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INHIBITION OF DIHYDROTESTOSTERONE FORMATION: AN EFFECTIVE MEANS OF BLOCKING ANDROGEN ACTION IN HAMSTER SEBACEOUS GLAND*

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The action of androgens in the skin is of special dermatologic interest because the responsiveness of skin to androgens may contribute to the pathogenesis of several common skin disorders, such as acne vulgaris, seborrhea, and male-pattern baldness. Studies in several laboratories have shown that the skin not only responds to androgens (Strauss and Pochi, 1969) but also is an active site of androgen metabolism (Wotiz et al., 1956; Cameron et al., 1966; Rongone, 1966; Gallegos and Berliner, 1967; Gomez and Hsia, 1968; Wilson and Walker, 1969; Faredin et al., 1969). Dihydrotestosterone (DHT), the major metabolite of testosterone in the skin (Gomez and Hsia, 1968), is a more potent androgen than testosterone; this was shown by bioassays on the ventral prostate and seminal vesicles of the rat (Vida, 1969). There is now convincing evidence that DHT formed from testosterone is an active form of androgen which mediates the hormone action by influencing gene expression in the prostate (Bruchovsky and Wilson, 1968; Anderson and Liao, 1968; Harper et al., 1970; Bruchovsky, 1971). A study by Takayasu and Adachi (1972) indicated that DHT may also be an active androgen in the sebaceous glands of hamsters. A study by Sansone and Reisner (1971) showed that acne-bearing skin on the face and back of both male and female patients converted testosterone into DHT at greater rates than skin from corresponding sites of normal controls. Excessive androgen formation may be related to disease and this excessive formation could be averted by blocking DHT formation in the target organ; this approach has the special advantage of delivering the blocking agent directly to the skin by topical application.

One of the enzymes of steroid metabolism in the skin studied in our laboratory during the past few years has been testosterone 5α-reductase, which mediates the formation of DHT. We discovered that a number of steroids can interfere with 5α-reductase activity and inhibit DHT formation. One of the most effective inhibitors is 4-androsten-3-one-17β-carboxylic acid (17βC), which also exhibits antiandrogenic activity after topical application to the flank organ of the hamster.

5α-REDUCTASE AND ITS INHIBITORS

The stereospecific reduction of testosterone with NADPH to form DHT in target tissues is mediated by testosterone 5α-reductase, an enzyme demonstrated in the nuclei of the prostate (Bruchovsky and Wilson, 1968; Moore and Wilson, 1972). We found in human skin that most of the enzymic activity was associated with the microsomes; little activity could be detected in the purified nuclear fraction (Voigt et al., 1970). We reasoned that the formation of DHT in the skin is in the cytoplasm and that after its formation it is probably transported into the nuclei, perhaps by a receptor protein, in the same way that estradiol is carried into uterine cell nuclei (Jensen et al., 1968). Several steroid hormones (progesterone, deoxycorticosterone, and androstenedione) inhibited the enzyme during the conversion of testosterone to DHT, probably by means of the structural resemblance of these steroids to testosterone and their ability to compete for the binding site on the enzyme molecule. This explanation was substantiated by results from mixed substrate experiments with progesterone and testosterone and by kinetic studies with the two steroids, in which microsomal preparations of human skin were used as the source of the 5α-reductase. The results indicated that progesterone as well as testosterone could serve as the substrate for the 5α-reductase and that the two steroids mutually inhibit each other from undergoing 5α-reduction, probably by competing for the same enzyme site (Voigt et al., 1970).

Competition among steroids for binding to enzyme sites is probably more common than we think and may be of fundamental endocrinologic significance. We found another example in the enzyme 17β-hydroxysteroid dehydrogenase of rat skin. This enzyme mediates the interconversion of estradiol-17β and estrone, as well as of testosterone and androstenedione. Estradiol-17β and testosterone were found to competitively inhibit each other in undergoing the dehydrogenation mediated by this enzyme at C17 (Davis et al., 1972). Since enzymic transformation of a steroid can lead to the formation of metabolites with either more or less hormonal potencies, such transformations are intimately related to the regulation of steroid hormone action. Therefore, interference with the enzymic transformation of a steroid hormone by another steroid hormone or metabolite may be a physiologic means of balancing hormonal activities.

