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IN VITRO INHIBITION OF 5α-3β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN RAT TESTES

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SUMMARY:

Three steroidal inhibitors of Δ_5-3β-hydroxysteroid dehydrogenase have been tested in incubations of teased testicular tissue for their ability to inhibit the activity of 5α-3β-hydroxysteroid dehydrogenase and 5α-3α-hydroxysteroid dehydrogenase. All three inhibitors significantly blocked the metabolism of 3β-diol. Additionally, small but significant reductions in the metabolism of DHT and 3α-diol were observed. These results demonstrate that these steroids are effective in blocking 5α-3β-hydroxysteroid dehydrogenase activity with minimal ability to block 5α-3α-hydroxysteroid dehydrogenase activity. These compounds may well be effective in helping differentiate in vivo biologic activity of 5α-reduced androgens.

INTRODUCTION:

The use of a steroidal compound which blocks Δ_5-3β-hydroxysteroid dehydrogenase both in vivo and in vitro has been extensively investigated by Goldman and coworkers (1-4). The major compound thus far investigated was 17β-hydroxy-4,4,17-trimethyl-3-oxoandrost-5-ene-2α-carbonitrile (cyanoketone, I). By inhibiting the activity of this enzyme, the conversion of Δ_5-3β-hydroxysteroids to Δ_4-3-ketosteroids is blocked. This prevents formation of any biologically active corticosteroids or estrogens. Additionally, androgens containing Δ_4-3-keto functions are not produced with a resultant increase in 3β-hydroxy-5-androstene-17-one (dehydroepiandrosterone), a weak androgen. Moreover, it would appear that formation of 5α-reduced androgens[1] will also be impaired, since this conversion must come from Δ_4-androgens.

It has been shown that DHT has biologic activity in the prostate glands, seminal vesicles and testes of rats (5-9). Additionally, biologic activity

[1] The following abbreviations have been used: DHT; dihydrotestosterone (17β-hydroxy-5α-androstan-3-one). 3α-diol (5α-androstane-3α,17β-diol). 3β-diol (5α-androstane-3β,17β-diol).

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of 3α-diol and 3β-diol on these same organs has also been shown (6,9-11). Evidence for the conversion of DHT to 3α-diol in rat testes has been presented by Rivarola and Podesta (12), Rivarola et al. (13), Folman et al. (14) and Sowell et al. (15). Additionally, we have recently demonstrated (16) that 3α-diol, 3β-diol and DHT are interconvertible in rat testes and ventral prostate glands and 3α-diol and DHT are metabolized in rat seminal vesicles.

Because all three of these 5α-reduced androgens show biologic activity, and since they are interconvertible, the active form(s) has yet to be established. The ability to block the conversion of any of these steroids to one of the others might yield information regarding the biologic activity of a particular 5α-reduced androgen. We have, therefore, attempted to assess the capability of inhibitor I as well as two other steroidal compounds as to their capacity to inhibit 5α-3β-hydroxysteroid dehydrogenase and 5α-3α-hydroxysteroid dehydrogenase in an in vitro system so that if inhibitory activity does exist in any of the compounds, then in vivo use might help establish the active configurations of 5α-reduced androgens.

MATERIALS AND METHODS:

Steroids

Tritiated 3β-diol, 3α-diol and DHT, as well as [^{14}C] DHT, were purchased from New England Nuclear, Boston, Massachusetts and purified before use on the chromatographic system described later. Labeled [^{14}C] 3α-diol and [^{14}C] 3β-diol were prepared by incubating the [^{14}C] DHT with Pseudomonas testosteroni, obtained from Sigma Chemical Company, St. Louis, Missouri, in Krebs-Ringer bicarbonate glucose (KRBG) buffer, pH 7.0 for 15 min. at 25°C. At the conclusion of the incubation period, the steroids were extracted with ether and the solvent was evaporated in a vacuum oven. The residue was chromatographed in the system described later. The radioactive peaks were then eluted and stored for later use as recovery standards.

The potential steroidal inhibitors of 5α-3β-hydroxysteroid dehydrogenase were a gift from the Upjohn Company, Kalamazoo, Michigan. The three compounds tested for their ability to inhibit 5α-3β-hydroxysteroid dehydrogenase were 17β-hydroxy-4,4,17α-trimethylandrost-5-eno- 2,3-d isoxazole (II), and 17β-hydroxy-17-methyl-3-oxo, Spiro(androst-5-ene-4,1'-cyclopropane)-2α-carbonitrile (III).

Incubation Procedure

Long-Evans male rats, 60 days of age, obtained from Simonson Laboratories, Gilroy, California, were used for all experiments. Animals were sacrificed by

decapitation, the testes removed, detunicated and placed in KRBG buffer, pH 7.4, maintained at 4° C. Portions of the testes were then blotted on filter paper and 100 mg of tissue weighed and placed in a 25 ml Erlenmeyer flask containing 2 ml of KRBG buffer, pH 7.4. The flasks were kept on ice until all samples had been prepared.

Six flasks for each inhibitor and each substrate were incubated with tissue as well as six flasks for each substrate without any inhibitor which served as a control to compare the action of the inhibitor. Additionally, six flasks for each inhibitor and each substrate but without tissue served as controls for nonspecific metabolism of the substrates.

At the beginning of the incubation, 50 µl of ethanol or ethanol containing 10 µg of an inhibitor were added to each control or sample flask. The incubation proceeded for 15 min. at 34°C in an atmosphere of 95% O_2 and 5% CO_2 at which time 1 µCi of the appropriate tritiated substrate was added in 50 µl of ethanol and the incubation continued for an additional 30 min. The incubation was terminated by addition of 1 ml of chloroform:methanol, 1:1 (v:v) and the samples stored at -20°C until further processing took place.

