method of Furchgott and Bhadrakom.[35] They were mounted in a 5-ml organ bath containing Krebs solution (NaCl, 11 mmol; $NaHCO_3$, 25 mmol; KCl, 5 mmol; NaH_2PO_4, 1 mmol; $MgCl_2$, 0.5 mmol; $CaCl_2$, 2.5 mmol, and dextrose, 11.5 mmol) at 37° and were aerated with a mixture of 95% O_2 and 5% CO_2. The strips were placed under 1 g passive tension and allowed to equilibrate for 1.5–2.0 hr. Isometric contractions were recorded using a Grass force displacement transducer (FT-03) on a Grass polygraph (Model 7).[4]

In order to evaluate the mechanism of antagonism, the criteria used by Schild were used.[36] First a log dose–response curve for angiotensin II was determined on aortic strips, before and in the presence of the analogs. Next, the dose ratio of angiotensin II was calculated in the presence of the various analogs at several molar concentrations. The calculated log K_2 values and pressor activities are given in Table I.

Acknowledgments. This investigation was supported in part by Grant No. HL-6835 and General Research Support Grant FR-5674. The authors wish to express their appreciation to Dr. S. Sen for determining the pressor activities and to Messrs. W. L. Maloy, R. A. Leese, and W. Brashear for their valuable assistance.

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Uptake of Androgen Analogs by Prostate Tissue†

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A study of the *in vitro* binding of the three radioactive androgens 1,2-[^{3}H]-5α-dihydrotestosterone (**3**), 17α-[^{14}C]methyl-5α-androstan-17β-ol (**2**), and 17α-[^{14}C]methyl-5α-androst-2-en-17β-ol (**10**) to minced rabbit ventral prostate is described. The competitive effect of several other steroids on the binding is also outlined. The resulting data are explained most readily by an assumption of the presence of three different binding sites for such steroids: the "classical" dihydrotestosterone site, as well as separate sites for the hydrocarbon and olefin derivatives. The implications of this for structure–activity relationships in androgen analogs are discussed.

In previous papers we evaluated the structural characteristics of ring A in steroidal androgens which have importance in eliciting the characteristic biological response to these substances.[1] We concluded that a six-membered or equivalent A ring, flattened at C-2 and/or C-3, is required for activity. However, certain steroids having an unsubstituted A ring or a single C-3 substituent, such as androstan-17β-ol[2] (**1**) and 5α-androstane,[3,4] represent androgens which do not fit into this hypothesis. One reason for this could be metabolic conversion, for example, oxygenation of an A-ring hydrocarbon to an active 3-keto derivative. Recently, we have investigated this possibility[5] and found that 17α-methyl-5α-androstan-17β-ol (**2**), which has clinically useful[6] anabolic

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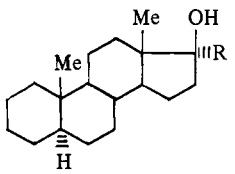
and androgenic activity,[7] is metabolized extensively by rabbit liver homogenate to 3-hydroxy compounds and the 3-ketone **4.** Moreover, the 3α-hydroxy derivative **6** is metabolized to the 3-ketone 17α-methyldihydrotestosterone **(4).** These findings are compatible with the possibility that the A-ring hydrocarbon and 3α-hydroxy androgens exert their effect through conversion to dihydrotestosterone derivatives. However, the results do not exclude the possibility that these compounds function directly as androgens. In order to explore this question, we examined the binding of such compounds directly to target organs.

The general subject of the mechanism of steroid action has been an area of intensive research during the past decade and has been reviewed.[8] In brief, upon entrance into a target cell, the steroid binds to cytoplasmic protein receptor(s) and the resulting complex is transported to the nucleus where it interacts with nuclear acceptor(s) and transcription processes are affected.



