Insecticide Inhibition of 5α-Dihydrotestosterone Binding in the Rat Ventral Prostate

Abstract. *The chlorinated hydrocarbon insecticides dieldrin and* o,p'-*DDT inhibit binding of 5α-dihydrotestosterone to specific receptor proteins in rat prostate cytosol. Dieldrin is less inhibitory than* o,p'-*DDT.*

Chlorinated hydrocarbon insecticides have hormonal and antihormonal activity (*1*). Experimental data with *o,p'*-DDT show estrogenic activity in rats (*2*) and chickens (*3*), while evidence of interrupted estrus in rats has been obtained with aldrin, another member of this insecticide family (*4*). A metabolite of *o,p'*-DDT, *o,p'*-DDD, produces atrophy of the zona fasciculata and zona reticularis of the adrenal cortex (*5*) and suppression of 17-hydroxycorticord secretion (*6*). It has been used in the treatment of adrenocortical carcinoma (*7*), Cushing's syndrome (*8*), and cancer of the breast and prostate (*9*). Administration of DDT has caused suppressed reproductive activity in male dogs (*10*) and retarded testicular growth and development of secondary sexual characteristics in young cockerels (*11*).

Recent evidence shows that DDT accumulates in the mouse prostate and inhibits uptake of testosterone by that organ (*12*). Men exposed to pesticides, including organochlorines, showed depression in sexual function which returned to normal after cessation of exposure and treatment with testosterone (*13*). Studies in our laboratory have shown that dieldrin antagonizes the reduction in prostatic secretion of dogs fed the antiandrogenic compound, chlormadinone acetate (6-chloro-17α-hydroxypregna-4,6-diene-3,20-dione-17-acetate) (*14*) and inhibits binding of 5α-androstan-17β-ol-3-one (5α-DHT) in the rat prostate cytosol and nucleus in vitro (*15*).

Target organs for sex steroid hormones are distinguished by receptor proteins which bind appropriate hormones with a high affinity and specificity. Several authors (*16–18*) have reported that 5α-DHT associates with specific receptor proteins in the rat prostate cytosol and nucleus. Our studies show that dieldrin and *o,p'*-DDT can directly affect the binding of 5α-DHT to these specific receptor proteins in rat prostate cytosol.

The presence of a 3.5*S* receptor protein for 5α-[^{3}H]DHT in rat prostate cytosol incubated in the presence of 0.4*M* KCl, is demonstrated in Fig. 1A. Cytosols incubated under the same conditions, with [^{14}C]dieldrin or *o,p'*-[^{14}C]-DDT also showed (Fig. 1A) a binding peak sedimenting at the same distance from the origin as 5α-[^{3}H]DHT. Polyacrylamide gel electrophoresis of similarly labeled cytosols showed that radioactivity of 5α-[^{3}H]DHT, [^{14}C]dieldrin, or *o,p'*-[^{14}C]DDT migrates an identical distance on the gels. Dieldrin and *o,p'*-DDT strongly inhibit binding of 5α-[^{3}H]DHT in the 3.5*S* region (Fig. 1B). *o,p'*-DDT reduces binding by 80 percent at a concentration of $1.4 \times 10^{-5}M$ (5 ppm), whereas the same percentage reduction in binding with dieldrin requires a concentration of 2.6 to $3.9 \times 10^{-5}M$ (10 to 15 ppm). Cyproterone acetate (1,2α-methylene-6-chloro-$\Delta^{4,6}$-pregnadien-17α-ol-3,20-dione-17α-acetate), which inhibits binding of 5α-DHT to specific receptors in the prostate (*19*), had no effect on this 3.5*S* binding. The binding was not saturated by concentrations of 5α-DHT up to $10^{-7}M$, and similar 3.5*S* binding fractions for 5α-[^{3}H]DHT were present in serum and in liver and kidney cytosols. The nonspecific 3.5*S* binding peak demonstrated under these conditions is not characteristic of the high-affinity specific hormone receptors present in the prostate.