Extraction, Separation and Quantification of Androgens

The samples were defrosted and 1000 dpm each of [^{14}C] 3α-diol, [^{14}C] 3β-diol and ^{14}C DHT were added to each flask. The samples were then homogenized in a Ten Broek homogenizer and extracted 3 times with 5 volumes of ether. The ether was evaporated in a vacuum oven and the residue dissolved in 10% chloroform in benzene and applied to 1 x 60 cm strips of Whatman #1 filter paper. The strips were placed in a chromatography tank and allowed to equilibrate for 3 hrs. in a Bush A_1 system, as described by Ewing et al. (17). The mobile phase was then added and the strips developed for 20 hrs. Steroid standards, obtained from Sigma Chemical Company (St. Louis, Mo.) were run on separate strips in each tank. All sample strips were then scanned for radioactivity on a Packard Radiochromatogram Scanner. The radioactive areas were then compared to the authentic standards and the corresponding 3α-diol, 3β-diol and DHT areas were cut from the strips and the androgens eluted with methanol. Definitive identification and assessment of purity of the androgens was established by selecting 3 samples of each steroid at random and recrystallizing a portion of the radioactive steroid with known pure compound to a constant specific activity. Aliquots of each sample were transferred to counting vials and counted with recovery standards. Corrections for losses were made from the ^{14}C recovery standards and the amount of each radioactive steroid expressed as the percent radioactivity recovered of the three metabolites.

RESULTS AND DISCUSSION:

Three samples, chosen at random, of each possible metabolite were recrystallized for positive identification and proof of purity of the steroid after chromatography. The samples subjected to recrystallization showed no significant change in specific activity from the starting material through six recrystallizations of the androgens.

All substrates were incubated without tissue in the presence or absence of one of the inhibitors. Since there were no significant differences in nonspecific conversion between substrates incubated with inhibitors or those

TABLE 1

Percent conversion of 5α-reduced androgens with and without inhibitors

Substrate	Metabolites	No Tissue	Control	I	II	III
[^{3}H]3β-diol	3β-diol	95.1 ± 0.7	21.4±1.3	90.0±0.2*	85.2±0.6*	92.1±0.3*
	3α-diol	0.5 ± 0.1	66.7±0.6	0.8±0.1*	1.4±0.1*	0.7±0.1*
	DHT	4.4 ± 0.6	11.9±0.2	9.2±0.2*	13.4±0.6**	7.2±0.3*
[^{3}H]3α-diol	3β-diol	2.3 ± 0.1	6.0±0.6	2.4±0.1*	3.6±0.3*	2.4±0.1*
	3α-diol	94.4 ± 0.6	82.9±1.7	93.7±0.3*	87.2±1.4*	95.2±0.1*
	DHT	3.3 ± 0.6	11.1±1.1	3.9±0.2*	9.2±1.2*	2.4±0.1*
[^{3}H]DHT	3β-diol	0.5 ± 0.1	9.6±0.5	4.9±0.2*	5.4±0.2*	4.1±0.2*
	3α-diol	0.4 ± 0.1	65.9±1.8	60.7±0.9*	52.7±2.0*	64.4±1.3
	DHT	99.1 ± 0.1	24.5±1.4	34.4±1.0*	41.8±2.2*	31.5±1.2*

* Significantly different than control $p < .01$
** Significantly different than control $p < .05$
6 samples for each substrate with each incubation were used.

without, the data for each substrate was pooled.

The results of the incubations are presented in Table 1. When [^{3}H] 3β-diol served as the substrate, only 21.4% of this steroid remained in control incubations as compared to 90%, 85.2% and 92.1% when inhibitors I, II or III were added. Only trace amounts of [^{3}H] 3α-diol were formed from [^{3}H] 3β-diol in the presence of inhibitors as compared to 66.7% in the control tissue. When [^{3}H] 3α-diol was incubated with testicular tissue, significantly less of the substrate was metabolized with the addition of any of the inhibitors as compared with controls. This reduction in metabolism was evident in conversion to either 3β-diol or DHT. When [^{3}H] DHT was utilized as the precursor, the addition of I or II to the incubation medium significantly suppressed conversion to 3α-diol and 3β-diol while III only blocked conversion to 3β-diol, not 3α-diol.

The fact that all three inhibitors dramatically blocked the metabolism of [^{3}H]3B-diol conclusively demonstrates that these compounds have the ability to block 5α-3β-hydroxysteroid dehydrogenase as well as Δ_5-3β-hydroxysteroid

dehydrogenase. Additionally, all three inhibitors limit to some degree the conversion of [^{3}H] 3α-diol to DHT and I and II block, to a limited extent, the conversion of DHT to 3α-diol. This leads to the conclusion that, while weak, there is 5α-3α-hydroxysteroid dehydrogenase inhibitory activity of I and II and very little of this activity in III. These results seem to indicate that these inhibitors may have potential use in specifically blocking the conversion of 5α-3β-ol-androstanes to 5α-3-keto-androstanes in in vivo systems.

It has been demonstrated that 3β-diol has the ability to maintain spermatogenesis in hypophysectomized rats (10) and promote the growth and secretory activity of the prostate glands and seminal vesicles (11) in hypophysectomized as well as castrated rats. Administration of a 5α-3β-hydroxysteroid dehydrogenase inhibitor to the hypophysectomized, 3β-diol treated rat will help elucidate if 3β-diol does indeed have activity of its own or must first be converted to a more active metabolite. Similarly, if a specific inhibitor of 5α-3α-hydroxysteroid dehydrogenase can be found, then an in vivo model can be utilized which may help differentiate the inherent biological activity of all three 5α-reduced androgens, namely 3α-diol, 3β-diol and DHT.

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