Bull. Environ. Contam. Toxicol. (1987) 38:911-916

Environmental Contamination and Toxicology

Lindane Effects on Testosterone Metabolism in Neuroendocrine Organs of Cockerel and Female Turkey

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The use of lindane (.-hexachlorocyclohexane) in agriculture without strict adherence to legislative end manufacturer's recommendations leads to its presence in raw and industrial food products. Ingesting such food makes possible lindane accumulation in tissues with high lipid content, including the brain (Vohland et al. 1981). The permanent presence of subtoxic doses of lindane is responsible for its long-lasting effect on various physiological systems in organisms, including the system for the regulation of hypothalamo-pituitary-gonadal axis.

Lindane decreases the egg-laying capacity of hens (Kosutzky et al. 1980) and reduces the number of good quality chicks hatched from fertile eggs (Kan and Jonker-den Rooyen 1978). Its possible adverse effects on the reproductive system in mammals were demonstrated through changes provoked in the metabolism of androgens in the neuroendocrine organs (Šimić and Kniewald 1985).

The aim of this work was to investigate **in vitro** the effect of lindane on the activity of enzymes responsible for testosterone metabolism in the pituitary and hypothalamus in birds.

MATERIALS AND METHODS

[4-^{14}C]-Testosterone (specific activity 59 mCi/mmol) was obtained from Radiochemical Centre Amersham, Bucks, U.K. It was purified before use by thin-layer chromatography. Unlabelled steroids were purchased from Sigma Chemical Co., St. Louis, Mo, USA. Lindane (purity 99.5%) was purchased from Chromos, Zagreb, Yugoslavia, and was used without further purification. All other chemicals were analytical grade commercial preparations.

The birds used in this study were cockerels of White Leghorn strain and female domestic turkeys of Nicholas strain. They were caged with food and water available at all times on the lighting schedule of 12 h light: 12 h darkness. Cockerels, aged 7 weeks,

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and female turkeys, aged 16 weeks, were killed by decapitation. The pituitaries and hypothalami (including the basal and preoptic regions) were removed immediately. After dissection, the tissues were separately collected and thoroughly rinsed in an ice-cold glucose Krebs-Ringer solution to remove blood. Tissue samples were prepared for incubation by cutting into small pieces and immersing in 2 ml of glucose Krebs-Ringer solution, pH 7.4, containing 35.96 nCi [4-^{14}C]-testosterone (about 0.59 nmol) and various amounts of lindane (0.344 - 0.516 μmol). Incubation procedure, isolation, identification and quantification of testosterone metabolites were performed according to the method described in detail elsewhere (Kniewald et al. 1984).

RESULTS AND DISCUSSION

The lindane effect on testosterone metabolism **in vitro** in the cockerel pituitary and hypothalamus is shown in Table 1. Results are expressed as pg of metabolite formed per mg of wet tissue for 3 h of incubation at 37 °C.

In the pituitary incubates 5β-androstane-3α,17β-diol (5β-diol), as the product of 5β-reductase (5β-R; EC 1.3.99.6) and 3α-hydroxysteroid dehydrogenase (3α-HSD; EC 1.1.1.50) activities, was formed in the largest amount (1297 ± 140.6 pg/mg). The amount of 5β-dihydrotestosterone (5β-DHT) which was formed from testosterone as a result of 5β-R activity was smaller (463 ± 33.7 pg/mg). The lowest activity was expressed by 17β-hydroxysteroid dehydrogenase (17β-HDS; EC 1.1.1.63), which was responsible for testosterone conversion to androst-4-ene-3,17-dione (androstenedione). 5α-Reductase (5α-R; EC 1.3.99.5) activity was not detected.

The addition of lindane (0.344 and 0.516 μmol) to pituitary incubates inhibited 5β-R activity up to 43% ($p<0.001$). 3α-HSD activity decreased to 49% ($p<0.05$) only in the presence of the lower lindane concentration, while 17β-HSD activity increased, but not significantly.

In the cockerel hypothalamus, the dominant enzymatic activities were shown by 5β-R and 3α-HSD, 5α-R activity was low, and 17β-HSD activity was not detected. The presence of lindane (0.344 and 0.516 μmol) in the hypothalamic incubates inhibited 3α-HSD activity up to 45% ($p<0.001$). 5β-R activity was inhibited up to 46% ($p<0.001$) only with the lower lindane concentration, whereas 5α-R activity remained within the range of control values.

Testosterone metabolism in the female turkey pituitary and hypothalamus in the presence of lindane is shown in Table 2. In the pituitary gland, 5α-R and 17β-HSD activities were demonstrated with the formation of 5α-dihydrotestosterone (5α-DHT) and androstenedione (91 ± 6.2 and 155 ± 14.8 pg/mg, respectively). 5β-DHT was not detected, but the conversion of androstenedione to 5β-androstane-3,17-dione (5β-dione) occurred. The presence of 0.344 μmol of lindane in the incubates increased 5α-R activity up to 95% ($p<0.01$) and 17β-HSD activity up to 68% ($p<0.05$). The

Table 1. [4-^{14}C]- Testosterone conversion to the metabolites in cockerel pituitary and hypothalamic incubates after the addition of lindane

