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EFFECTS OF LINDANE ON TESTOSTERONE METABOLISM IN NEUROENDOCRINE ORGANS OF MALE RAT

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Experiments were performed in male rats to study the influence of lindane on the activities of hypothalamic and pituitary enzymic systems (5α-R, 3α- and 17β-HSD) involved in testosterone metabolism. Lindane at a concentration of 0.17 .... 0.51 μmol significantly inhibited the activity of 5α-R (up to 48%), 3α-HSD (up to 41%) and 17β-HSD (up to 29%) in the anterior pituitary. At hypothalamic level the same concentrations of lindane inhibited 5α-R activity (up to 39%), while 3α- and 17β-HSD activities were not affected. The ability of lindane to inhibit the 5α-R, 3α- and 17β-HSD activities at neuroendocrine level suggest that its constant presence at a concentration likely to be found in the organism could have an impact on the reproductive system.

There exists an extensive evidence that most of the population, as well as domestic and wild animals, continuously ingest small amounts of chlorinated hydrocarbon insecticides. Because of their high liposubility they could be deposited in the body tissues and the fat. Data on human and animal body burdens of insecticides have led to the investigations of their possible influence on the mammalian endocrine system and consequently on reproduction (1, 2). The toxicology of chlorinated hydrocarbon insecticides, including lindane, has been well reviewed (3, 4), and some of their effects on the hormone-dependent organs have been reported (5, 6).

The aim of this study was to examine the influence of lindane on the activities of rat hypothalamic and pituitary enzymic systems involved in testosterone metabolism, and thereby in the regulation of the crucial physiological events in the reproductive system.

Most androgenic effects of testosterone at neuroendocrine level are accepted to be mediated by its 5α-reduced metabolite 5α-DHT (7, 8, 9). Testosterone is not only a substrate for 5α-R, it undergoes conversion to the other metabolites under the influence of 3α- and 17β-HSD activities in neuroendocrine organs and gonads (9, 10). The conversions to 5α-DHT, to 3α-Diol and to Dione are important for the regulation of the hormone-dependent physiological events in the organism and for the maintenance of regular hormonal status (11), which often can be modified by exogenous factors.

MATERIALS AND METHODS

Chemicals

Lindane (purity 99.5%) was purchased from Chromos, Zagreb, and was used without further purification.

/4-^{14}C/-Testosterone (specific activity 11.988 GBq/mmol) was obtained from the Radiochemical Centre, Amersham, U.K. and purified by thin layer chromatography (t.l.c.) before use. Unlabeled testosterone, 5α-DHT, 3α-Diol, Dione and 5α-Dione were purchased from Steraloids Inc., Pawling, N. Y., U.S.A.

All other chemicals were analytical grade commercial preparations. The water used for preparing the buffer solutions was demineralized and glass-distilled.

Animals and tissues

Male rats (Wistar strain) aged 28 and 90 days were kept 12 h in a light and 12 h in a dark environment, in temperature and humidity controlled rooms, and received food and water *ad libitum*. Under ether anesthesia, the rats (90 days old) were bilaterally orchidectomized by the scrotal route 48 h before experiments.

Animals were killed by decapitation, and the hypothalamic and anterior pituitary tissues were removed immediately. The hypothalamic tissue was cut out as a block (19.8 ± 0.81 mg from 28-day-old rats and 25.0 ± 1.14 mg from 90-day-old rats) limited anteriorly by the optic chiasma, laterally by the hypothalamic fissures and posteriorly by the mammillary body. The depth of the section was approximately 2.5 mm from the basal surface. The weights of anterior pituitaries (whole glands) were 4.5 ± 0.24 mg from 28-day-old rats, and 7.5 ± 0.16 mg from 90-day-old rats.

Incubation procedure

Tissue samples were immersed in 2 ml of glucose Krebs-Ringer solution, pH 7.4, containing 1.332 kBq /4-^{14}C/-testosterone (0.59 nmol) and various amounts of lindane (0.17 — 0.51 μmol). Incubation was carried out

for 3 h in an atmosphere of 95% O_2 and 5% CO_2 under continuous agitation, and it was terminated with 10 ml chloroform-methanol (2:1 v/v). The upper aqueous phase was separated and the organic phase dried in a stream of nitrogen. The aqueous phase did not contain any significant radioactivity. The dry residue was dissolved in ether and transferred to plates for t.l.c. on silica gel GF-254 (Kemika, Zagreb). The plates were first developed in n-heptane to remove impurities. Testosterone metabolites were separated by using the solvent system chloroform-acetone-n-heptane (4:1:3, by vol.) as described earlier (12). The fractions were identified by autoradiography, after covering the t.l.c. plates with X-ray film (Sanix, Fotokemika, Zagreb) for 7 days. The identity of metabolites was confirmed as described in detail elsewhere (8).

