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Interaction of Prolactin and Testosterone in the Human Prostate* **

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Summary. Three experiments were performed to determine whether human prolactin (hPr) affects prostatic uptake and metabolism of testosterone (T). 1) Patients with prostatic cancer were infused twice with radio-labelled androgens, the first time with basal hPr, the second time with oral thyrotrophin-releasing hormone (TRH)-elevated hPr. In 5/7, significant increases in metabolic clearance of dihydrotestosterone (DHT) and in conversion of T to DHT accompanied increased hPr. 2) The incorporation of labelled T into minced benign prostatic hypertrophy (BPH) tissue from subjects with high (40 ng/ml) hPr was measured and was found to be more than twice the uptake into tissue from those with low hPr (6.5 ± 1.9 ng/ml). 3) Uptake and metabolism in vivo of a bolus of ^{3}H-T by BPH and carcinomatous prostates was measured and was far greater in subjects whose hPr was elevated by chlorpromazine than in untreated controls. It is concluded that prolactin increases prostatic uptake and metabolism of T. It is suggested that the best management of prostatic cancer should include depletion of prolactin as well as androgen.

Key words: Prolactin, Prostate cancer, Benign prostatic hyperplasia, Androgen metabolism.

INTRODUCTION

In a series of papers Grayhack and co-workers (10-12) have demonstrated a synergistic action of prolactin on androgen-induced weight gain and citric acid secretion by rat prostate. Slaunwhite and Sharma (33) have confirmed these results. Using an entirely different approach, Asano and co-workers (1) and Hostetter and Piacsek (16) have reached the same conclusion. The former, using intact, adult rabbits and the latter using intact, immature rats showed that an immunologically-produced deficit of prolactin caused atrophy of the prostate and failure of the prostate to grow, respectively. Thus, the interaction of prolactin and androgen on the rat and rabbit prostate is firmly established by in vivo experiments. In man, administration of the anti-prolactin bromocryptine suppressed plasma hPr and the uptake of ^{3}H-T into prostatic carcinoma tissue (19).

We report here the results of three experimental studies of prolactin-androgen interaction in patients with advanced prostatic cancer (CA) or with BPH which support the hypothesis that prolactin promotes androgen uptake by the human prostate. In the first study patients were infused twice with radio-labelled androgens, the first time under basal conditions, and the second, in the presence of serum prolactin levels which had been significantly increased by treatment with TRH. Thus each patient served as his own control. It was anticipated that if more testosterone entered the prostate, more reduced steroid (DHT) (dihydrotestosterone; 17β-hydroxy-5α-androstan-3-one) would appear in the blood stream (8, 34). It is known that in elderly men over 50% of plasma DHT arises from secretion from extra-splanchnic organs (27). Since DHT levels are higher in the blood in prostatic veins than in peripheral veins, the prostate is one source of the steroid. While the magnitude of the prostatic contribution is un-

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known, the present working hypothesis assumes that the main respondents to changing prolactin levels are the prostate and related 5α-reductase-rich male reproductive accessory organs and tissues.

Next, the uptake in vitro of ^{3}H-T by excised prostatic tissue from unstimulated patients with widely differing plasma hPr was compared in order to establish whether the increased conversion of T to DHT seen in the previous experiment might be at least partially correlated to the extent of androgen uptake by the prostate. Finally, the in vivo uptake of ^{3}H-T into prostates of patients treated with chlorpromazine to raise their serum hPr was compared with that into those who did not receive the drug.

METHODS

Patient Population

All studies were carried out in patients admitted to the Urology Service of the Buffalo Veterans Administration Medical Center for treatment of either BPH or Stage IV CA. Four of the infused patients had received estrogen at some earlier point, but this had been discontinued at least one month before this study. Two were orchiectomized. All protocols and the texts of the two informed consent forms presented to all study subjects were reviewed and approved by the Medical Center Human Use Subcommittee of the Research Committee. The infusion protocol was also reviewed and approved by the Drug Safets Evaluation Committee. The infusion protocol was also reviewed and approved by the Drug Safety Evaluation Committee of Abbott Laboratories, who generously supplied the TRH, and by the Food and Drug Administration.