Addition of lindane (µmol)	Tissue wet weight (mg)	pg of steroid / mg wet tissue			
		5β-androstane-3α,17β-diol	5β-dihydro-testosterone	5α-dihydro-testosterone	androst-4-ene-3,17-dione
Pituitary					
control	7.8 ± 0.37 (13)	1297 ±140.6	463 ± 33.7	none	279± 24.6
0.344	8.3 ± 0.18 (6)	624 ± 77.0[b]	263 ± 10.8[a]	none	314± 25.5
0.516	6.5 ± 0.34 (5)	919 ±119.8	210 ± 20.6[a]	none	385± 69.3
Hypothalamus					
control	25.7 ± 2.83 (13)	1001 ± 52.9	700 ± 59.6	97 ±10.2	none
0.344	22.1 ± 1.48 (7)	551 ± 33.9[a]	376 ± 21.7[a]	104 ± 5.3	none
0.516	31.5 ± 2.04 (4)	549 ± 90.1[a]	542 ± 41.4[b]	73 ±10.2	none

Values are means ± S.E.;() = number of samples. Statistical evaluation was done with Student's t-test. Significantly different from the control: a = $p<0.001$; b = $p<0.05$

Table 2. [4-^{14}C]-Testosterone conversion to the metabolites in female turkey pituitary and hypothalamic incubates after the addition of lindane

Addition of lindane (μmol)	Tissue wet weight (mg)	pg of steroid / mg wet tissue			
		5β-dihydro-testosterone	5α-dihydro-testosterone	androst-4-ene-3,17-dione	5β-androstane-3,17 -diol
Pituitary					
control	15.7 ± 1.40 (7)	none	91 ± 6.2	155 ± 14.8	19 ± 2.9
0.344	12.5 ± 1.94 (6)	none	177 ± 19.5[b]	261 ± 45.8[c]	38 ±11.7
0.516	15.2 ± 1.89 (6)	none	117 ± 17.2	152 ± 28.8	20 ± 4.9
Hypothalamus					
control	28.1 ± 2.58 (4)	457 ± 19.4	38 ± 2.5	56 ± 9.4	28 ± 3.7
0.344	27.7 ± 1.54 (6)	191 ± 15.5[a]	33 ± 2.1	39 ± 3.9[c]	11 ± 1.4[c]
0.516	29.2 ± 3.48 (5)	175 ± 11.8[a]	34 ± 1.8	52 ± 5.8	15 ± 1.6[b]

Values are means ± S.E.;() = number of samples. Statistical evaluation was done with Student's t-test. Significantly different from the control: a = $p<0.001$; b = $p<0.01$; c = $p<0.05$.

higher lindane concentration (0.516 μmol) had no major effect on either enzyme. In the hypothalamus, 5β-R activity was the most evident, while all other enzymes showed very low activity. Both lindane concentrations decreased 5β-R activity down to 42% ($p<0.001$), but the effect on other enzymes was not appreciable.

In avian species, in target tissues testosterone is predominantly metabolized through 5β-reduction pathways and only to a lower extent by 5α-reduction (Steimer and Hutchison 1980; Massa and Sharp 1981; Kniewald et al. 1984). It is converted to 5α- and 5β-DHT, and their corresponding androstanediols, mainly 3α,17β-isomers. Another metabolic pathway leads to the formation of androstenedione, and further on to corresponding androstanediones (Bottoni and Massa 1981; Massa and Sharp 1981; Kniewald et al. 1984). These enzymatic conversions are presumed to be the regulatory mechanism at the cellular level of the testosterone action on sexual differentiation and behaviour and on gonadotrophin levels (Davies et al. 1980; Bottoni and Massa 1981; Massa and Sharp 1981; Balthazart and Ottinger 1984). It seems that 5β-reduction regulates the conversion of testosterone into the active metabolites (Massa and Sharp 1981; Balthazart and Ottinger 1984; Delville et al. 1984). The changes in the activities of 5β-R and other enzymes, caused by the presence of extraneous substances (lindane in our investigation), may have major consequences on the regular physiological process of reproduction in birds.

As present results show, in the female turkey pituitary testosterone is mainly metabolized to androstenedione, and in the hypothalamus to 5β-DHT. In the cockerel pituitary, as well as in the hypothalamus, 5β-diol is formed in the greatest amount, followed by 5β-DHT. Testosterone metabolism in the female turkey and cockerel, shows some differences. The formation of 5β-DHT was not detected in the female turkey, but 5β-R activity was present to a large extent in the cockerel at pituitary level.

5β-Dione formation in the female turkey pituitary could be an indication of the existance of 5β-R which acted on androstenedione, but not on testosterone. In contrast, 5α-DHT formation, which was evident in the female turkey pituitary, was not detected in the cockerel pituitary. The only common metabolite in the pituitary of both species was androstenedione.

The presence of lindane in the cockerel pituitary incubates inhibited significantly testosterone conversion to 5β-DHT and 5β-diol. At the same time the conversion to androstenedione increased. In the female turkey pituitary only the lower lindane concentration caused major changes. It increased the formation of 5α-DHT and androstenedione.

In the hypothalamus of the cockerel and female turkey 5β-DHT formation was higher than the conversion of testosterone to 5α-DHT. In the cockerel hypothalamus testosterone conversion to 5β-diol was expressive. The amounts of the other metabolites formed in the hypothalamus of both species were smaller. The

presence of lindane in the incubates inhibited the formation of 5β-DHT and 5β-diol.

It can be concluded that the inhibition of testosterone metabolism through 5β-DHT and 5β-diol formation provides more substrate (testosterone) for the other metabolic pathway, leading to androstenedione and 5β-dione formation. This implies a hazardous effect of lindane on the hormonal balance at the neuroendocrine level in birds.

Acknowledgments. This study was supported in part by the Self-Managed Community of Interest for Scientific Research of the S.R. Croatia and by the Yugoslav - U.S. Joint Fund for Scientific and Technical Cooperation, Grant No PN 649.

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Received September 29, 1986; accepted November 24, 1986.