

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activity in the supernatant was measured spectrophotometrically [9] and GPx activity was measured using tret-butyl hydroperoxide as the substrate [6]. The intensity of PPO was evaluated by the content of carbonyl (CO) amino acid derivatives in proteins by a modified method [12]. In experiments with toluene, the intensity of free radical oxidation in the brain was evaluated by activity of H_2O_2-induced chemiluminescence [10].

RESULTS

Catalase activity increased after inhalations of toluene in MPC, but inhalation of 10-fold MPC had no apparent effect; by contrast, exposure to 10-fold MPC considerably changed GPx activity. The intensity of PPO in experimental groups virtually did not differ from the control (Table 1). We did not evaluate the intensity chemiluminescence in ovarian tissue, but activity of free radical processes seemed to increase, which could be seen from increased GPx activity after exposure to 10 MPC toluene. A more pronounced activation of free radical oxidation was observed in the brain cortex: toluene in a dose of 10 MPC considerably increased the intensity of chemiluminescence and GPx activity, while activity of catalase and PPO remained unchanged (Table 1). Intensification of free radical oxidation did not lead to accumulation of modified proteins, which attested to high activity of the antioxidant system. It can also be concluded that additional capacity of the antioxidant defense in this case is related to activation of GPx and, presumably, other antioxidant components, but not catalase. Similar results were obtained in experiments with dioxane: inhalation of 10 MPC led to activation of GPx, but not catalase, the intensity of PPO being unchanged both in the ovaries and brain cortex (Table 1).

Hence, both toluene and dioxane stimulated free radical processes in the ovaries and brain cortex. The reaction of GPx to intoxication was not specific. According to published reports, the thiol-disulfide system is very sensitive to damaging factors due to vulnerability of SH group in proteins and low-molecular compounds to oxidation [5]. Brain tissue is more stable, which is explained by high resistance of the CNS to damage due to its important regulatory function for the whole organism.

No circadian rhythms of fluctuations of biochemical values were recorded in ovarian and cerebral tissues of intact animals (Figs. 1-3). After toluene inhalations (10 MPC) morning and evening activities of catalase and GPx in ovarian tissue differed considerably (Fig. 1). A similar rhythm of GPx activity was induced by toluene in the brain. Interestingly, daily rhythms of PPO intensity and chemiluminescence appeared after inhalation of 10 MPC toluene (Figs. 2, 3). Dioxane modulated daily rhythm of GPx activity: it appeared in the brain and disappeared in the ovaries (Figs. 1, 2). Hence, toluene induced more pronounced changes in circadian rhythms than dioxane in both cerebral and ovarian tissue. The appearance of circadian rhythms of the studied parameters after toluene inhalations is probably an adaptive reaction to chronic exposure to xenobiotic. The most pronounced response is the dose-dependent increase in the chemiluminescence intensity in cerebral tissue in the morning hours coinciding with the start of inhalation exposure. Both toxicants increased morning activity of GPx in the ovaries and brain cortex (Figs. 1, 2), which also indicates a response of the antioxidant system to in-

TABLE 1. Effects of Toluene and Dioxane Inhalations on Catalase and GPx Activities, PPO Intensity (per mg Protein), and Chemiluminescence in Rat Ovaries and Brain Cortex ($M\pm m$, n=6-9)

Organ	Toluene control	Toluene		Dioxane control	Dioxane	
		MPC	10 MPC		MPC	10 MPC
Ovaries						
Catalase, mmol H_2O_2/min	6.77±0.60	8.81±0.60*	7.10±0.48	6.27±0.32	6.74±0.29	7.03±0.31
GPx, mmol GSH/min	59.0±3.5	56.2±2.4	66.8±1.7*	38.4±1.3	39.1±2.5	45.9±3.1*
PPO, μmol CO derivatives	11.3±1.1	9.6±0.6	11.3±0.2	7.5±0.3	7.0±0.2	6.9±0.5
Brain						
Catalase, mmol H_2O_2/min	1.51±0.07	1.52±0.14	1.64±0.07	0.839±0.034	0.863±0.017	0.893±0.041
GPx, mmol GSH/min	38.7±1.3	40.4±1.2	46.5±2.5*	37.1±0.5	39.3±0.8	40.8±1.5*
PPO, μmol CO derivatives	4.89±0.22	4.36±0.33	4.74±0.36	2.33±0.16	2.60±0.17	2.44±0.15
Chemiluminescence intensity, arb. units/ml	261±36	335±22	421±70*	—	—	—

