UPTAKE OF RADIOLABELED TESTOSTERONE, 5-α-DIHYDROTESTOSTERONE, ESTRADIOL, AND PREGNENOLONE BY CANINE PROSTATE

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Twenty-five male dogs received injections into the common sacral artery of labeled testosterone, 5-α-dihydrotestosterone, estradiol, or pregnenolone. No advantage in prostatic percent uptake was achieved by injecting radiolabeled 5-α-dihydrotestosterone as compared with its "prohormone," testosterone. An increase in the specific activity of 5-α-dihydrotestosterone by an order of magnitude was accompanied by a similar increase in percent uptake. Attempts to increase the binding capacity or binding affinity by pretreatment with testosterone had no effect. Attempts to desaturate the binding sites of endogenous androgens by pretreatment with castration was without effect. Although the same percent uptake was achieved with labeled estradiol, the uptake of the common precursor pregnenolone with the same specific activity resulted in an order of magnitude less uptake in percent dose/gm of prostate. These uptakes were not achieved using the intravenous route of administration.

Early studies with ^{14}C-testosterone showed little or no localization in the prostate (*1–4*). When tritiated testosterone of very high specific activity was used, a specific uptake of radioactivity was shown to occur in rats in the ventral prostate and other androgen-dependent tissues (*5,6*). Farnsworth (*7*) found that testosterone was converted in vivo in the rat prostate to 5-α-dihydrotestosterone which was then retained selectively in the nuclei of prostatic cells (*8*). Morfin confirmed this finding in dogs by infusing physiological amounts of ^{14}C-4-testosterone into the common arterial supply of the prostate gland and urinary bladder of five adult dogs (*9*). Recoveries from the prostate and urinary bladder accounted for 1–5% and 0.93%, respectively, of the substrate radioactivity within 8 min. Half of the prostate radioactivity was associated with two major transformation products, 5-α-dihydrotestosterone (17B-hydroxy-5-α-androstane-3-one) and 5-α-androstane-3B,17B-diol. Baulieu (*10*) has reported that 5-α-dihydrotestosterone was the most active of a number of C_{19}-steroids in promoting cell division and inducing hyperplasia in rat prostate epithelium grown in cell culture.

We have infused ^{3}H-labeled 5-α-dihydrotestosterone intra-arterially using Morfin's technique to determine whether or not the radioactivity from the prostatic *metabolite* of testosterone might be concentrated more avidly in the canine prostate than the *precursor* testosterone radioactivity. We have also compared this radioactivity concentration with that after the intra-arterial infusion of ^{3}H-labeled estradiol and the steroid precursor ^{3}H-pregnenolone with the prostatic uptake of radioactivity after labeled testosterone. We report here that ^{3}H-labeled 5-α-dihydrotestosterone and ^{3}H-labeled estradiol, but not ^{3}H-labeled pregnenolone are concentrated equally to ^{3}H-labeled testosterone of equal specific activity.

METHODS

Dogs. Twenty-five male mongrel dogs, 10–28 kg in weight, were given intra-arterial injections under pentobarbital anesthesia using a modification of the method of Morfin (*9*) in such a manner that the labeled hormone flowed through the common sacral, the visceral branches of the internal iliac, and the urogenital arteries into the prostate gland and bladder. The operative procedure was to expose one external iliac artery, ligated distally, cannulate with a plastic catheter, and thread by palpation to the bifurcation of the internal iliacs. The contralateral external iliac

Received May 21, 1973; original accepted Sept. 5, 1973.

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was clamped and slow intra-arterial injection was made into the terminal aorta and both iliac arteries.

The distribution of dogs used in the various experiments are presented in Table 1. Twenty-two dogs received 40–60 μCi of either ^{14}C-4-testosterone (*3*), ^{14}C-5-α-dihydrotestosterone, ^{3}H-5-α-dihydrotestosterone, ^{3}H-estradiol, or ^{3}H-pregnenolone intra-arterially and were sacrificed at 5 min.

Three additional dogs each received ^{14}C-4-testosterone and were sacrificed at 1 hr.