To seek therapeutically useful inhibitors of the 5α-reductase, we tested structurally different steroids for their ability to inhibit the formation of DHT from testosterone by the microsomal 5α-reductase of human skin. Examination of effective inhibitors revealed some features that were common; these features were used as a guide for choosing from among many additional steroids, the

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effective inhibitors of the 5α-reductase (Voigt and Hsia, 1973a).

Figure 1 shows the structural formulas of 16 steroids which illustrate the relationship between structure and inhibitory activity on 5α-reduction of testosterone. Compounds I–IV are effective inhibitors because they all have the 3-keto-Δ^4 structure in ring A. This structural similarity to testosterone apparently enables them to compete with the latter for binding with the 5α-reductase. In a kinetic study, progesterone (I) was found to have slightly greater affinity than testosterone for the enzyme (Voigt et al., 1970). Pregnenolone (V) differs from progesterone (I) in having a 3β-hydroxy-Δ^5 instead of the 3-keto-Δ^4 structure; it has little inhibitory activity. Dehydroepiandrosterone (VI), which differs from androstenedione (II) in an analogous manner, also has no inhibitory activity. Having a 3β-hydroxyl in place of the 3-keto group, 4-androstene-3β,17β-diol (VII) is ineffective as an inhibitor. DHT (VIII), the product of 5α-reduction of testosterone, having a 3-keto and 5α-saturated ring A, also has no inhibitory activity. Thus the 3-keto-Δ^4 structure is a rigid requirement for effective inhibitors. Despite the 3-keto-Δ^4 structure, compounds IX, X, and XI are relatively poor inhibitors. They have another structural feature in common, i.e., a 17α-substituent. The binding of the steroid to the enzyme may be at the α (rear) side of ring D, so that a 17α-substituent may hinder the binding of the steroid to the enzyme and thus reduce its chance for binding.

Unlike deoxycorticosterone (III), corticosterone (XII) is a rather poor inhibitor. The binding of the steroid to the enzyme in the regions of rings B and C appears to be on the β (front) side, so that the 11β-hydroxyl group in XII is detrimental to binding and also to the inhibitory effect. Any alteration in the steroid nucleus, such as deletion of the 19-methyl group (in 19-nortestosterone, XIII) or C1 (in A-norprogesterone, XIV), weakens or destroys the potency of the steroid as an inhibitor.

Despite rigid structural requirements in various parts of the steroid molecule, the effective inhibitors of testosterone 5α-reductase vary considerably in the 17β-substituent group. This is evident in compounds I, III, and IV, each of which has a different hydrophilic 17β-side chain. In contrast, cholestenone (XV) with a hydrocarbon side chain is ineffective as an inhibitor. Apparently, the hydrophilic nature of the 17β-side chain in compounds I, III, and IV is a necessary feature, although within limits the size of the 17β-side chain may vary.

We tested many other steroids not listed here whose structures and inhibitory activity enforce the above generalization about the required structural features for an effective 5α-reductase inhibitor. To be therapeutically useful, however, a steroid must have additional features besides its effectiveness in inhibiting the 5α-reductase in the enzymic assay. It must not have undesirable hormonal activity and should not be a potential precursor of steroid hormones. For example, deoxycorticosterone (III) is a potent inhibitor of 5α-reductase, but its influence on mineral metabolism eliminates it as a useful antiandrogen. Although androstenedione (II) can compete with testosterone in the enzymic reaction, it can be transformed into testosterone in the tissue (Gomez and Hsia, 1968) and is therefore an androgen instead of an antiandrogen. Although progesterone (I) has antiandrogenic properties as evidenced by the chick comb test (Dorfman, 1965), it is a precursor for the biosynthesis of androgenic steroids and is therefore not an ideal antiandrogenic agent. Several ethers and esters of testosterone, which inhibited the 5α-reductase (Voigt and Hsia, 1973a), were also eliminated because of the possibility of their releasing testosterone after hydrolysis.

After considering many steroid inhibitors of 5α-reductase and finding them biologically undesirable as antiandrogenic agents, we found that 4-androsten-3-one-17β-carboxylic acid (17βC, XVI) fulfills all structural requirements for a 5α-reductase inhibitor and in our assay it was one of the most effective inhibitors. It has no known hormonal activity and is not found in any natural steroidogenic pathway. It has been found as a degradation product of deoxycortiscosterone in perfusates of rat liver (Levy and Maloney, 1962) and bovine adrenal (Picha et al., 1952). It would seem to be a useful antiandrogen if blocking DHT formation in the target tissue is an effective means of blocking androgen action.

