Effects of Paint Thinner Exposure on Serum LH, FSH and Testosterone Levels and Hypothalamic Catecholamine Contents in the Male Rat

Bayram YILMAZ,*[,a] Selim KUTLU,[a] Sinan CANPOLAT,[a] Süleyman SANDAL,[a] Ahmet AYAR,*[,b] Rasim MOGULKOC,[a] and Haluk KELESTIMUR[a]

Firat University Medical School, Departments of Physiology[a] and Pharmacology,[b] 23119 Elazig, TURKEY.
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We have investigated the effects of thinner inhalation on serum LH, FSH and testosterone levels together with changes in hypothalamic catecholaminergic system in the male rat. A control group inhaled normal air ventilation. The remaining animals were divided into two groups and exposed to paint thinner in a glassy cage for 15 or 30 d. Toluene concentration (the largest constituent in thinner, 66%) was set at 3000 ppm in the inhalation air. At the end, all animals were decapitated and blood samples obtained. Serum LH and FSH levels were measured by RIA and testosterone by enzyme immunoassay. Following removal of brains on dry ice, medial preoptic area, suprachiasmatic nucleus, median eminence and arcuate nucleus were isolated by micropunch technique. Noradrenaline, 3,4-dihydroxyphenylglycol (DHPG) and dopamine concentrations of these hypothalamic areas were determined by HPLC-ECD. Fifteen-day thinner inhalation significantly suppressed serum LH and testosterone levels in parallel ($p<0.001$) compared to control group values (LH: 0.77±0.07; testosterone: 2.67±0.39). Thirty-day exposure markedly decreased LH levels ($p<0.001$), but surprisingly had no significant effect on testosterone. Serum FSH levels were not significantly altered in either group. Thinner inhalation for 15 or 30 d did not cause any significant change in noradrenaline, DHPG or dopamine concentrations in the hypothalamic regions examined (except in the arcuate nucleus). These results suggest that paint thinner has an anti-gonadotropic effect and may cause long-term endocrine disturbances in the male. It is thought that the hypothalamic catecholaminergic system is not involved in thinner inhibition of LH and testosterone secretion.

Key words paint thinner; gonadotropin; testosterone; noradrenaline; dopamine

Paint thinner is a widely used industrial solvent. There is increasing concern about involuntary exposure of industrial workers to this organic solvent.[1,2] In addition, abuse of paint thinner among young sniffers has become a social and public health problem.[3,4] It is well recognised that solvent abuse can result in sudden deaths and also cause metabolic disturbances and pathological changes in various tissues.[5—7] Toluene is the largest constituent of thinner, which also contains acetone, isobutyl acetate, isobutanol and butyl glycol in varying amounts. Various neurotoxic effects of toluene or thinner such as memory impairment, behavioural, electroencephalographic and neuropathological changes have been reported in the rat.[8—10]

Several organic solvents have been suggested to impair neurochemical mechanisms in the brain which control hypophyseal gonadotropin release.[1,11,12] There is very limited literature on the effects of paint thinner inhalation on male reproductive functions. Svensson *et al.*[1] reported that chronic occupational toluene exposure in male subjects resulted in reduced luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Slight decrease in plasma testosterone levels was not statistically significant in the same epidemiological study. Immunohistochemical evidence has revealed that hypothalamic gonadotropin releasing hormone (GnRH) neurons are not affected by intraperitoneal administration of high doses of toluene.[13] Toluene exposure for 30 d resulted in a decrease in hypothalamic noradrenaline concentrations in the rat.[14] This finding is further supported by Shen *et al.*[13] that density of noradrenaline-containing fibers in the median eminence (ME) and paraventricular nucleus (PVN) was reduced in the toluene-treated group. An earlier study had shown that subacute exposure to high levels of toluene led to increased noradrenaline concentrations in the hypothalamic ME and PVN and elevated levels of plasma FSH.[15]

The reports outlined above have usually evaluated neurotoxic and reproductive effects of a single organic solvent, toluene. However, industrial solvent exposure usually involves exposure to a mixture of two or more different solvents. The present study was undertaken to investigate the effects of paint thinner inhalation on serum LH, FSH and testosterone levels in the rat. Noradrenaline and dopamine concentrations in different hypothalamic regions were also examined since these catecholamines have been reported to be involved in the central regulation of gonadotropic hormone secretion.