Mainwaring (*17, 18*) has demonstrated a region of nonspecific binding (3.5*S*) and a region of specific binding (8*S*), in sucrose density-gradient analyses of prostate cytosol incubated with 5α-[^{3}H]DHT without 0.4*M* KCl. We confirmed the presence of these two binding regions in the rat prostate cytosol (Fig. 2A). Binding in the 8*S* region was abolished by parahydroxymercuribenzoate and greatly reduced by cyproterone acetate. These two

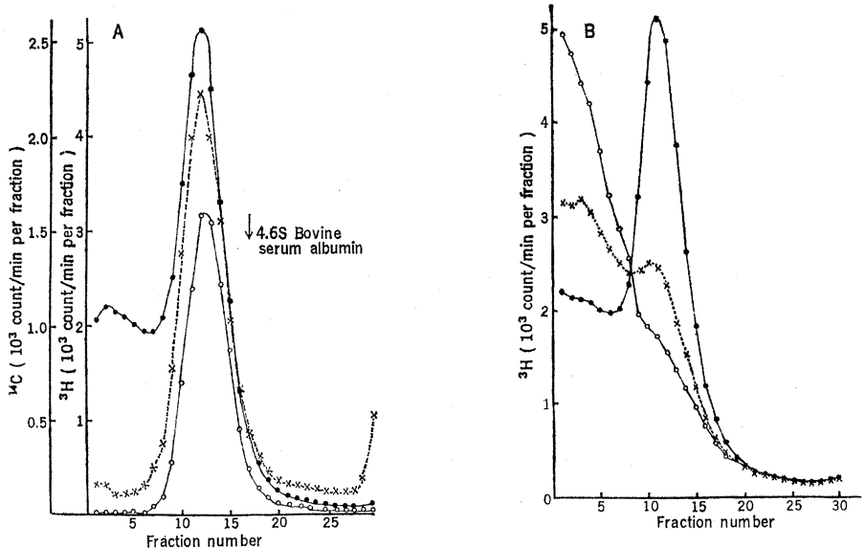

Fig. 1. (A) The 3.5*S* binding protein for 5α-[^{3}H]DHT in rat prostate cytosol. Prostates from rats castrated 24 hours previously were homogenized in three volumes of ice-cold HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, *p*H 7.4, containing 1.5 m*M* EDTA and 2.0 m*M* mercaptoethanol (HEM). The cytosol fraction, prepared by centrifugation of the homogenate at 105,000*g* for 1 hour at 0°C, was mixed with an equal volume of HEM containing 0.8*M* KCl and either 5α-[^{3}H]DHT (●), or *o,p'*-[^{14}C]DDT (X), or [^{14}C]dieldrin (○), and held overnight at −20°C. After the mixture was thawed, 0.2 ml of each cytosol was analyzed by centrifugation on a linear sucrose gradient (5 to 15 percent), made up in HEM and containing 0.4*M* KCl. Gradients were centrifuged at 45,000 rev/min for 19 hours at 0°C (SW-50.1 rotor; Beckman L2-65B centrifuge). Sedimentation is from left to right in all cases. The position of bovine serum albumin is included as a marker. (B) The effect of dieldrin and *o,p'*-DDT on the binding of 5α-[^{3}H]DHT by the 3.5*S* fraction of rat prostate cytosol. The procedure was that described above except that all samples contained 5α-[^{3}H]DHT and either 5 μl of ethanol (●), or 5 μl of ethanol containing $2.6 \times 10^{-5}M$ dieldrin (X), or 5 μl of ethanol containing $2.8 \times 10^{-5}M$ *o,p'*-DDT (○). These molar concentrations correspond to 10 ppm for the insecticides.

agents had little effect on the slower 3.5*S* peak (*20*). Designation of the 3.5*S* peak as nonspecific and of the 8*S* peak as specific binding was confirmed by the response to increasing concentrations of 5α-[^{3}H]DHT. The binding in the 3.5*S* region increased linearly with steroid concentration up to $4.3 \times 10^{-8}M$, whereas binding in the 8*S* region was saturated at $2.4 \times 10^{-8}M$ (Fig. 2A).

