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METABOLISM OF ANDROGENS BY THE BABOON KIDNEY

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SUMMARY

To study the role played by the kidney in androgen metabolism, a mixture of differently labeled testosterone (T) and dihydrotestosterone (DHT) was injected into the renal artery of adult male baboons and urine collected separately from each kidney. The excretory pattern obtained in the very early urine collections (5–45 min after injection) reflected the renal metabolism and/or conjugation of the androgens administered. A rapid rate of excretion of both labels occurred during the initial 15 min after injection, reflecting the renal handling of the androgens. The salient features consisted of glucuronidation of the bulk of excreted metabolites and conversion of significantly more DHT than T to 3α-androstanediol by the kidney and probable different pathways involved in the conversion of T and DHT to androsterone by extra-renal tissues. The possible significance of the results is discussed.

INTRODUCTION

That the kidney is under control of androgens, the so-called renotropic action of these hormones, has been indicated by the restoration by replacement therapy of kidney size in castrated mice [1–3]. Specific receptors for steroids, including androgens, as well as systems capable of metabolizing and/or conjugating steroids have been demonstrated in the kidneys of several species. Our laboratory has been engaged in an ongoing study whose aim is to try to answer the question as to whether there is an anatomic or other relationship between the presence of the receptors and the metabolizing and/or conjugating systems in the kidneys of various species, in view of the variability of these systems among animals.

To shed light on this question, we have compared the metabolism of T and DHT in the baboon kidney by injecting a mixture of both androgens into a renal artery and identifying the metabolites in urine collections from each ureter.

EXPERIMENTAL

[1,2-^{3}H]-DHT (40.3 Ci/mmol), [4-^{14}C]-T (57 mCi/mmol), [1,2-^{3}H]-T (40.0 Ci/mmol), [4-^{14}C]-Δ^4AD (57.5 mCi/mmol), [1,2-^{3}H]-androsterone (25.0 Ci/mmol), [1,2-^{3}H]-5αAD (42.7 Ci/mmol) and [1,2-^{3}H]-etiocholanolone (42.7 Ci/mmol) were purchased from New England Nuclear Corporation. These compounds were checked for purity by thin layer chromatography before use, as described below.

The following materials were also purchased: Unlabeled steroids from Steraloids, Inc., DEAE-Sephadex A-25 from Pharmacia Fine Chemicals, Inc., Lipidex 5000 from Packard Instruments, Inc., β-glucuronidase type B-1 from Sigma Chemical Co., and saccharolactone as an inhibitor of β-glucuronidase from Calbiochem. All other reagents were analytical grade.

Subjects and injection of steroids

[^{3}H]-DHT and [^{14}C]-T in an equimolar mixture (9.5×10^{-8} M) were injected into the left renal artery of adult male baboons (*Papio cyanocephalus*, 30–40 kg) as previously described [4]. Through bilaterial ureteral catheters inserted before-hand, urine was collected separately from each kidney 5, 15, 30, 45 min and 1, 2, 3, 4, 5 and 6 h after injection. Urine samples were adjusted to pH 9 with concentrated ammonia and kept at 4°C until analyzed.

To investigate the intra-renal fate of the injected androgens, both venous and urinary output of the injected side were stopped by clamping the renal vein and the ureter immediately after a mixture of [^{3}H]-T and [^{14}C]-Δ^4AD was injected into the renal artery. Five minutes later, the kidney was excised and the steroid extracted.

Analysis of urine samples

After determination of the amount of radioactivity in the urine, aliquots were applied to DEAE-Sephadex columns. The separated conjugates were hydrolyzed enzymatically and the liberated aglycones were separated by Lipidex 5000 column chromatography. The resulting peaks were further purified by TLC and were finally identified by co-crystallization to constant specific activity upon admixture with authentic standards.