Radiosteroids, including the 1,2-^{3}H dihydrotestosterone (50 Ci/mmole) and ^{14}C-testosterone (50 mCi/mmole) used in the infusion and the 1α, 2α-^{3}H-testosterone (50 mCi/mmole) used for the bolus experiments and the in vitro study, were obtained from New England Nuclear Corp. in solution in benzene-ethanol (9:1). After evaporation of the solvent under nitrogen, each steroid was dissolved in absolute ethanol. An aliquot of the solution was taken for re-crystallization to determine radiochemical purity and specific activity, and the remainder was stored in the original container at 20°C. For the bolus work, 60 μCi of ^{3}H-T in 60 μl ethanol was mixed with 6 ml sterile saline under aseptic conditions. For infusions, 40 μl (40 μCi) ^{3}H-DHT and 40 μl (10 μCi) ^{14}C-T were added to 50 ml sterile saline under aseptic conditions.

Infusions

Using a modification of our earlier protocol (29), seven patients with advanced carcinoma of the prostate were subjected to two infusions over a 2 h period with a saline solution of ^{3}H-DHT and ^{14}C-T. Before the second infusion each subject received oral TRH to raise the level of circulating prolactin.

Following breakfast (about 9:00 a.m.) 40 ml of blood was drawn from each supine subject for determination of plasma testosterone and prolactin. Then 5 ml of infusion solution was administered intravenously to prime the patient for the infusion. During the next 30 min a 16 gauge Teflon catheter (Angiocath) was placed in the brachial vein of one arm and closed with a three-way stopcock so that intermittent blood samples could be drawn into heparinized syringes and, after each sampling, the Angiocath could be rinsed with a dilute solution of heparin in saline. An Angiocath in the brachial vein of the other arm was made ready to infuse the steroid solution. Following the 30 min prime and preparation time, a 10 ml blood sample was taken for serum prolactin, and the infusion of the steroid mixture was begun from a gas-sterilized 50 ml syringe operated by a Harvard Infusion Pump at a rate of 0.25 ml/min. Further 10 ml blood samples were taken at 30, 60 and 90 mins to monitor prolactin concentration. Then at 120, 135 and 150 mins., 40 ml samples were drawn for prolactin assay and for the analysis of the infused steroids. After the infusion a series of 1 min collections were made from the pump in order to calibrate the infusion rate. When prolactin secretion was to be stimulated, the patient received 120 mg TRH orally at 7:00 a.m. and at 10:00 a.m. Such treatment is known to increase thyroid stimulating (TSH) secretion also but, since the peaks of thyroxine and triiodothyronine (T_3) secretion follow TRH administration by 4 to 8 hrs (30) it seemed unlikely that these hormones would have a significant effect on the ratio of 5α/5β reduction of the testosterone. Hellman and co-workers (15) had found it necessary to treat their patients with T_3 for a minimum of 10 days to demonstrate remarkable changes in the androsterone/etiocholanolone ratio.

Bolus Experiments

A pre-operative blood sample was first drawn for assay of endogenous plasma testosterone. Then, prior to initiation of transurethral resection, 50 μCi of 1α, 2α-^{3}H-T in 5 ml sterile saline was given as an intravenous bolus. At the approximate mid-point of the period of tissue excision (a time carefully noted), a second blood sample was drawn to determine the specific activity of the circulating testosterone. When endogenous prolactin concentration was to be raised, 50 mg chlorpromazine, i.m., was administered at 7:00 a.m. in lieu of other preoperative medications.

In vitro Experiments

A blood sample for prolactin assay and a gram (wet wt.) or more of tissue was collected from each of a series of patients undergoing transurethral resection for BPH. Each tissue specimen was minced, washed and incubated 30 min in 10 ml Ringer sodium phosphate buffer, pH 7.4, containing 0.1% glucose and 1.72×10^{-8}M ^{3}H-T. The tissue was then rinsed in saline and extracted as described below. The extracts were stored until the series was complete and all prolactin data were available.

Analytical Procedures

1. Extraction of Plasma. To a measured volume of plasma was added 10 µg portions of DHT and T in saline. After standing for 30 min to equilibrate carrier steroids with the plasma, 2 volumes of 95% ethanol were added, and the precipitated protein was sedimented by centrifugation. The supernatant solution was saved while the pellet was washed three times with 10 ml portions of 95% ethanol. The extract plus washings were pooled, evaporated to 2-3 ml under N_2 and diluted to 10 ml with water. The diluted extract was treated with 0.1 volume of 4 N NaOH for 30 min and then extracted three times with 3 volumes of methylene chloride. The pooled organic layer was washed twice with 2.5 ml portions of water, dried with anhydrous Na_2SO_4 and concentrated to a small volume under N_2 for chromatography (see below).