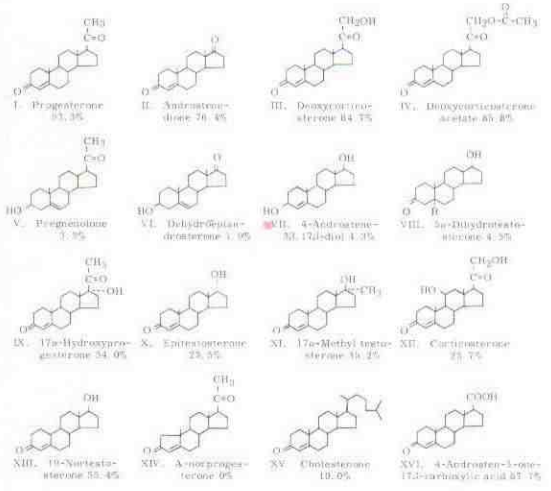

FIG. 1: Inhibitory effects of steroids on 5α-reduction of testosterone. The number under each steroid indicates percentage of inhibition. The assay mixture contained microsomes (10 mg protein) prepared from newborn preputial skin collected at circumcision, 4-^{14}C testosterone (10^5 dpm, 0.77 nmole), an NADPH-generating system, and the test steroid (10 nmole) in 1 ml of 0.1 M citrate–phosphate buffer, pH 5.6. 5α-reductase activity was measured by the amount of DHT and 5α-androstane-3α, 17β-diol formed in 10 min at 37°C. (Data taken from Voigt et al., 1970, and Voigt and Hsia, 1972a.)

ANTIANDROGENIC ACTIVITY OF 4-ANDROSTEN-3-ONE-17β-CARBOXYLIC ACID AND ITS METHYL ESTER ON HAMSTER SEBACEOUS GLAND

The flank organ, or costovertebral spot, of the hamster has been shown to be an androgen-dependent sebaceous structure (Hamilton and Montagna, 1950). Burdick and Hill (1970) reported that topical application of antiandrogenic agents, including chlormadinone acetate and cyproterone acetate, to the flank organ of the male animal had equivocal effects. However, Frost and Gomez (1972) showed that topical application of androgenic steroids to the gland of female hamsters caused enlargement of the gland and that inhibition of the androgenic effect by topical antiandrogens could be used as an assay for antiandrogen activity. This method was used to test the antiandrogenic property of 17βC and its methyl ester (Voigt and Hsia, 1973b).

Figure 2 shows a photo of the back of a female hamster whose left flank organ was treated with testosterone propionate in acetone daily for three weeks and whose right organ received acetone only. Note the enlargement in size and increase in pigmentation of the treated organ. Figure 3 shows the results in a female whose left flank organ received daily topical applications of testosterone propionate together with 17βC in acetone for three weeks and whose right flank organ received only acetone. The androgenic effect of testosterone propionate was completely blocked by the carboxylic acid. Parallel experiments were carried out with the methyl ester of the acid, and antiandrogenic activity was demonstrated in the same manner.

Histologic examination of the flank organ treated with testosterone propionate and of the organ treated with testosterone propionate plus 17βC revealed clear differences. The former had enlarged sebaceous glands which formed an almost continuous mass from follicle to follicle, and the sebaceous cells were replete with lipid droplets. In contrast, the latter showed undeveloped sebaceous glands, and the individual cells contained less lipids.

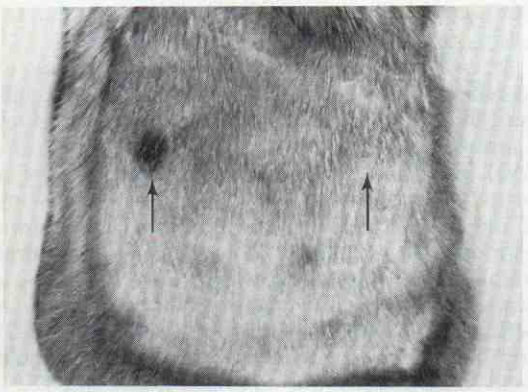

FIG. 2: Effect of testosterone propionate on the flank organ of a female hamster.