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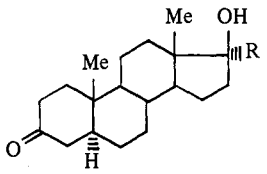

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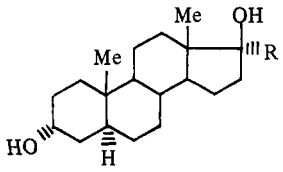

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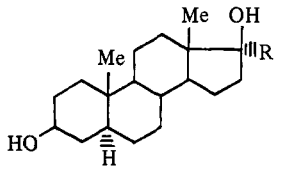

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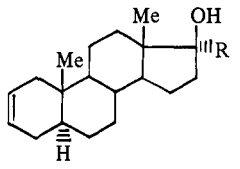

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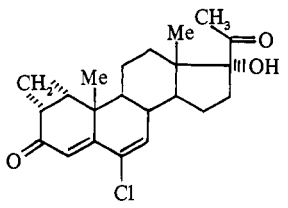

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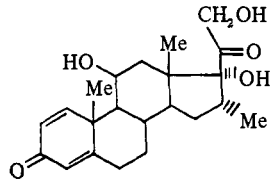

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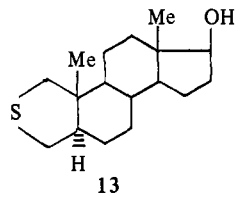

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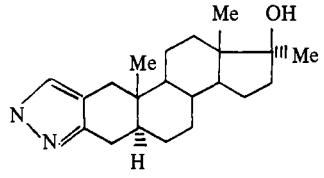

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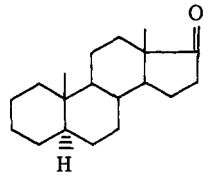

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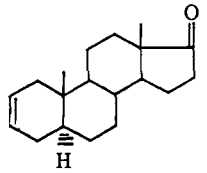

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Receptor proteins for androgens and the mode of action of androgens on gene transcription have been reviewed by Liao and Fang.[9] Ventral prostate, a target organ, selectively retains dihydrotestosterone formed from the metabolism of testosterone, in comparison to blood and other tissues.[10] Moreover, dihydrotestosterone is specifically retained by prostatic nuclei *in vitro.*[11] Despite much work in this area, it is still not clear whether testosterone and 5α-dihydrotestosterone have different or the same cytosol receptor sites and the site of testosterone binding in the nucleus is also unclear.[12] However, it is apparent that there is more than one androgen receptor even at the cytosol receptor level. We felt that a study on the binding of various synthetic androgens to target organs would be helpful in determining the type of receptor involved in binding various androgens.

We studied the *in vitro* binding of the three radioactive androgens 1,2-[^{3}H]-5α-dihydrotestosterone **(3)**, hydrocarbon **2** (^{14}C-17α-Me), and Δ^2 olefin **10** (^{14}C-17α-Me) to minced rabbit ventral prostate. Particular attention was given to determining whether the retention of these labeled steroids occurs at the same or different binding sites in the prostate. In competition experiments, the effect of several other nonradioactive steroids, such as 17β-hydroxy-17α-methyl-5α-androsten-3-one **(4)**, 17α-methyl-5α-androstane-3α,17β-diol **(6)**, 17α-methyl-5α-androstane-3α,17β-diol **(8)**, cyproterone **(11)**, and dexamethasone **(12)**, on the retention of these labeled androgens by rabbit ventral prostate was also investigated.

The effect of dexamethasone was examined since it has been shown[13] that cyproterone blocks the androgenic effect of androgen analogs whereas dexamethasone blocks the anabolic effects in levator ani preparations. Although these authors were unable to find effects on ventral prostate preparations under these conditions, our finding of several receptor sites in this preparation made it of interest to examine the action of these selective inhibitors.