o,p'-DDT inhibits the prostate cytosol 8*S* specific binding of 5α-[^{3}H]DHT (Fig. 2B). At a concentration of $2.8 \times 10^{-5}M$ (10 ppm), *o,p'*-DDT inhibited binding by 60.7 ± 10.4 percent in the presence of a saturating concentration of 5α-[^{3}H]DHT. Available concentration of 8*S* receptor was 2.4 and 2.9×10^{-10} mole per gram of cytosol protein in two control experiments, and was reduced to 1.5×10^{-11} mole/g in the presence of $2.8 \times 10^{-5}M$ (10 ppm) *o,p'*-DDT. When *o,p'*-[^{14}C]DDT replaced 5α-[^{3}H]DHT in the incubation, a small peak of radioactivity was present in the 8*S* region.

Dieldrin, at concentrations as high as $2.6 \times 10^{-5}M$ (10 ppm), produced no significant inhibition of 5α-[^{3}H]-DHT binding in the 8*S* region, in the presence of a saturating concentration of 5α-[^{3}H]DHT. No binding of [^{14}C]-dieldrin in the 8*S* region was detected after incubation of prostate cytosol with [^{14}C]dieldrin. However, in studies with a constant concentration ($2.6 \times 10^{-5}M$, 10 ppm) of dieldrin and increasing concentrations of 5α-[^{3}H]-DHT the available receptor concentration was reduced to 3.1×10^{-11} mole per gram of cytosol protein compared to control.

Measurements of 5α-[^{3}H]DHT binding to the specific receptor by Agarose gel chromatography (*21*) confirmed the results of the sucrose density-gradient studies. The available receptor concentration of rat prostate cytosol measured by this technique was 1.2×10^{-10} mole per gram of cytosol protein. This value was significantly depressed by both dieldrin and *o,p'*-DDT, the inhibition again being of greater magnitude for *o,p'*-DDT. The association constant for the prostate 8*S* 5α-[^{3}H]DHT receptor was 2.0 and $1.9 \times 10^{-9}M^{-1}$ measured by sucrose density-gradient and Agarose gel chromatography, respectively. These values are comparable with previous estimates for 5α-DHT binding in rat epididymis (*22*) and for estradiol-17β binding in the uterus (*23*).

Our studies demonstrate that chlorinated hydrocarbon insecticides inhibit in vitro binding of 5α-DHT to its specific receptor proteins in rat prostate cytosol. The results are characteristic of noncompetitive inhibition and may be due to a conformational change of the receptor protein induced by the insecticides. Direct competition of *o,p'*-DDT and dieldrin for hormone binding sites is not excluded however. It is possible that the concentrations of insecticides required in the present studies relative to 5α-DHT concentration would tend to obscure such an effect.

It has been suggested that insecticides produce endocrine responses in mammals by induction of liver microsomal steroid hydroxylase enzymes (*24*). We suggest, on the basis of available evidence (*10–15*) and the data presented here, that the inhibition of 5α-DHT binding to specific receptors in the rat prostate may be representative of a biochemical mechanism by which insecticides modify hormonal action in mammalian reproductive organs.