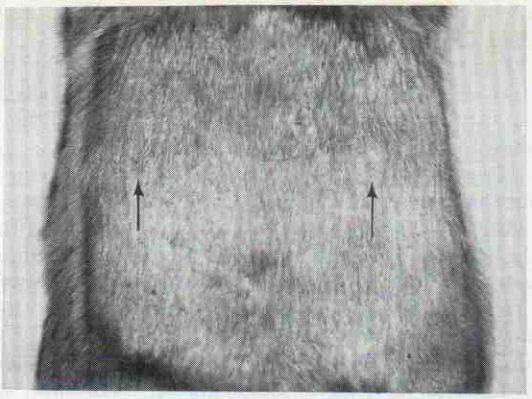

FIG. 3: Antiandrogenic effect of 17βC.

In another series of experiments, the flank organ was treated topically with DHT instead of testosterone propionate. As with testosterone propionate, the treated organ became enlarged and increased in pigmentation. The action of DHT could not be blocked by 17βC, an indication that 17βC acts in the flank organ as an antiandrogen by inhibiting DHT formation. This explanation was supported by experiments with homogenates of the flank organ. Testosterone 5α-reductase activity was demonstrated in the homogenates by the identification of [^{14}C]DHT and [^{14}C]5α-androstane-3α,-17β-diol after incubation with ^{14}C testosterone in the presence of an NADPH-generating system. 17βC added to the incubation medium inhibited the formation of these 5α-reduced products.

After our study on the flank organ was completed, a previous publication by Nayfeh and Baggett (1969) came to our attention. These investigators had demonstrated the inhibitory effect of the methyl ester of 17βC on the conversion of progesterone into androgenic steroids by homogenates of rat testis. Thus in addition to its ability to inhibit DHT formation in the target tissue, this compound can also inhibit androgen formation in the gonad.

CONCLUSION

Since DHT is an active form of androgen in target tissues, its formation is essential for androgen action. Our findings support the theory that inhibition of DHT formation is an effective means of blocking androgen action in target organs. The study of the 5α-reductase gave insight into the regulation of androgen action and led to the finding of a number of inhibitors for the 5α-reductase. Knowledge of the necessary structural features in these inhibitors assisted us in the selection of additional inhibitors, and 17βC was chosen both because of its ability to inhibit 5α-reductase activity and because of the lack of any apparent hormonal effects. Its antiandrogenic activity was demonstrated after topical application to the seba-

ceous gland of hamsters. The therapeutic usefulness of 17βC and its derivatives needs to be evaluated in further studies.

REFERENCES

Anderson, K. M., and Liao, S. (1968). Selective retention of dihydrotestosterone by prostatic nuclei. Nature (Lond.), *219:* 277–279.

Bruchovsky, N. (1971). Dihydrotestosterone—A common factor in the action of androgens. Clin. Res., *19:* 368.

Bruchovsky, N., and Wilson, J. D. (1968). The intranuclear binding of testosterone and 5α-androstan-17β-ol-3-one by rat prostate. J. Biol. Chem., *243:* 5953–5960.

Burdick, K. H., and Hill, R. (1970). The topical effect of the antiandrogen chlormadinone acetate and some of its chemical modifications on the hamster costovertebral organ. Br. J. Dermatol., *8*(Suppl. 6)*:* 19–25.

Cameron, E. H. D., Baillie, A. H., Grant, J. K., Milness, J. A., and Thomson, J. (1966). Transformation in vitro of [7α-^{3}H] dehydroepiandrosterone to [^{3}H] testosterone by skin from men. J. Endocrinol., *35:* xix–xx.

Davis, B. P., Rampini, E., and Hsia, S. L. (1972). 17β-hydroxysteroid dehydrogenase of rat skin. Substrate specificity and inhibitiors. J. Biol. Chem., *247:* 1407–1413.

Dorfman, R. I. (1965). Anti-androgens. In: *Methods in Hormone Research* (ed. by Dorfman, R. I.). Academic Press, New York, pp. 77–93.

Faredin, I., Fazekas, A. G., Toth, I., Kokai, K., and Julesz, M. (1969). Transformation in vitro of [4-^{14}C] dehydroepiandrosterone into 7-oxygenated derivatives by normal human male and female skin tissue. J. Invest. Dermatol., *52:* 357–361.