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Abbreviations: DHT = dihydrotestosterone = 17β-hydroxy-5α-androstan-3-one; T = testosterone = 17β-hydroxyandrost-4-ene-3-one; androsterone = 3α-hydroxy-5α-androstan-17-one; 3α-androstanediol = 5α-androstane-3α,17β-diol; etiocholanolone = 5β-androstane-3α-ol-17-one; etiocholanediol-5β-androstane-3α,17β-diol; Δ^4AD = Δ^4-androstenedione = androst-4-ene-3,17-dione; 5αAD = 5α-androstane-dione = 5α-androstane-3,17-dione

Extraction of steroids from the kidney

After dissecting the adherent connective tissue, the kidney was sliced with scissors and homogenized in four volumes of phosphate buffer (pH 7.4) in a Polytron Homogenizer (Model PT10-35, Brinkman Instruments). The homogenate was stored for 24 h in 80% acetone and the resulting pellet was removed by centrifugation (2500 rev./min for 10 min, *ca.* 1000 *g*). Extraction with three volumes of ethyl acetate (three times) separated free steroids (organic phase) from conjugates (aqueous phase).

Chromatography

1. DEAE-Sephadex A-25 column chromatography. To fractionate the conjugates, aliquots of the urine sample were directly applied according to methods reported previously [5]. A multiple column system was used (in order of flow-through: K9/60, K9/30 and K9/15, Pharmacia Fine Chemicals). The columns were eluted with 1200 ml of a linear NaCl gradient (0 to 0.4 M) with a flow rate of approximately 20 ml/h. Fractions (10 ml each) were collected.

2. Lipidex 5000 column chromatography. The aglycones resulting from enzymatic hydrolyses were separated into metabolite groups by this column chromatography. Lipidex columns (10 × 190 mm Pyrex No. 7282) were eluted with 450 ml of 10% benzene in hexane, followed by 100 ml of 20% chloroform in hexane for elution of polar metabolites. The flow rate approximated 60 ml/h, with 5 ml fractions being collected.

3. Instant thin layer chromatography (ITLC-SA, Gelman Instrument Co.). The system of chloroform–methanol (98:2, V/V) was used to purify the separated metabolites from the Lipidex 5000 column chromatography.

4. TLC with neutral alumina (Analtech, Inc.). The system used to separate androsterone from DHT [6] was: methylene chloride:ether (9:1, V/V). In our hands, a distinct separation (R_F 0.36 and 0.53 for androsterone and DHT, respectively) was obtained for two consecutive developments. Identification of the separated radioactive peaks by co-crystallization with authentic standards substantiated the reliability of this method.

Radioactivity was determined in Packard Instruments Tri-Carb liquid scintillation counters, models 3375 or 2450, with 10 ml of ACS, Amersham-Searle or Aquasol, New England Nuclear Corp. scintillation fluid. The efficiency for double label counting was about 40% for ^3H and 70% for ^{14}C under the conditions used. To determine the radioactivity on TLC plates, a Packard Radiochromatogram Scanner model 7201-C was used. The methods of enzymatic hydrolyses and details of principles of interpretation of excretion data have been reported elsewhere [4].

RESULTS

The urinary excretion of radioactivity after injection of [^3H]-DHT and [^{14}C]-T into the left renal artery is shown in Fig. 1. Within 15 minutes, the excretion rate from the injected side decreased appreciably, and thereafter, the rates from the injected and non-injected sides were almost the same. After the initial rapid decrease in excretion, radioactivity was eliminated slowly with a gradually diminishing rate over the collection period of 6 hours. Within this period of time, 62% of the ^{14}C and 68% of the ^3H of the injected labels were excreted.

Urine samples from either injected or non-injected sides were combined separately into groups for subsequent fractionation according to time of collection