2. Extraction of Tissue. To 2 gm (wet wt.) of tissue from a bolus experiment or about 1 gm of tissue from an in vitro experiment was added 100 µg each of T, DHT and 5α-androstane-3α, 17 β-diol (DIOL). Then the tissue was homogenized in 30 ml Delsal's reagent (dimethoxymethane/methanol/H_2O, 80:4:16) using a motor-driven Kontes glass homogenizer and centrifuged. The pellet was dissolved in 1 N NaOH and an aliquot used to assay protein. The Delsal's extract was de-fatted by equilibrating with 10 ml 70% methanol and then concentrated for chromatography.

3. Chromatographic Analysis of Tissue and Plasma Extracts for Androgens. Goldman's (9) partition chromatography on propylene glycol-impregnated Gelman ITLC-SA medium (2.5 sweeps with CCl_4/hexane 9:1) was used to separate T (R_t=1.0) from DHT (R_t=1.85) and DIOL (R_t=0.42). The appropriate regions of each chromatogram (determined by treating a reference strip with ethanolic H_2SO_4 (1:1) and heating to locate the standards) were cut out and eluted with methanol.

Small aliquots of each eluate were taken for determination of radioactivity and for measurement, as the trimethylsilyl ethers, of recovery by gas liquid chromatography (GLC). In the case of the dual-labelled plasma extracts, differential counting of ^{3}H and ^{14}C was done in a Beckman LS-100 beta counter employing narrow windows such that no ^{3}H appeared in the ^{14}C window and the efficiency of ^{14}C counting in the ^{3}H window was less than 10%. Each sample was counted with an external standard so that, with quench correction curves, the ^{3}H and ^{14}C activity of each sample could be computed readily.

The remainder of the eluted T and DHT was separately acetylated by treating the dried residues with 0.2 ml of a 1:1 mixture of pyridine and acetic anhydride overnight. After evaporating to dryness, the residue was dissolved in a few drops of methanol and chromatographed on unimpregnated ITLC strips using cyclohexane/ethyl acetate (9:1) as developing solvent. Rf's of testosterone acetate and DHT acetate were 0.33 and 0.67, respectively. The DIOL eluate obtained above was oxidized in acetone with Jones reagent (26.7 gm CrO_3 + 23.0 ml H_2SO_4 + H_2O to 100 ml) to 5α-androstane-3, 17-dione (DIONE) and chromatographed on the ITLC medium with cyclohexane/ethyl acetate (9:1), Rf=0.75. The acetates and the DIONE were eluted from their separate chromatograms with methanol. Separate aliquots were used for quantitation of radioactivity by counting and of mass by GLC.

Plasma testosterone was determined by radioimmunoassay using a kit from New England Nuclear Corp.

Calculations

1. Metabolic Clearance Rate (MCR). The data used for each metabolic clearance rate determination were the mean of six specific activity values obtained on each steroid (three samples X (free compound and derivative) and the mean of six calibration samples for infusion rate.

MCR = (dpm steroid/day)/(dpm steroid/litre) = litre/day. The specific activity of the three samples drawn at 120, 135 and 150 min were constant within experimental error, showing the attainment of a steady state in each case.

2. Conversion,

$$p = (MCR_{DHT} MCR_T) \times (\text{dpm } {}^{14}C_{DHT}/\text{dpm}_T) \times 100\%.$$

3. Incorporation of T (Bolus experiments). The specific activity of the testosterone in the plasma bathing the tissue was calculated from the plasma testosterone concentration determined by RIA and the radioactivity of the blood sample obtained at midsurgery attributable to testosterone.

S.A. = (cpm T/ml)/(ng T/ml) = cpm/ng T.

The uptake equals (cpm/gm tissue protein)/S.A. = ng T equiv/gm tissue protein.

Incorporation into T, DHT and DIOL was computed in the same way. This approach obviated consideration of dilution of specific activity result-

ing from mixing of the entering labelled material with endogenous tissue steroid.
4. Incorporation of T in vitro. Calculations were the same as for the bolus experiments except that the specific activity was pre-determined as indicated.
5. Serum prolactin was assayed initially in the laboratory of Dr. Henry Friesen (Winnipeg, Manitoba) and subsequently in that of Dr. Ulysses Seal (Minneapolis, Minn) using the methods of Hwang et al. (17) and Sinha et al. (31), respectively. Values from the two laboratories were comparable.
6. Statistical analyses were performed using a computer programme described in the Statistical Package for the Social Sciences (28).