Experimental Section

Steroids.§ **17α-[^{14}C]Methyl-5α-androstan-17β-ol (^{14}C-2)** and **17α-[^{14}C]methyl-5α-androst-2-en-17β-ol (^{14}C-10)** were obtained by a Grignard reaction of 5α-androstan-17-one **(15)** and 5α-androst-2-en-17-one **(16)**, respectively, with $^{14}CH_3MgI$. To $^{14}CH_3I$ (total 4 mCi, specific activity 5 mCi/m*M*; International Chemical & Nuclear Corp., Lot 554558) 1.82 ml of unlabeled CH_3I was added. The resulting CH_3I (total 30 m*M*) was then added to a stirred solution of 1.08 g of Mg (45 m*M*) in 30 ml of Et_2O over a 45-min period. The mixture was refluxed for 1.5 hr. A solution of 2 m*M* steroid (**15**, 548 mg; **16**, 544 mg) in 30 ml of anhydrous C_6H_6 was added to 12 ml of the Grignard solution prepared above. The solvent was distilled off until the boiling point reached 78° and refluxing was continued for 5 hr. After cooling the mixture, 10 ml of 3 *M* CH_3MgBr (Alfa Inorganics) in Et_2O was added, the solvent was again distilled until the boiling point reached 78°, and then the reaction mixture was refluxed for 3 hr. After the reaction mixture was stirred overnight at room temperature, a solution of NH_4Cl (1.4 g) in 25 ml of H_2O was added dropwise with stirring. The aqueous phase was extracted alternately with 30 ml of $CHCl_3$ and 20 ml of EtOAc. The combined extract and C_6H_6 phase were washed with dilute HCl (1:3), 5% $NaHCO_3$, and H_2O. The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to afford the crude products, 480 mg of **2** and 460 mg of **10**, respectively. Recrystallization twice from Me_2CO gave 230 mg of **2** and 200 mg of **10.** Further purification and chemical purity check of these radioactive products were carried out by preparative layer chromatography and tlc (C_6H_6–Et_2O, 5:1) to obtain radiochemically pure ^{14}C-**2** and ^{14}C-**10.** The specific activities of ^{14}C-**2** and ^{14}C-**10** were 7.38 × 10^5 dpm/mg (90.9 μCi/m*M*) and 8.12 × 10^5 dpm/mg (99.5 μCi/m*M*), respectively.

17α-Hydroxy-1,2-[^{3}H]-5α-androstan-3-one (^{3}H-3) was purchased

§We thank Dr. C.-T. Peng for advice concerning the synthesis of labeled compounds.

from New England Nuclear Corp., specific activity 44 Ci/m*M*, Lot 635-068. **Cyproterone** [6-chloro-17α-hydroxy-1α,2α-methylenepregna-4,6-diene-3,20-dione **(11)**] was a gift from Dr. U. Kerb, Schering A-G, Berlin, whom we thank. **Dexamethasone** [9α-fluoro-11β,17α,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione **(12)**] was a gift from Dr. Ralph F. Hirschmann, Merck Sharpe & Dohme, Rahway, N. J., whom we thank. Nonradioactive **17α-methyl-5α-androstan-17β-ol (2)**, **17α-methyl-5α-androstane-3α,17β-diol (6)**, **17α-methyl-5α-androstane-3β,17β-diol (8)**, and **17β-hydroxy-17α-methyl-5α-androstan-3-one (4)** were prepared as described in a previous publication.[5] **17α-Methyl-5α-androst-2-en-17β-ol (10)** was prepared by treatment of **16** in anhydrous C_6H_6 with MeMgBr. The procedures were essentially the same as described above in the preparation of ^{14}C-**10**.

In Vitro **Experiments.** Ventral prostates were excised from intact rabbits and minces of the prostatic tissue were suspended in Krebs–Ringer phosphate buffer (pH 7.4) containing radioactive steroids and incubated under an atmosphere of air for various intervals (15 min to 2 hr) at 37° in a shaking bath. Each incubation contained approximately 100 mg of the prostate minces in 4 ml of Krebs–Ringer solution containing the appropriate amount of radioactive steroid dissolved in EtOH. Control samples were prepared in a medium containing the same volume of EtOH (25–200 μl). The amounts of the labeled steroids incubated were 0.005 μg for ^{3}H-**3** and 10–200 μg for ^{14}C-**2** and ^{14}C-**10**. In the competition experiments, 25–300 μg of the appropriate nonradioactive steroid was added to the incubation mixture.

After the incubation was terminated, the prostate tissue was blotted with filter paper and washed in 5 ml of steroid-free Krebs–Ringer solution for 15 min to 2 hr at 37° in a shaking bath. The tissue was blotted, dried on filter paper to remove all moisture, transferred to weighed scintillation vials, and weighed. The weight of the tissue was obtained by difference. To each vial 3 ml of $CHCl_3$–MeOH (2:1) was added and the vial was left in a refrigerator overnight to extract radioactive components. The solvent was evaporated to dryness under N_2 or air flow and the sample was subjected to scintillation spectrometry to determine radioactivity.

