Bull. Environ. Contam. Toxicol. (1991) 46:92–99
© 1991 Springer-Verlag New York Inc.

Environmental Contamination and Toxicology

Reversibility of the Inhibitory Effect of Atrazine and Lindane on Cytosol 5α-Dihydrotestosterone Receptor Complex Formation in Rat Prostate

Branimir Šimić,[1] Zlatko Kniewald,[1] John E. Davies,[2] and Jasna Kniewald[1]

[1]Faculty of Food Technology and Biotechnology, University of Zagreb, 41000 Zagreb, Yugoslavia and [2]School of Medicine, University of Miami, Miami, Florida 33176, USA

The widespread use of pesticides produces residues in meat, dairy products, eggs and feed crops, which have become a matter of concern in respect to human health (Akhtar, 1985). Once entering the bloodstream, most toxic substances, including pesticides, can reach organs involved in the reproductive system. They can cross the placenta, as well as the brain barrier, posing various risks to the reproductive processes (Šimić and Kniewald, 1980).

It is generally accepted that testosterone must be converted into 5α-reduced metabolites in its target structures to become fully active. One of the steps which leads to the androgen effect is the formation of the androgen-active form: the specific receptor complex (DeMoor et al., 1975). 5α-Dihydrotestosterone has been identified as a major testosterone metabolite in the rat prostate (Bruchovsky and Wilson, 1968). The presence of its specific receptors in prostate cytosol has also been established (Mainwaring, 1969).The organochlorine insecticide lindane and the s-triazine herbicide atrazine produce changes in hormone-dependent reactions in the rat hypothalamus (Kniewald et al., 1980), anterior pituitary (Kniewald et al., 1987) and prostate (Kniewald et al.; 1980; Kniewald et al., 1987; Kniewald et al., 1989). Lindane also causes histological and biochemical alterations in the rat testis (Dikshith et al., 1978; Srinivasan et al., 1988). In vivo treatment with atrazine produces a markedly inhibitory influence on 5α-dihydrotestosterone - receptor complex formation in rat prostate cytosol (Kniewald et al., 1987; Kniewald et al., 1989). Therefore, the aim of this study was to investigate wheather such changes in this crucial step in the reproductive process are reversible. A parallel investigation using lindane was also undertaken.

Send reprint requests to J. Kniewald at the above address.

MATERIALS AND METHODS

/1,2,4,5,6,7-^{3}H(N)/-Dihydrotestosterone (^{3}H-DHT; sp. act. 5.217 TBq/mmol) was obtained from New England Nuclear and used without further purification. Atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine), purchased from Radonja, Sisak, Yugoslavia, was purified by recrystallization and TLC before use. All other chemicals were analytical grade commercial preparations. Lindane (γ-hexachlorocyclohexane) was purchased from Sigma Chemist GmbH, and was used without further purification. The buffer used was TEDG (Tris 0.05 mol/l, EDTA 0,0015 mol/l, dithiothreitol 0.0015 mol/l, containing 20% glycerol and adjusted to pH 7.4).The dextran-coated charcoal (DCC) suspension contained activated charcoal Norit A (1.0%, w/v) and dextran T_{60} (0.1%, w/v) in a TEDG buffer.

Fischer strain male rats (28 and 90 days old) were caged with food and water ad libitum on a lighting schedule of 12 h light : 12 h darkness. Treatment consisted of daily per oral intubation of the animals with the atrazine or lindane suspensions in paraffin oil. The atrazine dose was 12 mg/100 g bw and the lindane dose was 6 mg/100g bw daily for 7 days. Control groups only received the same volume of paraffin oil. At the end of the treatment period, the animals were divided into the groups and left from 1 to 22 days in ad libitum food and water conditions. The rats were then sacrificed by decapitation. Each treated group (from 8 to 12 rats depending on the age) had a corresponding control group.