RESULTS

The effects of treating seven patients with established diagnoses of adenocarcinoma of the prostate with TRH are shown in Table 1. Associated with the doubling of the circulating prolactin concentration were a 50 % increase in the conversion of T to DHT and some acceleration of the metabolic clearance rate of DHT. These differences were significant when analysed by a paired T-test (one tail). Steroid metabolism of Patient 7, who had the highest control hPr, did not respond to further increase in the hPr by TRH. Fig. 1a, b show that metabolic clearances of T and DHT per unit of circulating hPr decreased logarithmically with increase in serum hPr.

Table 1. Effect of TRH on serum prolactin, plasma testosterone, the metabolic clearance of T and DHT and conversion of T to DHT

Pat	Age	Orch	Prolactin (ng/ml)			Plasma T (ng/ml)		
			C	TRH	Δ	C	TRH	Δ
1	80	-	3.5±0.6[a]	30.3±2.6	26.8	3.3	3.3	0.0
2	54	12 days	6.1±0.6	21.6±2.0	15.5	1.8	1.6	-0.2
3	81	-	10.5±1.6	14.5±2.1	4.0	8.0	8.7	0.7
4	67	-	11.3±1.2	16.3±1.5	5.0	7.9	9.6	1.7
5	72	-	15.0±1.2	30.0±1.8	15.0	3.8	3.2	-0.6
6	80	1 month	19.7±1.2	30.6±3.3	10.9	0.9	0.7	-0.2
7	78	-	22.0±1.8	37.0±2.6	15.0	1.2	1.1	-0.1
($\bar{X}$±SD)					13.2±7.7			0.3±0.7
P					<0.005			ns

Pat = patient no.; Orch = orchiectomized; C = control: untreated patient; TRH = treated with TRH.

[a]Each tabulated value = mean ± SEM of n individual values; n = 7 for prolactin and 6 for all other measurements

Table 1. continued

Pat	MCR_T (1/day)			MCR_{DHT} (1/day)			PT → DHT		
	C	TRH	Δ	C	TRH	Δ	C	TRH	Δ
1	240±11	242±4	2	273±6	320±14	47	2.1±0.2	6.0±0.4	3.9
2	159±14	227±16	68	308±42	439±26	131	4.8±0.7	7.0±0.6	2.2
3	369±13	363±37	-6	414±127	504±55	90	4.7±0.3	11.9±2.8	7.2
4	466±38	565±40	99	433±42	520±129	87	5.2±0.2	6.8±1.7	1.6
5	556±66	542±25	-14	374±34	441±50	67	2.1±0.1	2.1±0.1	0.0
6	169±9	203±5	34	239±3	269±1	30	1.6±0.7	3.9±0.3	2.3
7	220±14	219±7	-1	335±42	301±8	-33	1.9±0.4	1.3±0.1	-0.6
($\bar{X}$±SD)			26±43			60±52			2.4±2.6
P			ns			0.01			0.025

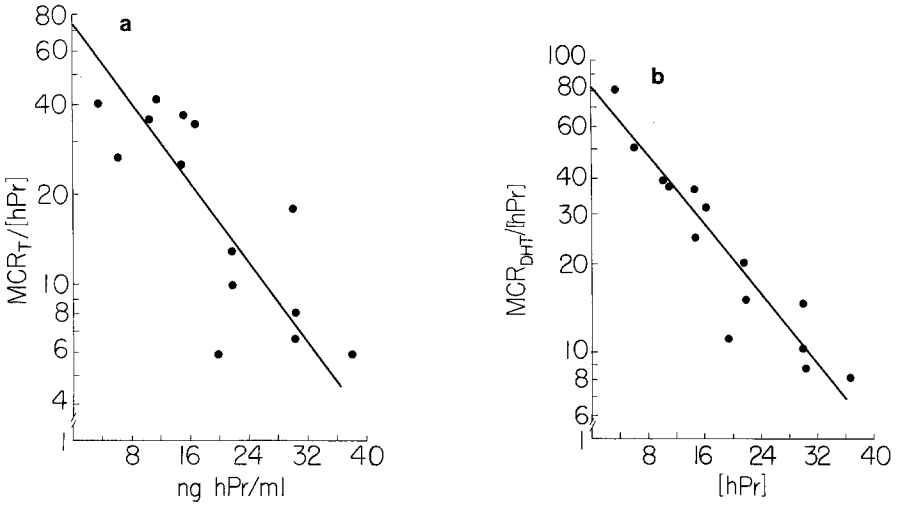

Fig. 1a, b. Metabolic clearance of T (a) and DHT (b) per unit of serum prolactin as a function of serum hPr. Metabolic clearance, MCR = 1/day. hPr = ng/ml. a Coef. of correlation, r = -0.87 (P<0.01). log MCR_T = -0.033 hPr + 1.865. b r = -0.9 (P <0.01). log MCR_{DHT} = -0.030 hPr + 1.924