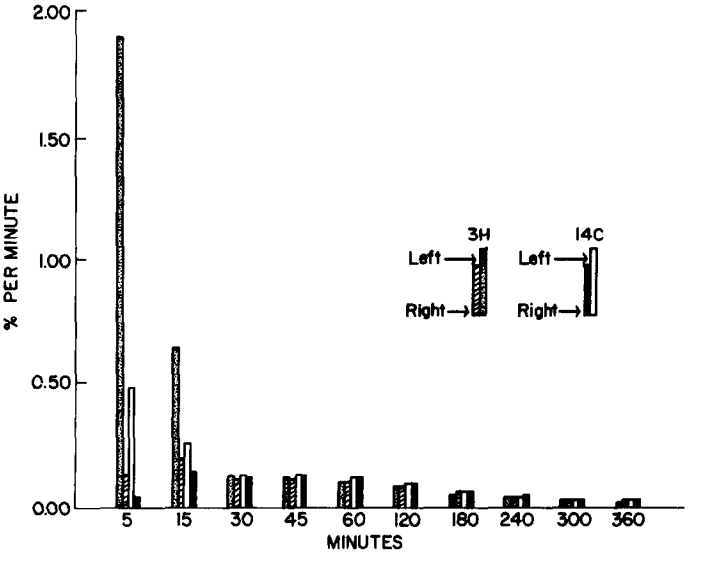

Fig. 1. Rate of excretion of ^3H and ^{14}C in the urine following the injection of [^3H]-DHT and [^{14}C]-T into the left renal artery of a baboon.

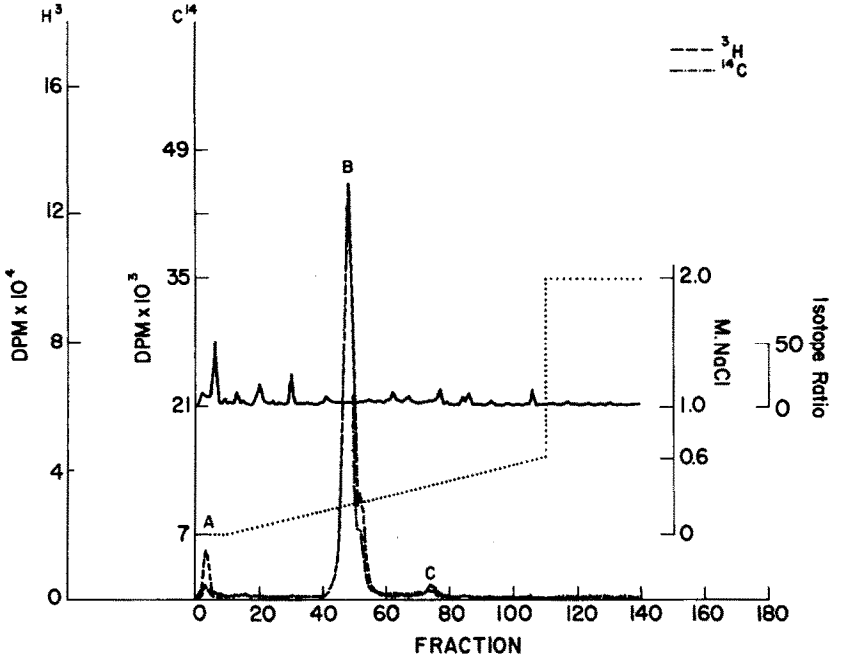

Fig. 2. Elution pattern after chromatography on DEAE-Sephadex of the 5–15 min urine from the injected side.

as follows; 0–5 min, 5–15 min, 15–120 min, 120–240 min and 240–360 min. Aliquots of each group were applied to DEAE-Sephadex columns directly for separating the conjugates. Figure 2 illustrates the elution pattern for one of the urine collection groups. In all fractionations, only three peaks of radioactivity were observed, with the preponderant amount (an average from all the urine fractions of 85% ^{3}H and 78% ^{14}C) occurring in the second peak (Peak B), which proved upon enzymatic hydrolyses to be the glucosiduronate fraction. Thus, 91% of ^{3}H and 94% of ^{14}C of the total radioactivity became ethyl-ether extractable following hydrolysis of the main peak (sample Nos 83–55) of Fig. 2 with β-glucuronidase and only 23% and 18%, respectively, when in the presence of saccharolactone. Negligible (0–3%) radioactivity was extracted in control samples. Since both free steroids found in the first peak (Peak A) and the sulfates found in the third peak (Peak C) were quantitatively minor, no further analyses of these two fractions were carried out.