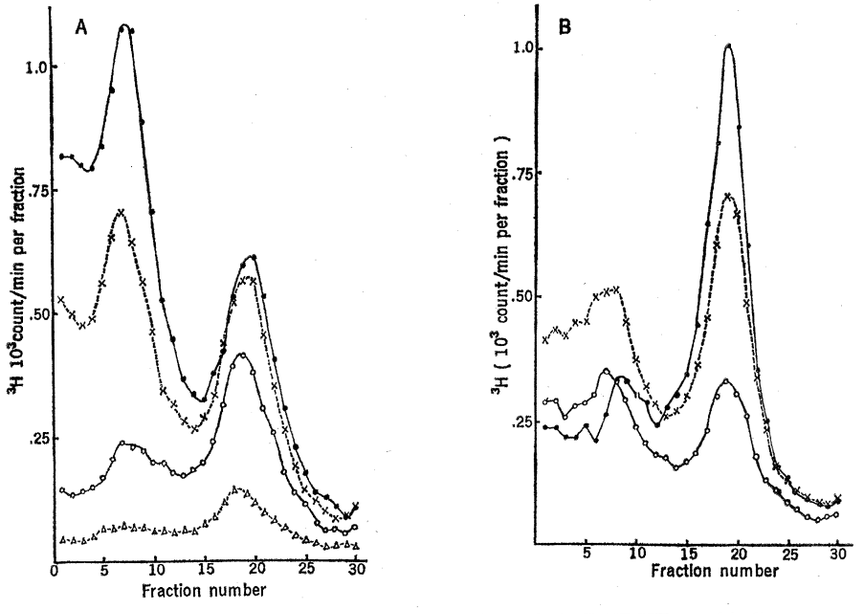

Fig. 2. (A) The effect of increasing concentration of 5α-[^{3}H]DHT on binding to rat prostate cytosol. Prostates were homogenized manually in an all-glass apparatus at 4°C in the cold room, in three volumes of 0.02*M* phosphate buffer, *p*H 7.4, containing 1.5 m*M* EDTA and 2.0 m*M* mercaptoethanol (PEM). Cytosol was prepared as described (Fig. 1A). Portions of the cytosol were incubated with increasing concentrations of 5α-[^{3}H]DHT added in 5 μl of ethanol. After incubation for 2 hours at 0°C, 0.2-ml portions were mixed with an equal volume of dextran-charcoal solution (0.25 percent dextran and 2.5 percent charcoal in PEM buffer) to remove unbound steroid. The charcoal was removed by centrifugation, and 0.2-ml portions of the supernatant were placed on linear sucrose gradients (5 to 20 percent) prepared in PEM buffer. The gradients were centrifuged at 30,000 rev/min (SW-50.1 rotor) for 20 hours at 0°C, to give a sedimentation approximately one-half that described in Fig. 1, A and B. The total steroid concentrations were $4.3 \times 10^{-8}M$ (●), $2.4 \times 10^{-8}M$ (X), $5.9 \times 10^{-9}M$ (○), and $1.4 \times 10^{-9}M$ (△). (B) The effect of *o,p'*-DDT upon binding of 5α-[^{3}H]DHT by rat prostate cytosol. The procedure was that described above for samples containing 5α-[^{3}H]DHT and 5 μl of ethanol, control (●), or 5 μl of ethanol containing *o,p'*-DDT to give a final concentration of $5.6 \times 10^{-6}M$ (X) or $2.8 \times 10^{-5}M$ (○).

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References and Notes

1. D. J. Ecobichon, *Can. Med. Assoc. J.* **103**, 711 (1970).
2. R. M. Welch, W. Levin, A. H. Conney, *Toxicol. Appl. Pharmacol.* **14**, 358 (1969).
3. J. Bitman, H. C. Cecil, S. J. Harris, G. F. Fries, *Science* **162**, 371 (1968).
4. W. L. Ball, K. Kay, J. W. Sinclair, *Arch. Ind. Hyg.* **7**, 292 (1953).
5. A. Cazorla and F. Moncloa, *Science* **136**, 47 (1962).
6. C. Cueto and J. H. U. Brown, *Endocrinology* **62**, 326 (1958).
7. D. M. Bergenstal, R. Hertz, M. B. Lipsett, R. H. Moy, *Ann. Intern. Med.* **53**, 672 (1960); A. M. Hutter and D. E. Kayhoe, *Amer. J. Med.* **41**, 581 (1966).
8. A. L. Southern, S. Tochimoto, L. Strom, A. Ratuschni, H. Ross, G. Gordon, *J. Clin. Endocrinol.* **26**, 268 (1966).
9. B. Zimmermann, H. S. Bloch, W. L. Williams, C. R. Hitchcock, B. Hoelscher, *Cancer* **9**, 940 (1956).
10. W. B. Deichmann, W. E. MacDonald, A. G. Beasley, D. Cubit, *Ind. Med.* **40**, 10 (1971).
11. H. Burlington and V. F. Lindeman, *Proc. Soc. Exp. Biol. Med.* **74**, 48 (1950); D. J. Ecobichon and P. W. Saschenbrecker, *Can. J. Physiol. Pharmacol.* **46**, 785 (1968).