Note. *p<0.05 compared to the corresponding control.

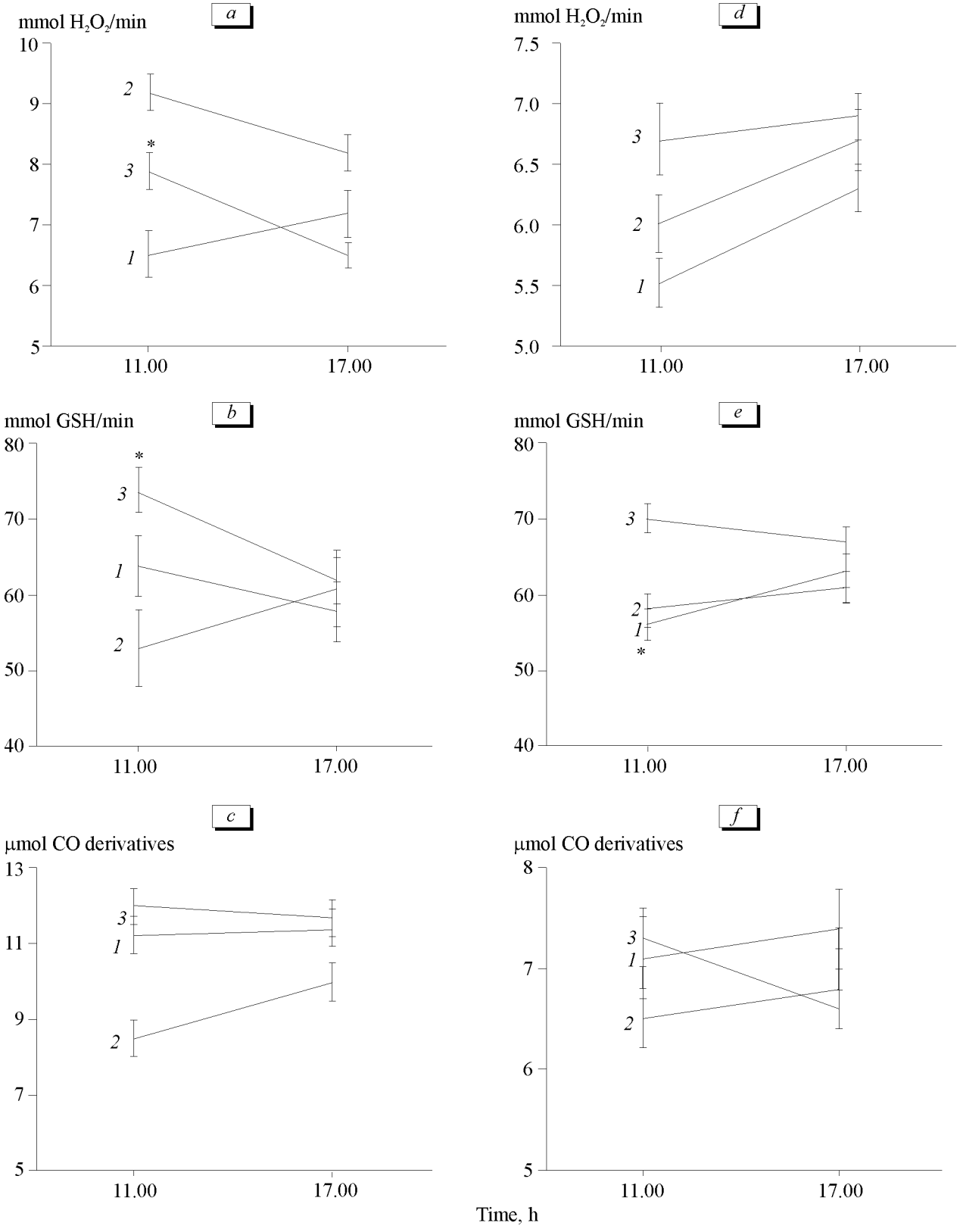

Fig. 1. Daily changes in catalase (*a, d*) and glutathione peroxidase (*b, e*) activities and protein peroxidation (*c, f*) in rat ovaries (per mg protein) after toluene (*a-c*) and dioxane (*d-f*) inhalations. Here and in Figs. 2 and 3: *1*) control; *2*) MPC; *3*) 10 MPC. *Differences between morning and evening values are significant (p<0.05).