MATERIAL AND METHODS

Adult male Wistar rats weighing 280—320 g (Firat University Biomedical Unit, Elazig) were used as subjects. They were maintained in a temperature-controlled environment (21±1 °C) with lights on between 07 : 00—19 : 00. Food (rat chow) and water were available *ad libitum*. The rats were divided into three groups. Groups I ($n=10$) and II ($n=10$) were exposed to paint thinner in a glassy cage for a period of 15 and 30 d, respectively. The control group inhaled ambient air ventilation ($n=10$). The paint thinner solution was comprised of toluene (66%), aceton (20%), isobutyl acetate (10%), butyl glycol (3%) and isobutanol (1%). Toluene concentration (the largest constituent in the liquid thinner) was set at 3000 ppm in the inhalation air. The glassy chamber was well-sealed. Liquid thinner was absorbed on a piece of cotton and a small ventilator was used to distribute a gas form of the solvent constituents evenly in the inhalation air within the cage. Toluene concentration in the exposure chamber was

* To whom correspondence should be addressed. e-mail: b.yilmaz@excite.com
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monitored using gas chromatography (UNICAM, Cambridge, U.K.).

Applications were carried out for 1 h in the morning (between 9—11 a.m.) and evening (between 16—18 p.m.) for 15 or 30 d. At the end, all animals were decapitated and trunk blood obtained. They were centrifuged at 4°C for 10 min at 3000 rpm. Serum samples were stored at −20°C until assayed. Brains were rapidly removed and frozen on dry ice, then 500 μm coronal brain slices were cut. Medial preoptic area (MPOA), suprachiasmatic nucleus (SCN), median eminence (ME) and arcuate nucleus (ARN) were micropunched using modified stainless steel hypodermic needles (0.7 and 0.3 mm internal diameter), according to the Stereotaxic Atlas of the Rat Brain.[16] This study was approved by the Firat University Medical School Animal Research Ethics Committee.

Liquid Chromatography The specific hypothalamic areas collected were kept at −80°C prior to the analysis. One hundred microliters of 0.1M HCl was added to each sample, along with 50 μl of 3,4-dihydroxybenzylamine (DHBA: 1 ng) as an internal standard. The samples were homogenised and then centrifuged at 4°C for 10 min at 3000 rpm. Twenty microliter aliquots of supernatant were injected on to a reverse phase High Performance Liquid Chromatographic (HPLC) column (S5ODS2-250A, 5 μm, 4.6 mm i.d.×25 cm, Waters) coupled to an electrochemical detector (ECD, Model 142, GILSON). The catecholamine content of the hypothalamic regions was simultaneously detected. The mobile phase consisted of 6.74 g citric acid, 4.81 g sodium citrate, 47 mg ethylenediamine tetra-acetic acid, 200 mg heptasulphonic acid, 1.15 ml glacial acetic acid, 3 ml tetrahydrofuran and 25 ml HPLC grade methanol. It was made up to 1 l with HPLC grade water and then brought to pH 4.9 using 10 M NaOH. The flow rate was set at 1 ml/min. The method has been previously described.[17]

Protein Estimation The protein content of the tissue pellet remaining after HPLC-ECD was determined from its absorbance at 595 nm after reaction with Coomassie Blue G250 (Pierce, Luton, U.K.). The weight of protein was calculated from a standard curve prepared using bovine serum albumin as detailed.[17]