Frost, P., and Gomez, E. C. (1972). Inhibitors of sex hormones: Development of experimental models. In: *Advances in Biology of Skin. Vol. XII. Pharmacology and the Skin* (ed. by Montagna, W., Van Scott, E. J., and Stoughton, R. B.). Appleton-Century-Crofts, New York, pp. 403–420.

Gallegos, A. J., and Berliner, D. L. (1967). Transformation and conjugation of dehydroepiandrosterone by human skin. J. Clin. Endocrinol., *27:* 1214–1218.

Gomez, E. C., and Hsia, S. L. (1968). In vitro metabolism of testosterone-4-^{14}C and Δ^4-androstene-3,17-dione-4-^{14}C in human skin. Biochemistry, *7:* 24–32.

Hamilton, J. B., and Montagna, W. (1950). The sebaceous glands of the hamster. Morphological effects of androgens on integumentary structures. Am. J. Anat., *86:* 191–233.

Harper, M. E., Pierrepoint, C. G., Fahmy, A. R., and Griffiths, K. (1970). The effect of prostatic metabolites of testosterone and other substances on the isolated deoxyribonucleic acid polymerase of the canine prostate. Biochem. J., *119:* 785–786.

Jensen, E. V., Suzuki, T., Kawashima, T., Stumpf, W. E., Jungblut, P. W., and De Sombre, E. R. (1968). A two-step mechanism for the interaction of estradiol with rat uterus. Proc. Natl. Acad. Sci. U.S.A., *59:* 632–638.

Levy, H., and Maloney, P. J. (1962). The isolation of 3-keto-4-etienic acid and allopregnan-21-ol-3,20-dione from bovine adrenal perfusions of deoxycorticosterone. Biochim. Biophys. Acta, *57:* 149–150.

Moore, R. J., and Wilson, J. D. (1972). Localization of the reduced nicotinamide adenine dinucleotide phosphate: Δ^4-3-ketosteroid 5α-oxidoreductase in the nuclear membrane of the rat ventral prostate. J. Biol. Chem., *247:* 958–967.

Nayfeh, S. N., and Baggett, B. (1969). Metabolism of progesterone by rat testicular homogenates. III. Inhibitory effects of intermediates and other steroids. Steroids, *14:* 269–283.

Picha, G. M., Saunders, F. J., and Green, D. M. (1952). An oxidative metabolite of deoxycorticosterone. Science, *115:* 705.

Rongone, E. L. (1966). Testosterone metabolism by human male mammary skin. Steroids, *7:* 489–504.

Sansone, G., and Reisner, R. M. (1971). Differential conversion rates of testosterone to dihydrotestosterone in acne and in normal human skin—A possible pathogenic factor in acne. J. Invest. Dermatol., *56:* 366–372.

Strauss, J. S., and Pochi, P. E. (1969). Recent advances in androgen metabolism and their relation to the skin. Arch. Dermatol., *100:* 621–636.

Takayasu, S., and Adachi, K. (1972). The in vivo and in vitro conversion of testosterone to 17β-hydroxy-5α-androstan-3-one (dihydrotestosterone) by the sebaceous gland of hamsters. Endocrinology, *90:* 73–80.

Vida, I. A. (1969). *Androgens and Anabolic Agents: Chemistry and Pharmacology*. Academic Press, New York.

Voigt, W., Fernandez, E. P., and Hsia, S. L. (1970). Transformation of testosterone into 17β-hydroxy-5α-androstan-3-one by microsomal preparations of human skin. J. Biol. Chem., *245:* 5594–5599.

Voigt, W., and Hsia, S. L. (1973a). Further studies on testosterone 5α-reductase of human skin: Structural features of steroid inhibitors. J. Biol. Chem. *248:* 4280–4285.

Voigt, W., and Hsia, S. L. (1973b). The antiandrogenic action of 4-androsten-3-one-17β-carboxylic acid and its methyl ester on hamster flank organ. Endocrinology *92:* 1216.

Wilson, J. D., and Walker, J. D. (1969). The conversion of testosterone to 5α-androstan-17β-ol-3-one (dihydrotestosterone) by skin slices of man. J. Clin. Invest., *48:* 371–379.

Wotiz, H. H., Mescon, H., Doppel, H., and Lemon, H. M. (1956). The in vitro metabolism of testosterone by human skin. J. Invest. Dermatol., *26:* 113–119.