Table 2. In vitro studies. Prostatic tissue (transurethral resection) from BPH patients was incubated for 30 min with 1.72 X 10^{-8}M ^{3}H-T. Patients were selected on the basis of their serum prolactin being low (4-9 ng/ml) or high (36-50 ng/ml). Tissue concentrations of radioactive steroids were calculated from the specific activity of the added testosterone

Pat	Age	hPr	Uptake[a]			Distribution[b]			sum[c]
		ng/ml	ng	ng/gm	%[d]	DIOL	T	DHT	ng
1	66	7	8.7	60	17	22	50	28	2.0
2	70	4	9.4	71	19	18	46	36	2.8
3	82	5.5	9.8	63	20	20	56	24	1.9
4	63	7	10.5	73	21	-	-	-	-
5	57	9	13.1	61	26	-	-	-	-
$\bar{X}$	68	6.5	10.3	66	21	20	51	29	2.2
SD		1.9	1.7	6					0.5
6	56	36	24.9	160	50	16	55	29	7.0
7	67	36	17.3	120	35	14	53	33	8.5
8	64	50	27.1	174	54	15	53	32	8.2
9	72	38	23.6	197	47	16	53	31	10.2
$\bar{X}$	65	40	23.2	163	47	20	53	31	8.5
SD		6.7	4.2	32					

[a]Based on radioactivity measured in the Delsal's extract

[b]% of sum

[c]sum of DIOL + T + DHT uncorrected for experimental losses

[d]percent of substrate incorporated

Uptake: P<0.001; sum P<0.001

The in vitro and bolus experiments permitted direct assessment of the effect of prolactin on prostatic uptake and metabolism of the androgen. Upon 30 min incubation of a fixed concentration (1.72×10^{-8}M) of ^{3}H-T with freshly excised benign hypertrophic tissue from patients with low and high serum prolactin concentrations (Table 2), uptake into the tissue of the high group was 2.5 times that into the tissue of the low hPr group. Nevertheless, the proportion of entering testosterone reduced to DHT + DIOL was essentially identical in the two groups.

Table 3 shows that the mean uptake (ng/gm wet wt.) of testosterone by prostatic tissue from the administered bolus was significantly greater in both chlorpromazine-stimulated groups than in the control (unstimulated) group. The rate of uptake of testosterone (ng/gm wet wt. /ng serum testosterone/ml) was also significantly higher (Fig. 2) in the stimulated than in the control BPH tissue, appearing to follow first order kinetics. A Pearson's correlation analysis (17) with a 2-tailed test of significance shows a highly significant ($P = 0.001$) correlation of time of exposure to the steroid with uptake and with concentrations of the three steroids in the 13 BPH subjects. Fig. 3 shows that, if three out-liers (patients 2, 6 and 9) are arbitrarily deleted, uptake is linearly related to hPr ($r = 0.74$; $p = 0.002$). Also, the rate of uptake per unit of prolactin is found to be inversely related to the concentrations of both serum T and hPr. The relationship to T is only

Table 3. Uptake of a bolus of ^{3}H-T into prostates of unstimulated BPH patients and into prostates of BPH and prostate cancer patients pretreated with 50 mg chlorpromazine, i.m.

Pat	Age (yrs)	hPr (ng/ml)	T (ng/ml)	Mean[a] exposure time (min)	^{3}H uptake[b] in T equiv. (ng/gm)	Tissue steroids		
						T	DHT	DIOL
Controls (BPH)								
1	85	5	4.2	14	0.9	0.19	0.50	0.08
2	83	20	4.3	20	1.0	0.04	0.63	0.14
3	81	6	2.8	26	1.1	0.05	0.53	0.11
4	83	11	6.6	30	2.1	0.09	1.82	0.06
5	80	6	2.4	34	1.6	0.21	0.62	0.17
6	71	19	3.8	36	2.1	0.14	1.16	0.17
7	67	4	3.5	37	2.9	0.16	1.09	0.25
8	82	4	1.5	43	3.5	0.33	2.23	0.16
$\bar{X}$	79	9.4	3.8	30	1.9	0.15	1.07	0.14
SD	6.4	6.6	1.4	9.6	0.9	0.10	0.65	0.06
Stimulated BPH								
9	84	28	7.2	15	1.0	0.32	0.14	0.02
10	70	15	4.6	50	10.0	0.32	1.83	0.70
11	62	25	6.8	53	10.9	0.86	3.59	0.98
12	62	10	3.9	61	13.5	1.97	2.70	0.78
13	60	20	8.2	65	14.4	1.60	2.62	0.93
$\bar{X}$	68	19.6	6.1	49	9.9	1.01	2.18	0.68
SD	9.9	7.3	1.8		5.3	0.75	1.29	0.39
Stimulated CA								
14	74	15	6.8	28	6.2	2.86	0.57	0.14
15	81	15	8.3	30	8.2	2.15	2.78	0.88
16	69	13	10.8	62	5.0	1.38	0.46	0.05
$\bar{X}$	75	14.3	8.6	40	6.5	2.13	1.27	0.36
SD	6.0	1.15	2.0		1.0	0.74	1.31	0.46