Radioimmunoassay Serum levels of rLH and rFSH were measured by radioimmunoassay (RIA) following the instructions given with the reagents generously provided by the National Hormone and Pituitary Program of NIDDK (Baltimore, Maryland, U.S.A.). The rLH reference preparation was rLH-RP-2 and the antiserum was anti-rat LH-RIA-11. The sensitivity (90% B/Bo) of this assay using a 100 μl sample is about 0.16 ng/ml. The rFSH reference preparation was rFSH-RP-2 and the antiserum was anti-rat FSH-S-11. The sensitivity of this assay using a 200 μl sample is about 2 ng/ml. Both antigens were radiolabelled with iodine-125 (IMS 30 from Amersham Life Science, Ltd., Bucks, U.K.). Bound and free radioiodinated antigens were separated using double antibody generously provided by the Scottish Antibody Production Unit, Law Hospital, Carluke, Lanarkshire, Scotland. The mean intra-assay coefficient of variation for two quality control samples was <5% in both assays.

Chemiluminescent Enzyme Immunoassay Serum testosterone levels were determined by chemiluminescent enzyme immunoassay (The Chiron Diagnostic ACS:180 Testosterone, Los Angeles, U.S.A.). This is a competitive immunoassay utilising chemiluminescent technology. Testosterone in samples competes with acridinium ester-labelled testosterone in the Lite reagent for a limited amount of polyclonal rabbit anti-testosterone antibody attached to monoclonal mouse anti-rabbit antibody. Testosterone releasing agent is used to release bound testosterone from endogenous binding proteins in the sample. There is an inverse relation between the amount of testosterone present in sample and the amount of relative light units detected by the system.

Statistics The results were statistically analysed by One-Way ANOVA (MINITAB, release 10 for Windows). Level of significance was set at $p<0.05$.

RESULTS

Serum LH, FSH and testosterone results are shown in Figs. 1—3. Fifteen-day thinner inhalation significantly suppressed serum LH and testosterone levels in parallel ($p<0.001$) compared to control group values (LH: 0.77±0.07; testosterone: 2.67±0.39). Thirty-day exposure markedly decreased LH levels ($p<0.001$), but, surprisingly, had no significant effect on testosterone. In the 30-d inhalation group, serum LH values were lower than in the 15-d exposure group. Serum FSH levels were not significantly altered in either group.

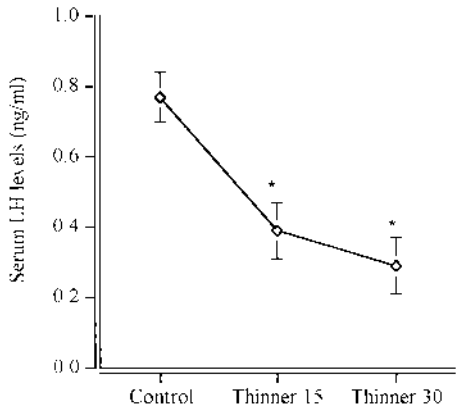


Fig. 1. Serum LH Levels (ng/ml±S.E.M.) in Male Rats Following Daily Exposure to Paint Thinner Inhalation for 15 and 30 d

*: $p<0.001$ compared to the control group, using One-Way ANOVA.

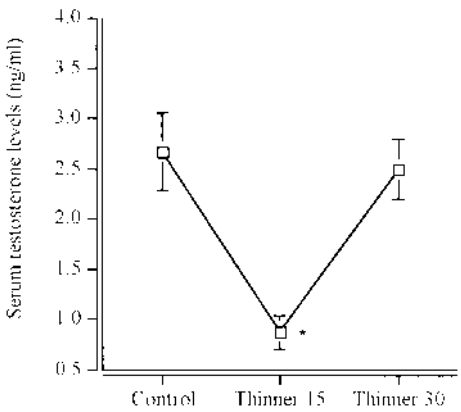


Fig. 2. Serum Testosterone Levels (ng/ml±S.E.M.) in Male Rats Following Daily Exposure to Paint Thinner Inhalation for 15 and 30 d

*: $p<0.001$ compared to the control group, using One-Way ANOVA.