Liquid Scintillation Counting. The radioactivity in each sample was counted in a Packard Tri-carb liquid scintillation spectrometer, Model 3375. Samples were deposited in nylon scintillation vials, and to each scintillation vial was added 14 ml of scintillation liquid consisting of 5 g of PPO (2,5-diphenyloxazole) and 100 mg of POPOP (1,4-bis[2-(5-phenyloxazolyl)]benzene) in 1 l. of scintillation grade toluene. Quenching due to the prostate tissue and trace amounts of organic solvent was corrected by the external standard ratio method. The counting efficiency of this system ranged from 26 to 32% for ^{3}H and from 88 to 91% for ^{14}C. The analyses of radioactive components were carried out by tlc and glpc.

Characterization of Radioactive Steroids. Separations of the labeled steroids in question were achieved on 6 × 20 cm silica gel tlc plates (2 mm thick, Brinkmann) along with authentic carrier steroids. The plate was developed with C_6H_6–$CHCl_3$–Me_2CO (80:15:5). After the development the plate was sprayed with ceric ammonium sulfate reagent consisting of 40 g of ceric ammonium sulfate and 70 ml of concentrated H_2SO_4 in enough water to make 400 ml and heated at 150° for 10–15 min. Each of the spraying reagent positive zones corresponded to the authentic standards which had been added to radioactive samples and the migration path on the plate was marked and divided into horizontal bands. Each band was scraped and placed into a scintillation vial, 14 ml of scintillation liquid was added to the vial, and the sample was assayed for radioactivity. External standardization was used to correct for quenching with silica gel in a counting vial. A Varian Aerograph Series 2100 gas chromatograph equipped with an ionization detector and a 1:1 glass splitter was used. A 1.8-m glass column (0.8 cm inside diameter) was packed with 3% OV-1 on 100–200 mesh Gas Chrom Q. Conditions employed were: column temperature 205°; injector 250°; detector 300°; He 30 ml/min; air 2.8 kg/cm^2; and H_2 1.4 kg/cm^2. The vapors eluted from the column were condensed in capillary tubes by use of a splitter, and each sample for radioactivity determination trapped from the effluents was washed into a counting vial containing 14 ml of scintillation fluid.

Results and Discussion

Although a number of studies on the binding of androgens like dihydrotestosterone to specific fractions of cytosol and nuclear proteins have been carried out, the present study describes the binding of androgens to prostatic minces. We felt it important to begin with these highly heterogeneous preparations in order to avoid the possibility of missing important binding components. This could happen if binding to only a few prostatic components were studied. There is, moreover, evidence that binding to whole prostatic tissue is subject to the same factors as binding to nuclear components. Thus, it has been shown that cyproterone acetate inhibits the formation of nuclear 5α-dihydrotestosterone protein complex during incubation of minced ventral prostate *in vitro* with tritiated 5α-dihydrotestosterone.[14] In short, we were interested in observing the retention of various androgens by a heterogeneous unfractionated target organ preparation.

The possibility of metabolism of the hydrocarbon **2** and the olefin **10** was examined by incubation of the minced prostate tissue with these labeled steroids for 2 hr at 37° In neither case was there significant radioactivity found other than from the parent compound when extracts of these homogenates were examined by thin-layer chromatography. The effect of time on the uptake and retention of the labeled hydrocarbon **7** was studied by incubating the minced rabbit prostate with this material for periods ranging from 15 min to 2 hr. Retention of radioactivity increased gradually during this time course as shown in Figure 1. The effect of varying the concentration of ^{14}C-**7** in the incubation medium was next examined. Radioactivity retained by prostate tissue increased with increasing concentration of steroid and reached a plateau at between 100 and 200 μg per experiment (Figure 2). Finally, the effect of washing on retained radioactivity was examined (Figure 3). Some of the retained radioactivity was removed upon washing, and the amount increased with time. A plateau level of about 83% retention was reached between 30 and 60 min and as much as 2 hr of washing did not decrease this value. These preliminary experiments, therefore, served to determine the amount of steroid incubated, the length of the incubation, and the amount of washing required. In all subsequent *in*

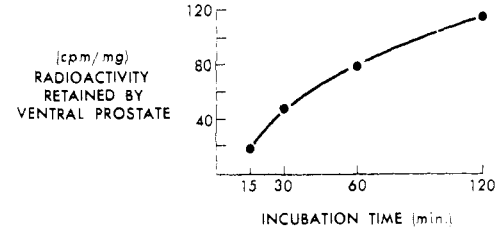

Figure 1. The uptake of radioactivity by minced rabbit ventral prostate at various time intervals following incubation with 100 μg of 17α-[^{14}C]methyl-5α-androstan-17β-ol at 37°. Each point on the curve represents the mean value of duplicate determinations.