Radiolabeled steroids. The ^{14}C-4-testosterone (58.2 mCi/m*M*), ^{3}H-5-α-dihydrotestosterone (49 Ci/m*M*), ^{3}H-2,4,6,7-estradiol (85 Ci/m*M*) and ^{3}H-7-α-pregnenolone (18 Ci/m*M*) were obtained from Amersham/Searle, Chicago, Ill. The ^{14}C-5-α-dihydrotestosterone (55 mCi/m*M*) and ^{3}H-5-α-dihydrotestosterone (44 mCi/m*M*) were obtained from New England Nuclear, Boston, Mass. Radiochemical purity >97% was documented by the suppliers by paper and thin-layer chromatography and by reverse isotopic dilution analysis by recrystallization.

All radiosteroids were obtained dissolved in benzene. The benzene was evaporated and the residue dissolved in absolute ethanol. Polysorbate 80, 0.05 ml, was added and sufficient normal saline to give a total volume of 5–10 ml of 20% ethanol solution (5–10 μCi/ml).

Radioassays. Upon termination of each dog study, the animal was sacrificed with i.v. pentobarbital, and 16 tissues were routinely removed (blood; prostate, dorsal and ventral; bladder-base and fundus; testis; urethra; vas; liver; spleen; kidney: medulla and cortex; adrenal; intestines; pancreas; fat, abdominal and periprostatic; muscle, perineal and leg; heart; and lung), cleaned of fat and extraneous material and weighed. An effort was made to keep the weight of samples between 10 and 50 mg. Routinely, duplicate samples of all tissues studied were processed. After weighing, all these samples were placed in counting vials, digested in either 3 ml 10% NaOH or NCS (Amersham/Searle) overnight and then heated 10–30 min in warm water to complete digestion. One-tenth milliliter benzoyl peroxide was added to samples requiring decolorization, and all samples were neutralized with 0.1-ml glacial acetic acid. Ten milliliters of liquid scintillation fluid was added (PCS, Amersham/Searle) and the radioactivity assayed in a Nuclear-Chicago (Mark II) liquid scintillation counter. Quench corrections were made using the channel-ratio method. Counts were accumulated for a sufficient period of time to insure a statistical counting error of less than 5% and at least 95% confidence level. Results were expressed in dpm/mg and percent administered dose/gm tissue.

RESULTS

Table 1 gives all the data. No increased concentration of radioactivity occurred in canine prostate after ^{3}H-5-α-dihydrotestosterone (as compared with after testosterone) that could not be accounted for by a difference in specific activity. When ^{3}H-5-α-dihydrotestosterone was used in a specific activity of 49 Ci/m*M* the mean concentration of radioactivity (0.35% dose/gm) was about three times greater than after ^{14}C-4-testosterone (0.13%) and seven times greater than after ^{14}C-5-α-dihydrotestosterone (0.05%) used with a specific activity roughly an order of magnitude less (49 Ci/m*M* as compared with 50 mCi/m*M* and 55 mCi/m*M*, respectively).

Pretreatment with a total of 200 mg of testosterone proprionate/day over a period of 3 days i.m. with dogs then killed at 4, 7, and 11 days post-treatment to increase the binding capacity of the prostate/gm of tissue did not enhance the uptake (0.07% dose/gm) and castration 3, 5, or 9 days previously to desaturate the binding sites did not significantly enhance this uptake (0.1% dose/gm).

Radioactivity uptake from ^{3}H-estradiol of approximately the same specific activity as the ^{3}H-5-α-dihydrotestosterone was associated with a similar, or intermediate, percent uptake in the prostate (0.24%).

Uptake from the steroid pregnenolone, a precursor common to all three hormones, testosterone, 5-α-dihydrotestosterone, and estradiol, showed an order of magnitude less uptake (0.05%) than with the androgens and with estradiol. This low order of magnitude of uptake was also seen using ^{14}C-dihydrotestosterone with a specific activity an order of magnitude less than in all other experiments, and after pretreatment with testosterone proprionate for 4–11 days, and in dogs castrated 3–9 days previously.

DISCUSSION

The initial question that prompted this study was whether or not the intraprostate conversion product of testosterone, namely, 5-α-dihydrotestosterone, would concentrate more avidly in the canine prostate when given intra-arterially than when its labeled "pro-hormone", testosterone, was given intra-arterially. No advantage in percent uptake/gm of prostate of radioactivity was achieved by giving the conversion product of testosterone.