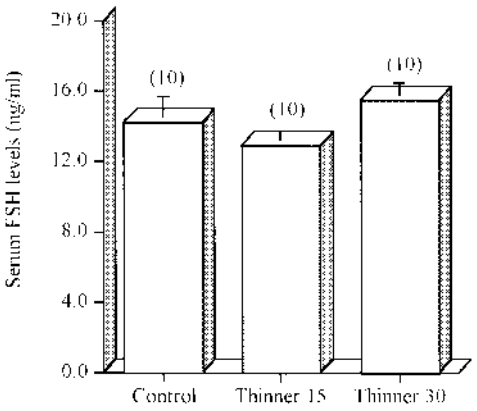


Fig. 3. Serum FSH Levels (ng/ml±S.E.M.) in Male Rats Following Daily Exposure to Paint Thinner Inhalation for 15 and 30 d

Number of animals in each group is given in brackets.

Table 1. Noradrenline, DHPG and Dopamine Concentrations (pg/μg Protein±S.E.M.) in the apothalamic Median Preoptic Area (MPOA), Suprachiasmatic Nucleus (SCN), Median Eminence (ME) and Arcuate Nucleus (ARN) Following Daily Exposure to Paint Thinner for 15 and 30 d in Male Rats.

	MPOA	SCN	ME	ARN
Noradrenaline				
Control	41.5±12.0	158.9±15.3	53.6±12.8	36.7±7.1
Thinner 15	52.9±8.8	140.4±10.0	66.3±6.5	74.8±8.4 *
Thinner 30	78.5±15.6	164.8±19.6	59.3±8.6	140.6±33.0 *
DHPG				
Control	125.1±35.4	374.9±63.7	97.5±11.2	75.3±13.3
Thinner 15	206.5±41.6	415.9±71.2	90.8±12.3	108.3±20.3
Thinner 30	114.0±33.7	334.5±53.3	79.6±13.9	105.9±22.8
Dopamine				
Control	7.7±3.4	8.3±3.1	0.8±0.2	2.5±0.1
Thinner 15	0.83±0.2	3.5±0.8	0.92±0.3	2.2±0.6
Thinner 30	1.9±0.6	4.3±2.2	2.3±1.1	10.3±3.3 **

*: $p<0.05$, **: $p<0.01$ compared to the control group values, using One-Way ANOVA.

Concentrations of noradrenaline and 3,4-dihydroxyphenylglycol (a noradrenaline metabolite, DHPG) and dopamine in the hypothalamic MPOA, SCN, ME and ARN are summarised in Table 1. Thinner inhalation for 15 or 30 d did not cause any significant change in noradrenaline, DHPG and dopamine concentrations in the hypothalamic MPOA, SCN and ME. Noradrenaline concentrations were significantly increased in only the ARN by 15 and 30 d thinner inhalation ($p<0.05$). Dopamine levels were higher than the control group values in only the ARN in the 30-d exposure group ($p<0.01$).

DISCUSSION

The present findings indicate that daily exposure to high concentrations of paint thinner for 15 d causes a reduction in LH secretion from the anterior pituitary which, in turn, inhibits testosterone production. It has previously been suggested that toluene has no significant effect on testosterone secretion or spermatozoa levels in the epididymis.[18] In contrast, epidemiological studies have shown that occupational exposure to toluene reduced serum LH and testosterone levels[19] and semen volume.[2] We suggest that thinner, containing a mixture of organic solvents (66% toluene), has a negative influence on male reproductive functions by acting at a hypothalamo-hypophyseal level. Serum LH levels were reduced in a dose-dependent manner in the 30-d exposure group. Surprisingly, these decreases were not accompanied by reduced testosterone levels. The enzyme immunoassay for testosterone was repeated in a separate assay, but the results proved to be the same as before. It is speculated that the Leydig cells might have sensitised to low levels of LH over the four week period of exposure.