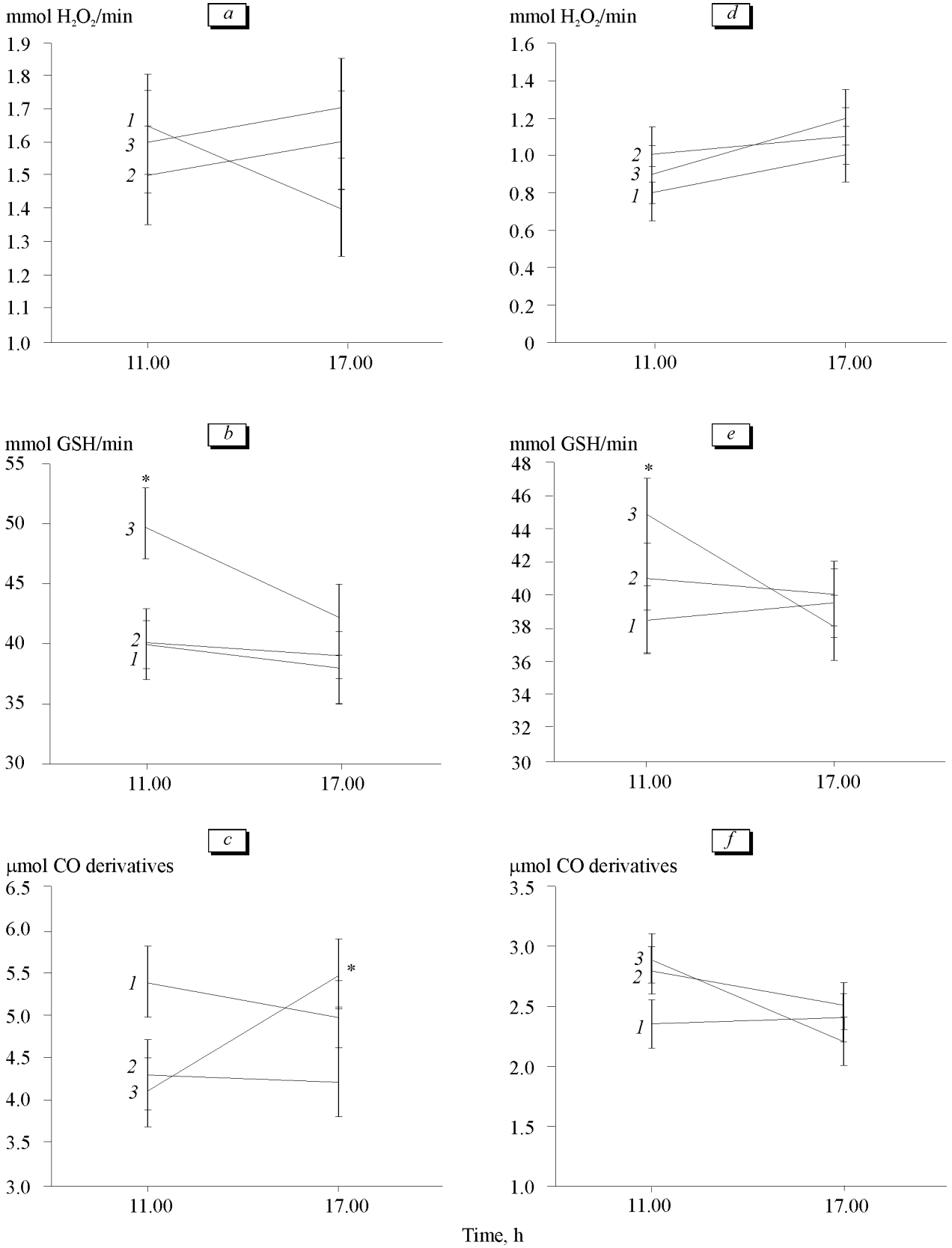

Fig. 2. Daily changes in catalase (*a, d*) and glutathione peroxidase (*b, e*) activities and protein peroxidation (*c, f*) in rat brain cortex (per mg protein) after toluene (*a-c*) and dioxane (*d-f*) inhalation.

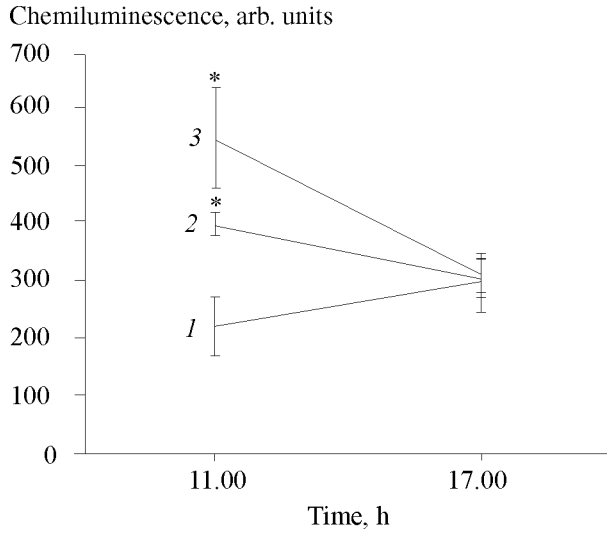

Fig. 3. Daily changes in chemiluminescence intensity in rat brain cortex after toluene inhalations.

toxication. Changes in GPx activity were observed only after exposure to toluene and dioxane in a dose of 10 MPC and were absent in the evening. Decreased evening chemiluminescence intensity in the brain after toluene inhalation attested to inhibition of free radical processes during 4-h inhalation procedure. The corresponding decrease of evening GPx activity after inhalation of toluene and dioxane in doses of 10 MPC is therefore understandable (Fig. 3).

When evaluating the daily rhythms of biochemical parameters in ovarian tissue, it should be noted that the weight of ovaries in control animals changed significantly: from 61±6 mg in the morning to 75±5 mg in the evening ($p<0.05$). Toluene exposure smoothened these daily fluctuations. Dioxane did not eliminate the daily rhythm of ovarian weight. These data also attested to higher toxicity of toluene for the ovaries compared to dioxane.

Changes in the daily dynamics of the studied parameters under the effects of the studied toxins indicates their influence on the suprachiasmatic hypothalamic nuclei, the main generators of biorhythms, and presumably on the epiphysis producing melatonin. Melatonin, an indole hormone, is now believed to play an important role in the regulation of daily activity of various physiological functions and metabolic processes [12,13].

Hence, toluene and dioxane induced similar changes in free radical processes in the brain cortex. In the ovaries, both toxicants intensified free radical processes, but the effect of toluene on the ovaries was more pronounced: it modifies not only GPx, but also catalase activity and PPO intensity. Toluene modifies circadian rhythms of catalase and GPx activities in the ovaries and activity of GPx and chemiluminescence intensity in the brain, leading to appearance of normally absent differences between the morning and evening values of the studied parameters.

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