The areas corresponding to testosterone metabolites were removed from t.l.c. plates and transferred to counting vials. After the addition of 10 ml PPO-POPOP scintillation fluid, radioactivity was counted in a Mark II Nuclear Chicago liquid scintillation counter. The counts obtained for each fraction were corrected for counting efficiency by external standardization, for recovery and sample weight.

RESULTS

The influence of lindane on the conversion of testosterone to its main metabolites in the anterior pituitary and hypothalamus is shown in Tables 1 and 2. Results are expressed as picogram of the steroid formed per milligram of wet tissue during 3 h. Testosterone was mainly converted to 5α-DHT under the influence of 5α-R activity and further on to a somewhat lower extent into 3α-Diol under the influence of 3α-HSD activity in both tissues.

Table 1 shows that the anterior pituitary of male rats (28 days old or 90 days old castrated 48 h earlier) converted testosterone to its metabolites to a higher degree than the hypothalamic tissue (Table 2). The addition of lindane in the incubation medium significantly inhibited the pituitary 5α-R, 3α- and 17β-HSD activities (Table 1). The degree of inhibition was for 5α-DHT formation up to 48%, for 3α-Diol up to 20% for Dione up to 29% in 90-day-old rats. In the anterior pituitary of 28-day-old rats the formation of 5α- and 3α-reduced metabolites was strongly suppressed.

The results of lindane influence at hypothalamic level (Table 2) show a significant inhibition of 5α-DHT formation at the highest insecticide concentration (about 31% in 28-day-old rats and 34% in 90-day-old rats). The inhibition occurred even after the addition of the lowest amount of lindane to the incubates (18% and 9%). The formation of 3α-Diol was not affected significantly in the presence of lindane. The conversion of testosterone to Dione and further to 5α-Dione was at a rather low level,

and was not affected by the addition of the insecticide in the incubation medium (Table 2). The results suggest that lindane interferes with the activity of 5α-R, but not with 3α- and 17β-HSD enzyme systems in the hypothalamus of male rats.

Table 1

Conversion of (4-^{14}C)-testosterone to the metabolites in male rat anterior pituitary incubates after the addition of lindane

Age (days)		Addition of lindane (μmol)	pg of steroid / mg wet tissue			
			3α-Diol	5α-DHT	Dione	5α-Dione
28	(4)	none (control)	704 ± 24.9	830 ± 11.0	213 ± 6.5	184 ± 17.6
		0.17	640 ± 20.5	640 ± 12.0[a]	182 ± 4.4[a]	115 ± 15.5[c]
		0.34	527 ± 18.5[a]	558 ± 10.7[a]	179 ± 4.4[a]	113 ± 12.6[b]
		0.51	412 ± 21.6[a]	514 ± 14.6[a]	204 ± 6.7	135 ± 18.2
90 castrated 48 h earlier	(3)	none (control)	602 ± 18.6	795 ± 9.1	252 ± 3.3	97 ± 11.3
		0.17	556 ± 16.6	467 ± 4.9[a]	190 ± 12.8[a]	91 ± 5.1
		0.34	576 ± 42.9	463 ± 7.0[a]	206 ± 7.5[a]	93 ± 6.4
		0.51	480 ± 21.7[b]	414 ± 5.0[a]	177 ± 7.3[a]	102 ± 10.4

Values are mean ± S.E.; () = number of samples.
Statistical evaluation was done with the Student's t-test. Significant difference compared to control: [a]$P<0.01$; [b]$P<0.02$; [c]$P<0.05$.