The aglycones liberated by β-glucuronidase hydrolysis were separated into different groups by means of Lipidex 5000 column chromatography. Despite the almost identical elution patterns following DEAE-Sephadex chromatography, Lipidex chromatography successfully reflected the changes in metabolite profiles with time of collection (Fig. 3). Upon comparing these patterns with those obtained after identical chromatography of standard steroids, urinary metabolite peaks I, II, III, IV and V were tentatively identified as DHT plus androsterone, etiocholanolone, T, 3α-androstanediol and etiocholanediol, respectively. These identifications were confirmed, following thin layer chromatography (as described below) and co-crystallization with standards. Table 1 gives results of the quantitative determinations of the amounts of these compounds in the various urine collections.

Since DHT and androsterone standards, in our hands, eluted from Lipidex columns in the identical volume and thus could not be separated, Peak I from each group was further separated by alumina thin layer chromatography. From Table 1, it can be seen, that all DHT identified was singly labeled with ^{3}H; Peak I of the 0–5 min urine from the injected side was composed only of [^{3}H]-DHT, whereas Peak I of the 5–15 min collection (Fig. 3 and Table 1) was composed of DHT *and* doubly labeled androsterone. In the latter peak, 56% of the ^{3}H was associated with DHT; the remaining ^{3}H and all of the ^{14}C were associated with androsterone. Peaks II through V were purified by ITLC prior to crystallization.

From Table 1, it can be seen, that unchanged T (Peak III) and DHT (Peak I) were excreted rapidly with a rate maximizing in the 0–5 min urine collections, reflecting elimination mostly from the injected side. Androsterone, which was quantitatively the major urinary metabolite identified, reached a maximum output rate in the 15–20 min collection, probably reflecting the rate of metabolic formation of this compound, and its elimination from both injected and non-injected sides. Peak II, etiocholanolone, was only labeled with ^{14}C, leading to the conclusion that the compound was derived solvely from T. Peak IV, 3α-androstanediol, was derived, on the other hand, solely from DHT, but this compound was excreted both from the injected and non-injected sides (Table 1

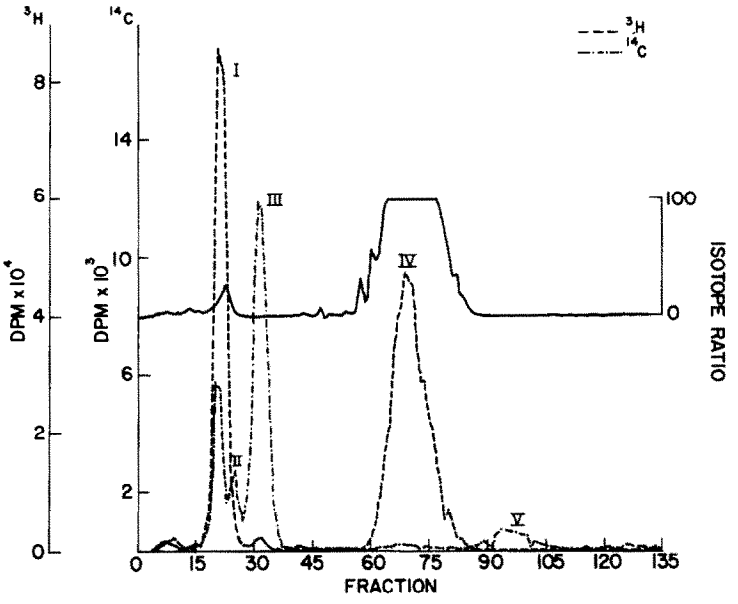

Fig. 3. Elution pattern after Lipidex chromatography for the glucosiduronate aglycones of the 5–15 min urine from the injected side. Peak I is DHT (56% of ^3H) and androsterone (44% of ^3H, 100% of ^{14}C). Peak II consists of etiocholanolone, Peak III of T, Peak IV of 3α-androstanediol and Peak V of etiocholanediol.

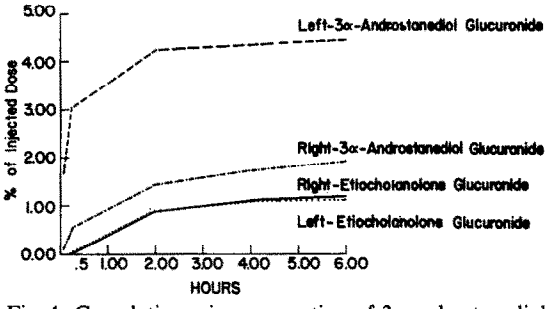

Fig. 4. Cumulative urinary excretion of 3α-androstanediol glucuronide (^3H) and etiocholanolone glucuronide (^{14}C).

and Fig. 4): the excretion rate from the injected side maximized in the 0–5 min collections, whereas that from the non-injected side maximized in the 15–120 min collections, similar to the excretion of androsterone. These data reflected the renal and extra-renal origin of the diol. The last peak (Peak V), etiocholanediol, was singly labeled with ^{14}C, being derived solvely from T.