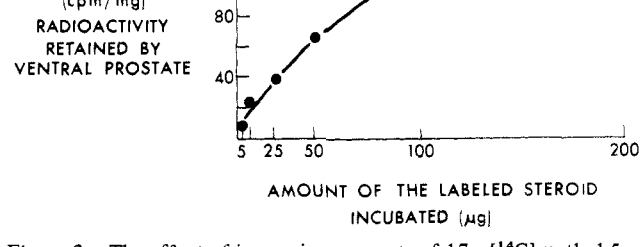

Figure 2. The effect of increasing amounts of 17α-[^{14}C]methyl-5α-androstan-17β-ol on the retention of radioactivity in minced rabbit ventral prostate after incubation with 100 μg of the steroid for 2 hr at 37° followed by washing in a hormone-free medium at 37° for 30 min. Each point on the curve represents the mean value of duplicate determinations.

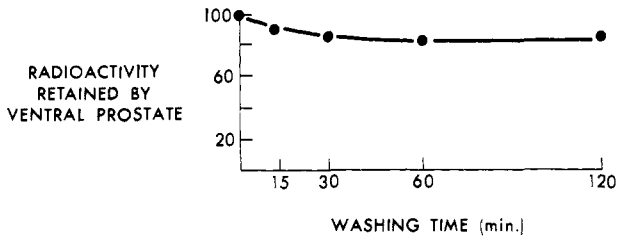

Figure 3. The amount of radioactivity remaining in the minced ventral prostate in per cent of the controls at different tiine of washing in a hormone-free medium at 37°. Each point on the curve represents the mean value of duplicate determinations.

Table I. Binding of 17α-[^{14}C]Methyl-5α-androstan-17β-ol (2) to Rabbit Prostate[a]

Nonlabeled steroids added	Radioactivity in the minced ventral prostate (cpm/mg of tissue) Without nonlabeled steroid (control)	With nonlabeled steroid (competition)	% of inhibition of radioactivity uptake
HC (2)	117.5 ± 10.3	52.2 ± 5.5	63.2
3α-OH (6)	119.8 ± 6.8	65.9 ± 3.2	45.0
3β-OH (8)	106.9 ± 9.2	60.4 ± 4.0	43.5
CYP (11)	88.6 ± 5.2	90.4 ± 7.3	0
DEX (12)	102.8 ± 10.7	97.1 ± 9.8	0
17α-Me-DHT (4)	93.3 ± 10.0	101.5 ± 10.4	0
Δ^2 (10)	110.4 ± 9.6	115.4 ± 11.2	0
2-Thia (13)	93.5 ± 7.4	98.7 ± 5.0	0
Stanazolol (14)	100.7 ± 10.6	111.7 ± 9.4	0

[a]The effect of unlabeled steroids on the uptake of 17α-[^{14}C]-methyl-5α-androstan-17β-ol by minced rabbit ventral prostate. For each set of competition experiments, 100 μg of the labeled steroid was coincubated with 200 μg of unlabeled steroid. The results are presented as cpm/mg wet tissue ± standard error for four determinations.

vitro competition experiments, the incubation was carried out for 2 hr and the time of washing with steroid-free medium was 30 min at 37°