After the sacrifice of the animals, the prostate glands were dissected free of adhering fat, homogenized as a pool of glands in TEDG buffer using a glass-Teflon tissue homogenizer (Kontes Glass Co., N.J.), and the homogenate was centrifuged at 105,000 g for 60 min at 4 ^{o}C. A supernatant with a protein concentration of approximately 2.5 mg/ml was used for the further determination of DHT receptor. Cytosol (200 µl) was incubated with 25 µl ^{3}H-DHT (concentrations from 1 to 9 nM) for 20 h at 4 ^{o}C. Parallel incubations contained a 200-fold excess of unlabeled DHT as the competitor. Bound ligand was determined by exposing the incubates to 500 µl of the DCC suspension in order to strip the unbound steroid. The mixture was agitated vigorously for 30 min at 4 ^{o}C, and then centrifuged at 1,100 g for 15 min. An aliqoute of the supernatant was taken for determining the radioactivity count in the liquid scintillation counter. The results were evaluated by Scatchard analysis (Scatchard, 1969).

RESULTS AND DISCUSSION

Tables 1 and 2 summarize the results of atrazine influence (dose of atrazine: 12 mg/100 g bw daily for 7 days) on the DHT-receptor complex formation in the rat prostate cytosol in young and adult rats during 22 and 14 day periods following herbicide exposure. Using Scatchard analysis, the apparent K_d values and concentrations of binding sites ("n") for ^{3}H-DHT receptors in the prostate cytosol were determined. Following the termination of the atrazine treatment (values for the first day after treatment in Tables 1 and 2), the number of binding sites on the DHT-receptor molecules were significantly decreased ($p<0.001$) in both cases compared to each corresponding control value. Three and seven days following the termination of treatment (Table 1), there were still significant decreases of "n" ($p<0.001$) in the young rat prostate cytosol. Three, seven and nine days following the termination of the treatment of adult rats with atrazine (Table 2), also a significant decreases ($p<0.001$ and $p<0.01$) of "n" were determined. Differing from adult rats, 14 days after the termination of atrazine treatment, young rats still exhibited a detectable and significant ($p<0.01$) change in the number of DHT binding sites in the prostate cytosol (Table 1). There was no significant change in "n" on the 14th day after the cessation of the treatment of adult rats (Table 2), and on the 22nd day after the cessation of the treatment of young rats (Table 1).

The reversibility of the inhibitory effects of atrazine on DHT-receptor complex formation in the prostate cytosol of 28 and 90-day-old rats is schematically presented in Figure 1A. For the control groups, "n" is expressed as a constant value, i.e. as 100% of the possible DHT-receptor complex formation determined under normal physiological and experimental conditions. The corresponding data for the treated groups of animals, sacrificed on different days after the cessation of treatment, are presented as percentage of determined values in relation to the controls.

According to the experimental results presented, it is evident that treatment with atrazine produces (Figure 1A) a stronger inhibitory influence upon young animals (treatment initiated at a prepubertal age) than on adult, sexually mature rats (90 days old). The inhibition of DHT-receptor complex formation on the 1st and the 3rd days after the cessation of treatment with atrazine was 34.6% and 35.0% in the rat prostate cytosol of young rats, and 31.7% and 18.9% in adult rats, as compared to control values. A similar rate of inhibition was determined until the 7th day after

Table 1. Apparent K_d values and concentrations of binding sites (n) for ^{3}H-dihydrotestosterone receptors in prostate cytosol after atrazine treatment of 28-day-old rats.

Treatment		Days after treatment	K_d ($Mx10^{-9}$)	n (fmol/mg of protein)
CONTROL	(31)		2.5±0.24	26.3±0.34
ATRAZINE	(6)	1	1.9±0.54	17.2±0.70[a]
12 mg/100 g bw	(4)	3	2.4±1.01	17.1±1.07[a]
daily for	(6)	7	2.9±0.76	18.6±0.53[a]
7 days	(6)	14	2.9±0.69	24.0±0.70[b]
	(4)	22	1.9±0.81	27.4±1.81

( ) Number of determinations.
[a]$p<0.001$; [b]$p<0.01$ - vs control
Statistical evaluation was performed using Student's t-test

Table 2. Apparent $K_d$values and concentrations of binding sites (n) for ^{3}H-dihydrotestosterone receptors in prostate cytosol after atrazine treatment of 90-day-old rats.