Androsterone was the only doubly labeled metabolite identified (Table 1) and was excreted relatively slowly (compared to T and DHT), indicating its extra-renal origin. Furthermore, it was formed equally from

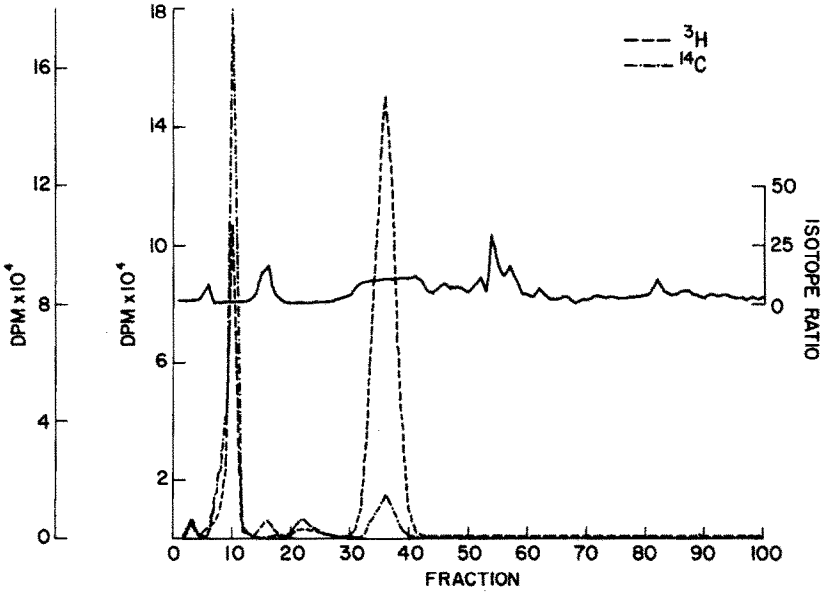

Fig. 5. Elution pattern from Lipidex 5000 column of extract from kidney.

Table 1. Excretion of androgens and their metabolites in baboon urine (% of injected dose)

Peak	Steroids†	Min.	0–5				5–15				15–120				120–240				240–360				Total			
		Side of kidney	L*		R		L		R		L		R		L		R		L		R		L		R	
		Label	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C
I	DHT		2.22	—	0.15	—	0.51	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.73	—	0.15	—
	Androsterone		—	—	—	—	0.40	0.26	0.55	0.32	4.34	4.58	4.59	5.18	0.47	0.58	1.47	1.66	1.40	1.43	0.85	0.93	6.61	6.85	7.46	8.09
II	Etiocholanolone		—	—	—	—	—	0.08	—	0.08	—	0.85	—	0.77	—	0.09	—	0.23	—	0.15	—	0.17	—	1.17	—	1.25
III	T		—	1.48	—	0.09	—	0.65	—	0.19	—	0.09	—	0.14	—	—	—	—	—	—	—	—	—	2.22	—	0.42
IV	3α-Androstanediol		1.76	—	0.10	—	1.28	—	0.49	—	1.24	—	0.86	—	0.10	—	0.32	—	0.20	—	0.16	—	4.58	—	1.93	—
V	Etiocholandiol		—	—	—	—	—	0.10	—	0.15	—	ND	—	0.76	—	ND	—	0.12	—	ND	—	0.06	—	ND	—	1.09

L = left kidney, R = right kidney.
† All the metabolites were excreted as glucosiduronates.
ND = not done‡.

both T and DHT, since its average isotope ratio in the urine collections was found to be 4.17, a value very similar to that of the injected mixture (4.02) and since its total excretion as a percent of the injected dose was 14% ^{3}H and 15% ^{14}C. 3α-Androstanediol had an intra-renal origin, but was also possibly formed extrarenally by conversion from DHT, which was absorbed from the kidney. Etiocholanolone and etiocholanediol were derived from T, since their precursor has to be a 4-ene bond prior to 5β-reduction, a reaction which probably occurs predominantly extrarenally. We have found no reference in the literature on the occurrence of 5β-reductase activity in the kidney.