The next question was how the prostatic uptake of 5-α-dihydrotestosterone related to its specific activity. We have found that, in the range of specific activities used here, an increase in the specific activity of 5-α-dihydrotestosterone by an order of magnitude was associated with an increase in percent uptake by an order of magnitude.

We then wondered whether or not we could increase the percent uptake by increasing the number of binding sites (binding capacity) or the binding affinity of these sites, by pretreatment with testosterone proprionate. Such treatment is known to cause rapid enlargement of the canine prostate. Under the conditions of our experiment, the percent uptake/gm of prostate was an order of magnitude less than when no pretreatment was given. It is possible that the binding sites were saturated by the testosterone proprionate given for treatment.

We then investigated the possibility that castration 3–9 days previously would desaturate the binding sites of endogenous androgens. The percent uptake in dose/gm of prostate was an order of magnitude less than when no castration was used.

TABLE 1. RELATIVE TISSUE DISTRIBUTION OF RADIOACTIVITY IN DOGS FROM LABELED STEROIDS (UPTAKE OF RADIOACTIVITY IN % DOSE/GM TISSUE)*

Dog weight (kg)	Prostate	Urinary bladder	Urethra	Testes	Adrenal	Liver	Kidney	Blood	Fat	Lung	Pancreas
					^{14}C-4-testosterone (58 mCi/mM, 40–50 μCi) (5 min)						
11	0.28	0.02		0.00	0.05	0.01	0.03	0.01	0.03	0.00	0.01
14	0.08	0.04		0.05	0.03	0.01	0.02	0.01	0.00	0.01	0.00
15	0.03	0.03	0.02	0.04	0.03	0.06	0.01	0.01	0.03	0.03	0.01
Mean	0.13	0.03		0.03	0.04	0.03	0.02	0.01	0.02	0.01	0.01
					(1 hr)						
—	0.17	0.13	—	0.06	0.10	0.14	0.14	0.02	0.08	0.05	0.09
10	0.01	0.03	—	0.00	0.01	0.01	0.02	0.00	0.01	0.01	0.01
—	0.03	0.02	—	0.02	0.01	0.02	0.01	0.00	0.03	0.00	0.01
Mean	0.07	0.06		0.03	0.04	0.06	0.06	0.01	0.04	0.02	0.04
					^{3}H-5-α-dihydrotestosterone (49 Ci/mM, 40–50 μCi doses) (5 min)						
—	0.63	0.53	—	0.03	0.04	0.03	0.02	0.00	0.01	0.01	0.01
13	0.10	0.10	0.10	0.20	0.35	0.18	0.32	0.17	0.00	0.02	0.01
11	0.33	0.20	0.18	0.02	0.13	0.05	0.06	—	0.04	0.03	0.01
Mean	0.35	0.28	0.14	0.08	0.17	0.09	0.13	0.09	0.02	0.02	0.01
					^{14}C-dihydrotestosterone (55 mCi/mM, 40–50 μCi) (5 min)						
—	0.04	0.09	0.03	0.01	0.02	0.03	0.01	0.01	0.00	0.01	0.01
23	0.02	0.02	0.02	0.05	0.05	0.03	0.02	0.01	0.01	0.00	0.01
15	0.09	0.08	0.06	0.00	0.02	0.04	0.01	0.01	0.00	0.01	0.01
Mean	0.05	0.06	0.04	0.02	0.03	0.03	0.01	0.01	0.00	0.01	0.01
					^{3}H-5-α-dihydrotestosterone after pretreatment with 200 mg testosterone proprionate i.m. daily (44 Ci/mM) (4, 7, 11 days respectively)						
—	0.05	0.02	0.02	0.00	0.03	0.04	0.02	0.02	0.00	0.01	0.01
—	0.07	0.06	0.06	0.01	0.03	0.04	0.02	—	0.02	0.01	0.03
13	0.08	0.06	0.04	0.01	0.03	0.07	0.01	0.06	—	0.00	0.01
Mean	0.07	0.05	0.04	0.01	0.01	0.05	0.02	0.04	0.01	0.01	0.02
					^{3}H-5-α-dihydrotestosterone after castration (44 Ci/mM) (3, 5, 9 days, respectively)						
14	0.20	0.17	0.12	—	0.10	0.05	0.18	—	0.08	0.02	0.01
29	0.01	0.00	0.00	—	0.02	0.01	0.02	—	0.03	0.06	0.02
12	0.08	0.03	0.11	—	0.03	0.09	0.03	0.31	0.02	0.05	0.03
Mean	0.10	0.07	0.08		0.05	0.05	0.08	0.31	0.06	0.06	0.02
					^{3}H-estradiol (85 Ci/mM) (5 min)						
12	0.01	0.02	0.06	—	0.12	0.00	0.01	0.00	0.02	0.00	0.01
10	0.12	0.03	0.09	—	0.07	0.07	0.02	0.00	0.02	0.01	0.03
11	0.22	0.06	0.30	0.01	0.10	0.02	0.07	0.02	0.01	0.01	0.03
16	0.59	0.08	0.36	0.00	0.06	0.04	0.01	0.01	0.01	0.01	0.01
Mean	0.24	0.05	0.21	0.01	0.09	0.03	0.02	0.01	0.02	0.01	0.02
					^{3}H-7-α-Δ5-pregnenolone (18 Ci/mM) (5 min)						
11	0.04	0.02	0.02	0.02	0.02	0.03	0.01	0.01	0.00	0.02	0.01
8	0.08	0.02	0.04	0.04	0.07	0.04	0.07	0.07	0.02	0.05	0.04
11	0.04	0.01	0.02	0.01	0.09	0.01	0.06	0.03	0.00	0.04	0.03
Mean	0.05	0.02	0.02	0.02	0.06	0.03	0.05	0.02	0.07	0.04	0.03