In Table I, the binding of the 17α-methyl hydrocarbon **2** to minced prostate is recorded. The binding of the labeled material is reduced by addition of unlabeled steroid. Moreover, the addition of the 3α-hydroxy compound **6** and 3β-hydroxy steroid **8** effects significant reductions in the binding of labeled **2**. It is noteworthy that neither cyproterone **(11)** nor dexamethasone **(12)** nor any of the other steroids examined affects the binding of labeled **2**. By contrast, in Table II is shown the results of studying the binding of labeled Δ^2 compound **10** to the prostate tissue. In this case, only the addition of unlabeled **10** or the addition of the 2-thia compound **13** inhibits the binding. All of the other compounds examined, including cyproterone **(11)** and dexamethasone **(12)**, are without effect on the binding. Finally, in Table III is shown the effect of various compounds on the binding tritiated dihydrotestosterone **(3)** to the tissue. In this case, 17α-methyldihydrotestosterone **(4)**, as well as cyproterone **(11)** and dexamethasone **(12)**, reduces the binding. In addition, the 2-thia compound **13** and stanazolol **(14)** reduce the binding.

It is seen that an interesting pattern of displacement activ ities results. The data are explained most readily by an assumption of the presence of three different binding sites for these materials. One of these is the "classical" site for dihydrotestosterone. This binding site binds both dihydrotestosterone, as well as the 17α-methyl analog. The inhibition of binding at this site by cyproterone has already been observed by Fang and Liao[14] who found that cyproterone

Table II. Binding of 17α-[^{14}C]Methyl-5α-androst-2-en-17β-ol to Rabbit Prostate[a]

Nonlabeled steroids added	Radioactivity in the minced ventral prostate (cpm/mg of tissue) Without nonlabeled steroid (control)	With nonlabeled steroid (competition)	% of inhibition of radioactivity uptake
Δ^2 (10)	108.0 ± 8.5	68.0 ± 5.0	57.8
2-Thia (13)	116.5 ± 6.6	97.3 ± 4.3	16.5
3α-OH (6)	97.0 ± 7.6	94.4 ± 6.1	0
3β-OH (8)	102.4 ± 8.1	106.0 ± 6.8	0
HC (2)	112.6 ± 6.4	109.2 ± 10.4	0
17α-Me-DHT (4)	100.2 ± 9.8	98.8 ± 5.4	0
CYP (11)	91.7 ± 8.4	93.7 ± 6.2	0
DEX (12)	89.2 ± 6.3	82.5 ± 8.0	0
Stanazolol (14)	127.1 ± 10.7	120.4 ± 9.1	0

[a]The effect of unlabeled steroids on the uptake of **10** by minced rabbit ventral prostate. For each set of competition experiments, 100 μg of the labeled steroid was coincubated with 200 μg of unlabeled steroid. The results are presented as cpm/mg net tissue ± standard error for four determinations.

Table III. Binding of 17β-Hydroxy-1,2-[^{3}H]-5α-androstan-3-one (3) to Rabbit Prostate[a]

Nonlabeled steroid added	Radioactivity in the minced ventral prostate (cpm/mg of tissue) Without nonlabeled steroid (control)	With nonlabeled steroid (competition)	% inhibition of radioactivity uptake
17α-Me-DHT (4)	1284.9 ± 68.6	875.0 ± 49.1	31.9
CYP (11)	1075.7 ± 24.4	689.5 ± 40.3	35.9
DEX (12)	1166.2 ± 75.6	939.8 ± 31.8	19.4
CYP + DEX[b]	1003.1 ± 56.0	623.1 ± 25.2	38.0
2-Thia (13)	1183.3 ± 88.2	778.8 ± 36.7	25.8
Stanazolol (14)	1428.2 ± 135.6	1030.0 ± 58.2	27.9
3α-OH (6)	1015.5 ± 73.4	1026.3 ± 61.4	0
3β-OH (8)	972.4 ± 58.5	946.6 ± 44.2	0
HC (2)	1074.9 ± 106.4	1048.0 ± 50.5	0
Δ^2 (10)	1027.4 ± 34.2	1013.6 ± 48.7	0

[a]The effect of unlabeled steroids on the uptake of **3** by minced rabbit ventral prostate. For each set of competition experiments, 100 μg of the labeled steroid was coincubated with 200 μg of unlabeled steroid. The results are calculated at cpm/mg net tissue ± standard error four determinations. [b]The amounts of CYP and DEX were 100 μg each.

suppressed the uptake of radioactive androgens *in vivo* by the ventral prostate of rats. By contrast, the Δ^2 binding site apparently does not interact with any of the steroids which interact with the dihydrotestosterone site with the exception of the 2-thia compound **13**. This interaction with the 2-thia compound would be expected on the basis of isosteric theory since **10** and **13** are isosteric. Still a third site, the hydrocarbon site, does not interact with any of the substances which interact with either the Δ^2 site or the dihydrotestosterone site.