Treatment		Days after treatment	K_d ($Mx10^{-9}$)	n (fmol/mg of protein)
CONTROL	(30)		2.7±0.29	34.4±1.05
ATRAZINE	(4)	1	5.4±1.40	23.5±0.67[a]
12 mg/100 g bw	(3)	3	2.0±0.70	27.9±0.54[a]
daily for	(5)	7	3.8±0.67	28.9±0.85[a]
7 days	(4)	9	3.0±0.63	30.7±0.32[b]
	(4)	14	1.9±0.87	34.5±0.16

( ) Number of determinations
[a]$p<0.001$; [b]$p<0.01$ - vs control
Statistical evaluation was performed using Student's t-test

the cessation of the treatment of young rats as on the 1st and the 3rd days. Between the 7th and the 14th days, the rate of inhibition decreased to 8.7% vs. control. On the 22nd day after the cessation of the treatment of young rats with atrazine, an inhibitory effect was not detected. The number of binding sites reached the control values (Table 1 and Figure 1A). In adult rats, the inhibitory effect of atrazine was expressed by the degree of

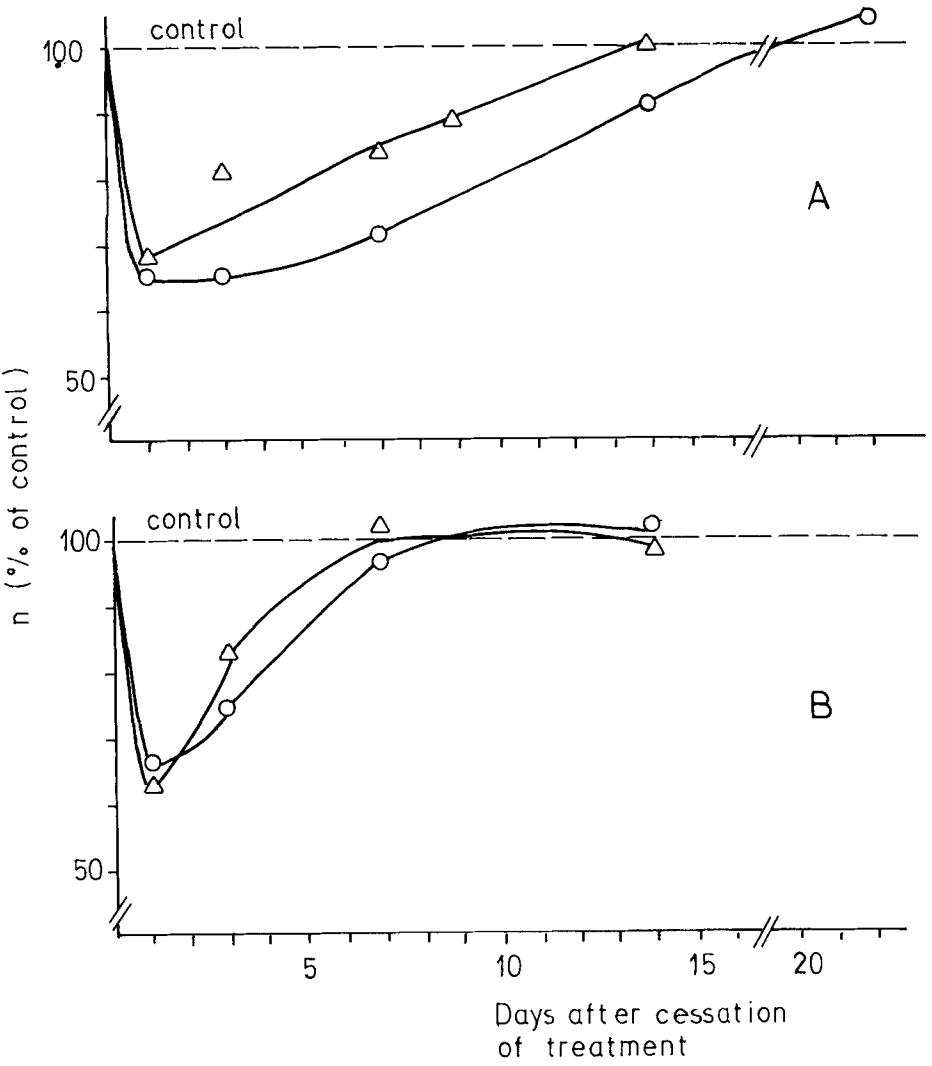

Figure 1. Effects of atrazine (A) and lindane (B) on the DHT-receptor complex formation in the prostate cytosol of 28-day-old rats (○–○) and 90-day-old rats (△–△). Rats were treated with 12 mg of atrazine, or 6 mg of lindane/100 g bw daily, for 7 days. The number of binding sites (n) was determined after the cessation of treatment, and expressed as a % of the corresponding control values.

18.9% as early as the 3rd day after treatment, and decreased until the 9th day to 10.8%. On the 14th day after the cessation of the treatment of adult rats with atrazine, no significant change in "n" was found (Table 2 and Figure 1A).

Tables 3 and 4 summarize the results of lindane influence (lindane dose: 6 mg/100 g bw daily for 7 days) on the DHT-receptor complex

Table 3. Apparent K_d values and concentrations of binding sites (n) for ^{3}H-dihydrotestosterone receptors in prostate cytosol after lindane treatment of 28-day-old rats.