* Figures rounded off to two places.

The estradiol was given to determine whether or not the uptake of androgens by the prostate in vivo was a nonspecific effect of intra-arterial infusion near the prostate. When we achieved the same order of magnitude of uptake of radioactivity with labeled estradiol, we gave the common steroid precursor pregnenolone to check further on the specificity of uptake. The fact that the percent uptake after the labeled pregnenolone was an order of magnitude less than after labeled androgen and estrogen with the same order of specific activity was evidence that the uptake of androgens and estradiol observed under the conditions of our experiments was specifically related to these three steroids and not a nonspecific effect from presacral intra-arterial injection.

We have seen no published work on the prostatic uptake of labeled estradiol and pregnenolone.

The work was initially undertaken to evaluate the possibility that we could achieve a percent dose/gm uptake from radiolabeled steroids equal to the percent uptake we have achieved with ^{14}C-cholesterol (*11–12*) and ^{131}I-19-iodocholesterol (*13*) in the adrenal cortex and with ^{14}C-dopamine in the adrenal medulla (*14*) and in tumors of the adrenal medulla (*15,16*).

Although the uptakes achieved here with high specific activity testosterone, 5-α-dihydrotestosterone and estradiol after intra-arterial injection are comparable to the uptake achieved in our previous work on the adrenal with labeled cholesterol (adrenal cortex) and dopamine (adrenal medulla) given intravenously, repeated experiments of ours with intravenous route of administration of 5-α-dihydrotestosterone failed to produce a concentration within this order of magnitude although there was significant concentration in the prostate only.

Although the lack of clinically utilizable uptake of these labeled compounds when given intravenously makes further efforts to label them with ^{131}I unpromising for diagnostic use in prostatic tissue, two further efforts in this area are deserving of further consideration. The uptake of ^{3}H-labeled testosterone, 5-α-dihydrotestosterone, and estradiol in metastatic prostatic carcinoma in humans should be evaluated. It is also conceivable that a labeled competitive inhibitor of androgen or estrogen might concentrate in prostatic tissue similar to the concentrations achieved in our present intra-arterial study. It is also possible that a clinically useful prostatic uptake of these competitive inhibitors might occur after intravenous injection.

ACKNOWLEDGMENT

This work was supported in part by the USPHS Training Grant CA-05134-10 and -11, USAEC Contract AT (11-1) 2031, The Elsa U. Pardee Foundation and the Nuclear Medicine Research Fund.

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