Whether these are receptors in the cytosol or nucleus, or both, and whether they are different sites on the same macromolecule or represent different macromolecules is, of course, still an open question. It is clear, however, that the different androgens are involved in binding at different sites. This must, of course, be considered in connection with the structure-function requirements for androgens.

These results also suggest future lines of research. One of these is to fractionate the cytosol proteins and to try to localize the binding sites more distinctly. Another is to study a wider range of androgens and their interaction with these sites. The result of such studies should give a better

insight than we have at present regarding the structure-function relationships in androgen molecules.

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Solid-Phase Synthesis and Some Pharmacological Properties of Deamino-4-threonine Analogs of the Vasopressins and Vasotocin and [Deamino]arginine-vasotocin†

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[Deamino,4-threonine]arginine-vasopressin (I), [deamino,4-threonine]lysine-vasopressin (II), [deamino,4-threonine]arginine-vasotocin (III), and [deamino]arginine-vasotocin (IV) were synthesized by the solid-phase method and tested for their biological activities. Compounds I–III exhibited a general reduction in rat uterus, fowl vasodepressor, and rat vasopressor potencies but possessed a selective enhancement of antidiuretic potencies when compared to the parent [1-amino-4-threonine] analog in each case. Thus, I–III exhibited diminishments of 45, 80, and 70% in rat uterus activity; 40, 27, and 5% in fowl vasodepressor activity; 70, 80, and 70% in rat vasopressor activity. I–III exhibited enhancements of 230, 250, and 25% in antidiuretic activity. This selectivity has resulted in the following antidiuretic to pressor ratios: I, 25; II, 54; and III, 11. The corresponding ratios in arginine-vasopressin, lysine-vasopressin, and arginine-vasotocin are 0.9, 1.1, and 1.4. Upon bioassays, IV was found to possess 251 ± 12 units/mg of rat uterus activity, 1174 ± 24 units/mg of fowl vasodepressor activity, 890 ± 100 units/mg of antidiuretic activity, and 256 ± 6 units/mg of rat vasopressor activity. These represent increases of 100, 138, 280, and 60% over the corresponding values for arginine-vasotocin.

In a preceding paper[2] we have shown that substitution of the glutamine residue in position 4 of the basic neurohypophysial peptides by a threonine residue resulted in a series of analogs exhibiting on the one hand a selective enhancement of oxytocin-like characteristics and on the other hand a selective diminishment of vasopressin-like characteristics, while at the same time giving rise to an increase in the ratio of antidiuretic to rat pressor activities in each of the three analogs studied.

It was speculated that an enhancement of the overall lipophilicity of each 4-threonine-substituted molecule as compared to the parent molecule might contribute to the observed antidiuretic-pressor selectivity. Earlier experiments had shown that the removal of the amino group from the [4-threonine] analogs of oxytocin and mesotocin had given rise to deamino analogs possessing greatly enhanced lipophilic properties.[3] It was thus considered worthwhile to try to explore this speculation further by preparing the analogous [deamino,4-threonine] analogs of the basic neurohypophysial peptides, *i.e.*, [deamino,4-threonine]arginine-vasopressin, [deamino,4-threonine]lysine-vasopressin, and [deamino,4-threonine]arginine-vasotocin. The present investigation was further prompted by the surprising properties exhibited by both [deamino,4-threonine]oxytocin and [deamino,4-threonine]mesotocin.[3] Instead of possessing the enhancement of activities which markedly characterized previously prepared deamino oxytocin analogs,[4-8] both analogs were found to possess markedly diminished potencies, by comparison with the parent [4-threonine] analog in each case, in all of the characteristic assay systems. The present investigation was therefore also undertaken to determine whether or not removal of the amino group from the 4-threonine analogs of the basic neurohypophysial peptides would bring about these same diminished effects[3] or an enhancement similar to that observed upon deamination of arginine-vasopressin[9] and lysine-vasopressin.[10]

The synthesis of [deamino]arginine-vasotocin has recently

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