Table 2

Conversion of (4-^{14}C)-testosterone to the metabolites in male rat hypothalamic incubates after the addition of lindane

Age (days)		Addition of lindane (μmol)	pg of steroid / mg wet tissue			
			3α-Diol	5α-DHT	Dione	5α-Dione
28	(4)	none (control)	93 ± 2.7	179 ± 12.0	44 ± 8.2	31 ± 7.1
		0.17	86 ± 2.8	146 ± 5.3[b]	51 ± 2.9	42 ± 10.0
		0.34	82 ± 2.5[c]	142 ± 5.8[b]	63 ± 10.2	46 ± 10.2
		0.51	86 ± 2.4	124 ± 9.5[a]	44 ± 6.6	38 ± 8.4
90 castrated 48 h earlier	(3)	none (control)	115 ± 12.0	184 ± 8.9	40 ± 5.5	13 ± 2.0
		0.17	95 ± 6.9	168 ± 21.0	44 ± 4.2	18 ± 2.7
		0.34	86 ± 8.4	111 ± 6.4[a]	38 ± 3.1	18 ± 2.2
		0.51	102 ± 8.6	122 ± 3.3[a]	38 ± 3.3	22 ± 5.3

Values are mean ± S.E.; () = number of samples.
Statistical evaluation was done with the Student's t-test. Significant difference compared to control: [a]$P<0.01$; [b]$P<0.02$; [c]$P<0.05$.

DISCUSSION

Many studies have been concerned with establishing body burdens of organochlorine pesticides in mammals, and their influence upon the endocrine system has been also investigated (1, 2, 5, 6).

The results of this investigation indicate that in male rats lindane, an γ-isomer of hexachlorocyclohexane, significantly inhibits the 5α-R activity involved in androgen metabolism at anterior pituitary and hypothalamic levels. Also, it supresses the activities of 3α- and 17β-HSD in the anterior pituitary independently of the age of animal.

There is evidence (13) that lindane inhibits the activities of liver microsomal drug metabolizing enzymes including the steroid hydroxylase activities in the rabbit. Although no evidence of any teratogenic effect of lindane administered during pregnancy to rabbits and rats has been reported (14), the present paper shows that lindane inhibits the activities of enzymes included in the androgen metabolism in rat hypothalamus and pituitary. It is well established that the conversions of testosterone to 5α-DHT and 3α-Diol are essential premises for androgenic actions in the neuroendocrine organs (11, 12). The ability of lindane to inhibit the 5α-R, 3α- and 17β-HSD activities suggests that its constant presence in the organism could have an impact on the reproductive system.

The present evidence concerning the influence of lindane on the mammalian endocrine system (4, 14), including the mode of action in the central nervous system (15), together with the present results of lindane influence on the enzymic systems responsible for androgen action at the neuroendocrine level, should suggest an extensive interest for investigations of such pollutant. The results obtained may stimulate further research for the sake of better understanding of a possible mechanism of lindane action in humans.

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Abbreviations:

lindane, γ-hexachlorocyclohexane; 5α-R, 5α-reductase (EC 1.3.99.5); 3α-HSD, 3α-hydroxysteroid dehydrogenase (EC 1.1.1.50); 17β-HSD, 17β-hydroxysteroid dehydrogenase (EC 1.1.1.63); testosterone, 17β-hydroxyandrost-4-ene-3-one; 5α-DHT (5α-dihydrotestosterone), 17β-hydroxy-5α-androstan-3-one; 3α-Diol, 3α,17β-dihydroxy-5α-androstane; Dione, androst-4-ene-3,17-dione; 5α-Dione, 5α-androstan-3,17-dione

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Sažetak

UTJECAJ LINDANA NA METABOLIZAM TESTOSTERONA U NEUROENDOKRINIM ORGANIMA ŠTAKORA

Djelovanje lindana na aktivnost enzimskih sistema (5α-R, 3α- i 17β-HSD) uključenih u metabolizam testosterona na nivou hipotalamusa i hipofize mužjaka štakora, proučavano je u *in vitro* pokusima. U hipofizi lindan inhibira aktivnost 5α-R — do 48%, aktivnost 3α-HSD do 41% i 17β-HSD do 29%. Na nivou hipotalamusa lindan inhibira 39% aktivnosti 5α-R, dok aktivnosti 3α- i 17β-HSD nisu bile promijenjene. Mogućnost da lindan inhibira enzimske aktivnosti uključene u metabolizam testosterona u neuroendokrinim organima (hipotalamus i hipofiza), pokazuje da prisutnost tog pesticida u organizmu može uzrokovati promjene u biokemijskim procesima odgovornim za proces reprodukcije.

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