In Vitro Uptake of Labeled Androgens by Prostate Tissue in the Presence of Dieldrin

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Many studies have shown that certain steroidal and non-steroidal compounds can affect the in vivo and in vitro uptake of estrogens and androgens by target organs, BRUCHOVSKY and WILSON (1968), JENSEN et al. (1969), WU and BLEND (1972), THOMAS and LLOYD (1973). Diethylstilbesterol and cyproterone acetate have been shown to inhibit the in vitro uptake and retention of various androgens by prostatic tissues, WHALEN et al. (1969), LERNER (1964). It is now known that some of the chlorinated hydrocarbon insecticides have hormonal and anti-hormonal activity, ECOBICHON (1970). Recent evidence shows that DDT accumulates in the mouse prostate and inhibits the in vivo uptake of testosterone by that organ, SMITH et al. (1972). The studies described in this paper were conducted to determine if dieldrin can affect the in vitro uptake of testosterone-7-^{3}H and 5α-dihydrotestosterone-1,2-^{3}H by rat prostatic tissues.

MATERIALS AND METHODS

The in vitro culture system described by FANG et al. (1969) was used in all 10 experiments. Minimum Essential Medium 199 with Hank's Salts plus glutamine and non-essential amino acids[1] with 0.5 I.U. insulin/ml served as the basic culturing medium. Stock solutions of the following hormones were employed: insulin, 80 I.U. per ml[2]; testosterone-7-^{3}H, 1.82 µg/ml in 95% ethanol[3];

1. Grand Island Biological Company, Grand Island, New York (GIBCO).

2. Eli Lilly and Company, Indianapolis, Indiana. Iletin Insulin, 80 I.U./ml.

3. New England Nuclear Corporation, Boston, Mass. Compound NET-171, Testosterone-7-^{3}H with a specific activity of 45 C/mM and a radiochemical purity greater than 98%. Compound NET-208, Progesterone-1, 2-^{3}H with a specific activity of 40 C/mM and a radiochemical purity greater than 98%.

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and 5α-dihydrotestosterone-1,2-^{3}H,[4] 9.2 μg/ml in 95% ethanol. Recrystallized dieldrin[5] dissolved in acetone and later partitioned into 95% ethanol was added directly to the basic culture medium to make final concentrations of 8.0 X 10^{-4}, 6.0 X 10^{-3}, 0.17, 1.7, 17.0 and 170.0 μg of dieldrin/ml of culture medium (80 mμmoles to 2.2 mmoles/liter). One hundred μl of hormone and insecticide stock solutions were added to 50 ml Erlenmeyer flasks containing 3 ml of the basic culture medium and mixed for 3 hours at 37°C in a Dubnoff water bath at 75 oscillations per minute. Control flasks received 100 μl of 95% ethanol. Isotope studies indicated that greater than 99% of the hormone content and 98% of the insecticide content went into the basic culture medium during this three hour pre-incubation period.

Sections of ventral lateral prostate were obtained under aseptic conditions from 30 Carworth CFE Strain adult male rats that had been castrated 8 days earlier. Each experiment employed tissues from 3 rats. The sections of tissues which approached 1 mg wet weight were incubated with their assigned treatment drugs for 60 minutes. At the end of the incubation period, all tissues were weighed, washed three times with 10 ml of 8.5% NaCl solution and recovered by centrifugation at 1000g for 20 minutes. The washed tissues were then digested in 1 ml of Protosol Solution[6] and added to 15 ml of scintillation fluid[7]. Radiochemical assay was performed with a Packard 2000 Series Tri-Carb Liquid Scintillation Spectrometer System having a ^{3}H counting efficiency of 46%.

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4. New England Nuclear Corporation, Boston, Mass. Compound NET-302, Dihydrotestosterone-1,2-^{3}H with a specific activity of 44 C/mM and a radiochemical purity greater than 98%.

5. Recrystallized dieldrin (hexachloroepoxyoctahydro-endo-exo-dimethanonaphthalene) of greater than 99% purity was donated by the Shell Chemical Company, Princeton, New Jersey.

6. New England Nuclear Corporation, Boston, Mass. Compound NEF-935.

7. Scintillation fluid: 4 g of 2,5-diphenyloxazole and 100 mg of 1,4-bis-2-(5-Phenyloxazolyl)-Benzene dissolved in one liter of toluene.

The data were statistically analyzed for differences between group means by the analysis of variance procedure followed by the Dunnetts multiple range test, STEEL and TORRIE (1964). Since the data in Table 1 represents pooled samples, statistical analysis was not employed.