It has been reported that *n*-hexane can cause testicular damage including testicular atrophy, cessation of sperm production and decreased serum testosterone levels,[20] and it was suggested that simultaneous exposure to *n*-hexane and toluene prevented the toxic effects of *n*-hexane exposure. However, the same authors did not study the effects of toluene alone. Recently, Ono *et al.*[21] have reported that inhalation exposure to toluene decreased weights of epididymis and spermatic count in the rat. Similarly, toluene exposure reduced percentage of motile spermatozoa, semen volume and testosterone levels in the male workers.[2] In our study, male gonads, sperm volume and motility were not examined. However, short term exposure to paint thinner at high doses would probably bring about similar defects in the testes since toluene constitutes the largest proportion in the liquid thinner.

There is a bulk of evidence to indicate that the brain noradrenergic system plays an important role in the regulation of GnRH and thus LH release.[22,23] A minor role of the central dopaminergic system has also been implicated in this mechanism.[24,25] In our study, we determined concomitant changes in noradrenaline and dopamine contents in the MPOA, SCN, ME and ARN, the hypothalamic regions thought to participate in the control of pulsatile LH secretion, together with circulating levels of LH and FSH. Andersson *et al.*[15] reported that toluene exposure increased noradrenaline concentrations in the subependymal layer, the paraventricular nucleus and the anterior periventricular area and dopamine levels in the ME. In contrast, decreased hypothalamic concentrations have been reported following toluene inhalation for 30 d in the rat.[14] The present results showed that thinner inhalation had no significant effect on catecholamine contents in the hypothalamic regions examined (except in the ARN). Neurons of the tuberoinfundibular dopaminergic tract (TIDA) originate in the ARN and terminate in the ME whereas nerve terminals of the incertohypothalamic tract are distributed in the other hypothalamic areas.[26] Increased dopaminergic activity in the ARN of the 30-d exposure group appears to be in line with previously reported inhibitory effects of TIDA on LH secretion in the rat.[25] Similarly, elevated levels of noradrenaline in the ARN could be attributed to an increase in the activity of dopamine β-hydroxylase which converts dopamine to noradrenaline. However, it seems difficult to propose a role for the noradrenergic system in thinner inhibition of LH release without providing evidence in the MPOA, SCN and ME. We suggest that paint thinner affects GnRH secreting neurons directly or by other neuromodulatory systems. It might also reduce responsiveness of the pituitary gonadotrops to hypothalamic GnRH.

There are contradictory reports on the effects of toluene on FSH release. It has been reported that occupational toluene

exposure reduced plasma FSH levels in male subjects.[1] Wu *et al*[27] have reported increased FSH levels in workers exposed to trinitrotoluene. In an experimental study, toluene exposure resulted in increased FSH concentrations.[15] In our study, organic solvent used for experiments was paint thinner which had no significant effect on plasma FSH levels in any of the groups.

It is generally believed that release of both FSH and LH is regulated by the hypothalamic decapeptide, GnRH.[28] However, existence of a separate hypothalamic FSH-releasing hormone (FSH-RH) has been proposed.[29] Administration of antiserum to GnRH abolishes LH release, but has no effect upon FSH secretion.[30] Electrochemical stimulation of hypothalamic ME evokes FSH but not LH release following GnRH antagonist treatment both *in vivo* and *in vitro*.[31] In the present study, neither 15 nor 30-d thinner inhalation had any significant effect on serum FSH levels. Thus, these results provide indirect evidence for differential regulation of LH and FSH.

In conclusion, these results suggest that paint thinner has an anti-gonadotropic effect. It would appear that the hypothalamic catecholaminergic system is not involved in thinner inhibition of LH and testosterone secretion. Its effects may be exerted by direct action on the GnRH neurons or by reducing the pituitary responsiveness to GnRH. Voluntary or involuntary exposure to paint thinner may cause long-term disturbances in the male reproductive axis.

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