The above results indicated that injected [^{3}H]-DHT was reduced to 3α-androstanediol promptly by the kidney and further converted to androsterone in extrarenal sites, whereas the injected [^{14}C]-T was metabolized to androsterone in the general circulation. The lack of urinary excretion of any ^{14}C-labeled androsterone intermediates indicated that there might be different pathways in the formation of ^{3}H- and of ^{14}C-labeled androsterone. To obtain evidence concerning this speculation, intra-renal steroids were analyzed after the administration into the renal artery of a mixture of [^{3}H]-T and ^{14}C[Δ^{4}AD] (see Experimental). After extraction of the kidney, 98% and 99% of the recovered ^{3}H- and ^{14}C-labels, respectively, were in the unconjugated fraction. Upon Lipidex 5000 chromatography, the elution pattern (Fig. 5) showed an extensive conversion of T to Δ^{4}-AD, but very little of the reverse reaction. Minute amounts of DHT and of 3α-androstanediol were detected. Further purification of the peaks on ITLC yielded two peaks (R_F 0.69 and 0.81, respectively). One peak was Δ^{4}AD, labeled with both ^{3}H and ^{14}C; the other was a less polar compound, labeled with only ^{14}C and which was identified as 5α-AD.

DISCUSSION

In contrast to estriol, which is excreted rapidly by the baboon kidneys predominantly conjugated as the 16α-glucosiduronate, it appears that the fate of androgens in these organs is analogous to those of estrone and estradiol [4], viz., they are excreted at a much lower rate than estriol, and are conjugated as glucosiduronates and sulfates, after being metabolized to other compounds.

The results presented indicate two different metabolic pathways involved in the biosynthesis of androsterone from T or DHT. It is probable that the rate-limiting step in the metabolism of T to androsterone, as delineated by the renal artery injection experiments, might be the 17β-hydroxysteroid oxidoreductase, whereas that of DHT to androsterone is the 3α-hydroxysteroid oxidoreductase. These metabolic pathways might be involved in inactivating the potent androgens (T and DHT) to the biologically "weak" androgen, androsterone. In addition, since the kidney is greatly dependent upon androgens for growth as well as function [7, 20], it is interesting to note that the androgens excreted by the kidney after renal injection of T and DHT are rather biologically inactive.

3α-androstanediol plays a unique role in the kidney. In the rat, Verhoven *et al.*[28, 29] identified three heterogenous 3α-hydroxysteroid oxido-reductases that interconvert DHT and 3α-androstanediol. They pointed to the utility of these enzymes as parameters of androgen activity and to the microsomal NADH-dependent oxido-reductase, which catalyzes the 3α-dehydrogenation of 3α-androstanediol; and which, in turn, enables the kidney to use the diol as an efficient precursor for the local formation of DHT. Based on kinetic studies in man, Kinouchi and Horton[10] speculated on the possible role of high affinity binding and metabolism of 3α-androstanediol in extrahepatic tissue. In elderly men with benign prostatic hyperplasia, Ishimaru *et al.*[11] reported that more than 50% of blood DHT was derived from secretion or production of that androgen by the testis and, more likely, by the prostate. The data in the present work support the findings that 3α-androstanediol is unique in that it has an unusually high extrasplanchnic metabolism. Furthermore, from observations that 3α-androstanediol is more potent in inducing growth of the prostate in the castrated dog than DHT, some unique role and androgenic activity was postulated for 3α-androstanediol [12–14].

In the present study insignificant amounts of DHT were present in the baboon urine following the injection of T and DHT. It is possible that receptors with high affinity for DHT exist in the kidney and this combined with a high rate of conversion of DHT to 3α-androstanediol by the renal tissue and its rapid excretion may account for the lack of DHT in the urine.

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