Treatment		Days after treatment	K_d ($Mx10^{-9}$)	n (fmol/mg of protein)
CONTROL	(31)		2.5±0.24	26.3±0.34
LINDANE	(6)	1	2.2±0.99	17.2±0.85 [a]
6 mg/100 g bw	(3)	3	0.7±0.17 [b]	19.5±0.73 [a]
daily for	(6)	7	1.8±0.19 [c]	25.3±0.67
7 days	(4)	14	1.9±0.30	26.7±0.60

( ) Number of determinations.
[a] $p<0.001$; [b] $p<0.01$; [c] $p<0.05$ - vs control. Statistical evaluation was performed using Student's t-test.

Table 4. Apparent K_d values and concentrations of binding sites (n) for ^{3}H-dihydrotestosterone receptors in prostate cytosol after lindane treatment of 90-day-old rats.

Treatment		Days after treatment	K_d ($Mx10^{-9}$)	n (fmol/mg of protein)
CONTROL	(31)		2.7±0.29	34.3±1.05
LINDANE	(8)	1	2.2±0.56	21.8±0.76 [a]
6 mg/100 g bw	(3)	3	2.1±0.54	28.6±1.10 [b]
daily for	(6)	7	2.1±0.48	35.1±1.23
7 days	(3)	14	4.7±0.45	34.1±0.61

( ) Number of determinations.
[a] $p<0.001$; [b] $p<0.01$ - vs control. Statistical evaluation was performed using Student's t-test.

formation in rat prostate cytosol in young and adult rats, respectively, during the 14 day period after exposure to the insecticide. On the 1st day after the cessation of the treatment of young rats, 34.6% ($p<0.001$) inhibition was detected, and in adult rats, the degree of inhibition was 36.6% ($p<0.01$). Three days after the termination of treatment with lindane, marked inhibition of DHT-receptor complex formation was still detected - 25.9% in young rats ($p<0.001$), and 16.9% ($p<0.01$) in adults. Seven days after the cessation of treatment with lindane, no detectable change in the number of specific binding sites for DHT in the prostate cytosol was found, either in young rats or adults. A schematic presentation of the effects of lindane on the DHT-receptor complex formation in the rat prostate cytosol is given in Figure 1B.