RESULTS

Table 1 illustrates the effects of 1.17, 1.70, 17.0 and 170.0 µg/ml of dieldrin on the *in vitro* uptake of testosterone-7-^{3}H by rat prostate tissue. In all cases the presence of dieldrin in the culture medium was able to decrease the incorporation of labeled testosterone by prostatic tissues. Also, as the concentration of insecticide increased in the culture medium, the degree of inhibition of androgen uptake increased indicating a dose-response relationship. The degree of inhibition ranged from as little as 4% in the case of 0.17 µg dieldrin/ml of culture medium to greater than 99% in the case of the 170.0 µg dieldrin/ml of culture medium treatment group.

TABLE 1

IN VITRO UPTAKE OF TESTOSTERONE-7-^{3}H BY RAT PROSTATE TISSUES WITH AND WITHOUT DIELDRIN

CPM/mg of Tissue

DIELDRIN CONCENTRATION (µg/ml)	Experiment Number					
	1	2	3	4	5	6
0.0	163.9[a]	215.5	378.4	156.7	252.9	648.4
0.17	154.5	200.6	344.8	147.6	267.0	625.0
1.70	123.2	186.2	250.2	106.2	200.4	570.9
17.0	109.1	---	218.3	87.4	138.8	398.8
170.0	63.7	29.9	23.6	27.9	57.1	30.8

[a]Each value represents the total amount of radioactivity from tissues in one treatment group (three separate flasks).

Table 2 illustrates the effects of 8.0×10^{-4}, 6.0×10^{-3}, 0.17, 1.70, 17.00 and 170.00 µg/ml of dieldrin on the in vitro uptake of 5∝-dihydrotestosterone-1,2-^{3}H by rat prostate tissue. In all cases the presence of dieldrin in the culture medium was able to decrease the incorporation of labeled 5∝-dihydrotestosterone by prostatic tissues. Again, as the concentration of insecticide increased in the culture medium, the degree of inhibition of androgen uptake increased indicating a dose-response relationship. Concentrations as low as 8.0×10^{-4} µg dieldrin/ml of culture medium significantly inhibited ($P < 0.05$) the uptake of labeled hormone. The inhibition ranged from 22 to 96%.

TABLE 2

IN VITRO UPTAKE OF 5∝-DIHYDROTESTOSTERONE-1,2-^{3}H BY RAT PROSTATE TISSUES WITH AND WITHOUT DIELDRIN

CPM/mg Tissue

DIELDRIN CONCENTRATION (µg/ml)	Experiment Number 7	8	9	10
0.0	411.9[a] ± 9.5	164.7 ± 7.3	213.6 ± 9.0	705.5 ±13.8
8.0×10^{-4}	329.4* ± 4.4	99.6* ± 9.5	180.1 ± 9.1	570.8* ±11.1
6.0×10^{-3}	232.7* ± 9.2	102.7* ± 9.0	128.3* ± 6.7	564.9* ±12.9
0.17	202.8* ± 7.9	88.2* ± 6.7	103.4* ± 0.6	473.5* ±13.3
1.70	213.4* ± 9.9	50.1* ± 4.6	73.7* ± 6.3	386.0* ±10.0
17.00	117.4* ± 6.7	12.6** ± 1.1	67.0* ± 4.3	292.2 ±10.5
170.00	20.4** ± 1.8	24.5** ± 2.9	9.5** ± 0.6	63.9** ± 1.7

[a]Each value represents the mean and its associated standard error of three separate flasks containing the tissues from three separate rats.

*$P < 0.05$

**$P < 0.01$

SUMMARY

These studies demonstrate that the chlorinated hydrocarbon insecticide, dieldrin, can inhibit the *in vitro* incorporation of labeled androgens by the rat prostate. These data are in substantial agreement with the work of THOMAS AND LLOYD (1973) who showed that the *in vivo* incorporation of labeled androgens by mouse prostate tissue is significantly impaired when the animals are previously exposed to dieldrin. The findings of WAKELING et al. (1972) demonstrate that dieldrin can inhibit the *in vitro* binding of 5α-dihydrotestosterone to its specific receptor protein(s) in rat prostate cytosol and are in agreement with the findings in this present study.

Hormone-responsive tissues in the male contain components which show a high affinity for testosterone and 5α-dihydrotestosterone *in vivo* and *in vitro*. These androgen-binding substances in target tissues have been called "androgen-receptors" and are responsible for the retention of male hormones in certain target tissues. This reaction appears to be essential for the proper functioning of the male reproductive system. The present data coupled with the findings of WAKELING et al. (1972) seem to indicate that dieldrin may bind or associate with the androgen receptor proteins in such a way as to decrease the uptake and retention of androgens. Studies are presently underway to determine some of the characteristics of this possible insecticide-receptor protein interaction.

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