12. M. T. Smith, J. A. Thomas, C. G. Smith, M. G. Mawhinney, J. W. Lloyd, *Toxicol. Appl. Pharmacol.* **23**, 159 (1972).
13. M. L. E. Espir, J. W. Hall, J. G. Shirreffs, D. L. Stevens, *Brit. Med. J.* **1**, 423 (1970).
14. M. J. Blend and W. J. Visek, *Toxicol. Appl. Pharmacol.* **23**, 344 (1972).
15. A. E. Wakeling, T. J. Schmidt, W. J. Visek, *Fed. Proc.* **31**, 725 (Abstr.) (1972); A. E. Wakeling, T. J. Schmidt, W. J. Visek, *Toxicol. Appl. Pharmacol.*, in press.
16. S. Fang, R. M. Anderson, S. Liao, *J. Biol. Chem.* **244**, 6584 (1969); S. Fang and S. Liao, *ibid.* **246**, 16 (1971).
17. W. I. P. Mainwaring, *J. Endocrinol.* **44**, 323 (1969).
18. ———, *ibid.* **45**, 531 (1969).
19. E. E. Baulieu and J. Jung, *Biochem. Biophys. Res. Commun.* **38**, 599 (1970).
20. The designation of the slower sedimenting peak at 3.5*S* identifies this peak with that previously recorded (Fig. 1A) but does not represent a significant difference from that peak designated by Mainwaring (*18*) as 4*S*.
21. A. C. Notides, D. E. Hamilton, J. H. Rudolf, *Biochim. Biophys. Acta* **271**, 214 (1972).
22. M. E. Ritzen, S. N. Nayfeh, F. S. French, M. C. Dobbins, *Endocrinology* **89**, 143 (1971).
23. B. M. Sanborn, B. R. Rao, S. G. Korenman, *Biochemistry* **10**, 4955 (1971).
24. A. H. Conney, R. M. Welch, R. Kuntzman, J. J. Burns, *Clin. Pharmacol. Ther.* **8**, 2 (1967).
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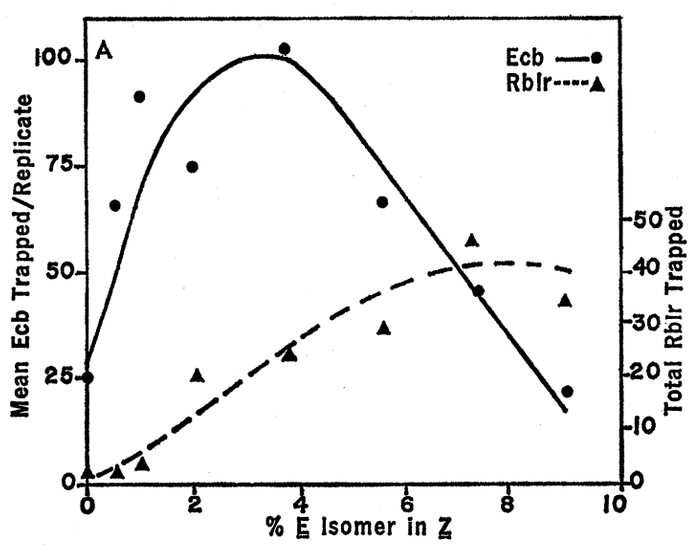
Insect Sex Pheromones: Minor Amount of Opposite Geometrical Isomer Critical to Attraction