It can be concluded that both atrazine and lindane interfere with the physiological process of specific DHT-receptor complex formation in the rat prostate cytosol, but that the effects are reversible. The process was restored after 22 days in young rats and after 14 days in adults following the cessation of atrazine influence, while following lindane influence, restoration was complete after 7 days for both age groups. The same rate of inhibition was detected on the 1st day after the cessation of the treatment of young rats, either with atrazine or lindane, despite the great difference in the toxicities of the applied pesticides (lindane - group II, and atrazine - group IV of toxicity) and the relations to the applied doses. The atrazine or lindane doses used in the present study can occur in some accidental situations or through the continuous use of pesticides, e.g. field or storage exposure without adequate protection. The differences between the inhibitory rates exerted after the treatments with atrazine or lindane could be partially explained by the intrinsic chemical characteristics of the pesticide molecules. Atrazine treatment exerted a stronger effect than lindane. The explanation could be in part, that besides lipophilic properties, lindane also possesses a certain hydrophilic property, which may be responsible for its faster absorption, metabolism, excretion and lower fat accumulation (Akhtar, 1985) than that of atrazine.

Acknowledgments. This study was supported in part by the Self-Management community of Interest for Scientific Research of SR Croatia and the Yugoslav-USA Joint Fund for S&T Cooperation, Grants No PN 649 and PN 873.

REFERENCES

Akhtar MH (1985) The fate of insecticides in economic animals. In: Hutson DH, Roberts TR (eds) Progress in Pesticide Biochemistry and Toxicology, vol. 4. John Wiley & Sons Ltd., (1985) p 219

Bruchovsky N, Wilson JD (1968) The conversion of testosterone to 5α-andostan-17β-ol-3-one by rat prostate in vivo and in vitro. J Biol Chem 243:2012-2021

Dikshith TSS, Tandon SK, Datta KK, Gupta PK, Behari JR (1978) Comparative response of male rats to parathion and lindane: Histopathological and biochemical studies. Environ Res 17:1-9

DeMoor P, Verhoeven G, Heyns W (1975) A comparative study of the androgen receptor apparatus in adult rodents. J Steroid Biochem 6:301-309

Kniewald J, Mildner P, Kniewald Z (1980) Effects of s-triazine herbicides on 5α-dihydrotestosterone receptor complex formation in hypothalamus and ventral prostate. In: Genazzani E, DiCarlo F, Mainwaring WIP (eds) Pharmacological Modulation of Steroid Action, Raven Press, New York, p 159

Kniewald J, Peruzović M, Gojmerac T, Milković K, Kniewald Z (1987) Indirect influence of s-triazines on rat gonadotropic mechanism at early postnatal period. J Steroid Biochem 27: 1095-1100

Kniewald J, Gojmerac T, Šimić B, Kniewald Z (1989) Biodegradation of atrazine and the impact on reproductive system in mammals and avians - comparative study. In: Lokke H, Tyle H, Bro-Rasmussen F (eds) Proceedings of the 1st European Conference on Ecotoxicology, The Technical University of Denmark, Lyngby, p 93

Mainwaring WIP (1969) A soluble androgen receptor in the cytoplasm of rat prostate. J Endocr 45:531-541

Scatchard G (1969) The attractions of proteins for small ions and molecules. Ann N Y Acad Sci 51:660-672

Srinavasan K, Ramesh HP, Radhakrishnamurty R (1988) Changes induced by hexachlorocyclohexane isomers in rat liver and testis. Bull Environ Contam Toxicol 41:531-539

Šimić B, Kniewald J (1980) Effects of pesticides on the reproductive system. Acta Pharm Jugoslav 30:59-72

Received March 5,1990; Accepted June 5,1990.