Abstract. *(Z)-11-Tetradecenyl acetate is the reported sex pheromone of European corn borer and redbanded leafroller moths. However, geometrically pure preparations of the compound are weakly attractive to these species. Presence of the* E *geometrical isomer in the* Z *is necessary for maximum sex attraction and these moths are "tuned" to respond optimally to specific proportions of* Z *to* E. *This discovery is important to considerations of moth pheromonal specificity, evolution, and in application of knowledge of the pheromones to insect-pest suppression.*

(*Z*)-11-Tetradecenyl acetate has been reported as the sex pheromone of two economically important insect pests—the European corn borer, the Iowa strain *Ostrinia nubilalis* (Hübner) (*1*), and the redbanded leafroller, *Argyrotaenia velutinana* (*2*). However, in studies at Ankeny, Iowa, the European corn borer was only weakly attracted and the redbanded leafroller was not attracted to geometrically pure (*Z*)-11-tetradecenyl acetate (*3, 4*). In contrast, when small amounts of (*E*)-11-tetradecenyl acetate were added to (*Z*)-11-tetradecenyl acetate, the attraction for both species was dramatically enhanced although neither species showed any behavioral response to the *E* isomer alone. Moreover, a defined concentration of the *E* isomer in the *Z* isomer is required for maximum attraction of European corn borer and redbanded leafroller males, and each species responds optimally to different proportions of the geometrical isomers. The finding that the maximum attraction in these species depends on the isomeric proportion provides a useful concept of pheromonal specificity and speciation in Lepidoptera and suggests a potentially practical method of disrupting the chemical communication between the sexes of these two species.

The requirement of the *E* isomer for optimum attraction became obvious in a preliminary field test (*5*). In the test, a total of 66 European corn borer males were caught in traps baited with the pure *Z* isomer, and 171 were caught in the traps baited with an isomeric mixture. Results with the redbanded leafroller were even more striking. None were attracted to traps baited with the pure *Z* isomer, and 127 were attracted to the isomer combination. In a definitive test of the effect of varying proportions of *Z* to *E*, samples of pure (*Z*)-11-tetradecenyl acetate (100 μg) mixed with pure (*E*)-11-tetradecenyl acetate (0 to 10 μg) (*6*) on rubber septa were exposed in the field traps. The results are shown in Fig. 1A. The presence of as little as 0.5 percent of the *E* isomer caused the attraction of European corn borer males to rise sharply, and maximum numbers were caught with the mixture containing 100 μg of *Z* plus 4 μg of *E*. Further increase in the *E* isomer produced decreased attraction, which is in accord with the known inhibitory effect of high concentrations of (*E*)-11-tetradecenyl acetate (*7*). In contrast, the redbanded leafroller showed a continuous rise in captures as the concentration of *E* was in-



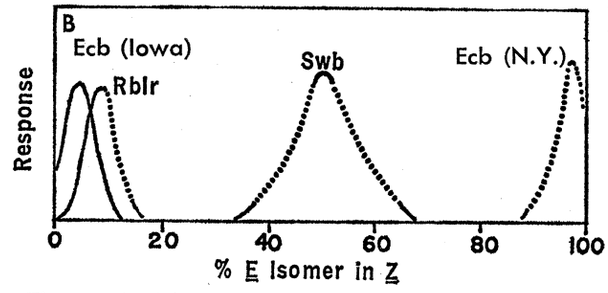


Fig. 1. (A) The response of male European corn borers (*Ecb*) and redbanded leafrollers (*Rbir*) to increasing amounts of (*E*)-11-tetradecenyl acetate added to the *Z* isomer. The experiment was conducted 10 to 31 August 1972 in a randomized block design with three subsamples of each concentration of *E* isomer, and four replications. The standard error for Ecb means plotted is 10.4. Statistical analyses showed quadratic effects were highly significant for both Ecb and Rblr. (B) Sex attraction response maxima of four moth species to different proportions of the *Z* and *E* isomers of 11-tetradecenyl acetate. Dotted lines represent concentrations where no data was obtained; *Swb*, smartweed borer.