[a] The time between injection of ^{3}H-T and the estimated midpoint of excision

[b] Uptake of steroid calculated from plasma T specific activity and expressed as testosterone equivalents. The specific activity of plasma T at the midpoint of surgery naturally varied with the time and endogenous pool of T; it varied from 1200 dpm/ng at 14 min to 70 dpm/ng at 65 min

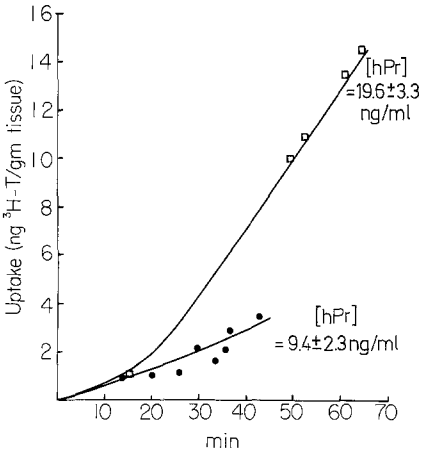

Fig. 2. Uptake of ^{3}H-T by benign hyperplastic prostatic tissue from chlorpromazine-stimulated (mean hPr = 19.6 ± 3.3 SE ng/ml) and unstimulated (mean hPr = 9.4 ± 2.3 SE ng/ml) patients. Uptake = ng ^{3}H-T/gm wet wt. Abscissa: Time in minutes from injection of bolus of ^{3}H-T to excision of the prostatic tissue

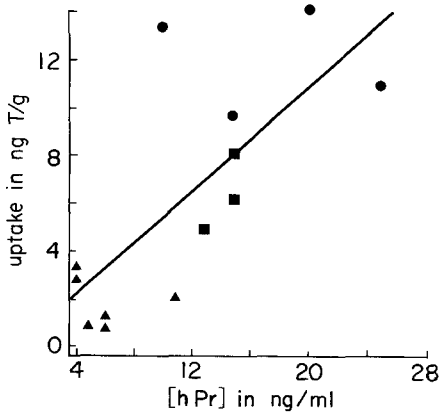

Fig. 3. Uptake of ^{3}H-T (ng/gm tissue) as a function of hPr. Data of Table 3 are plotted, arbitrarily deleting those from Patients 2, 6 and 9. Coef. of correlation, r = 0.74 (P = 0.002). Uptake = 0.54 hPr. Triangles: unstimulated BPH patients; circles: chlorpromazine-stimulated BPH patients; squares: chlorpromazine-stimulated cancer patients

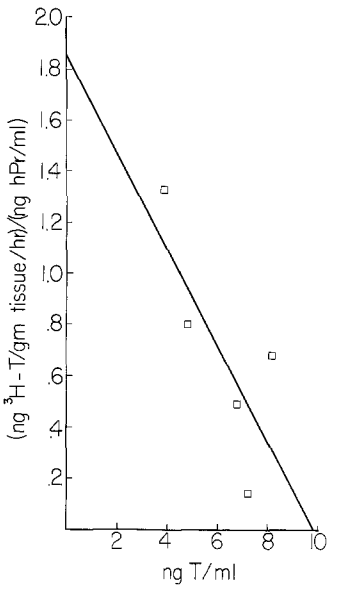

Fig. 4. Uptake sensitivity, ng ^{3}H-T uptake/gm tissue/hr per unit of hPr, ng/ml, as a function of (T), ng/ml. Coef. of correlation, r = -0.83 (P<0.01). Sensitivity = -0.19 (T) + 0.186

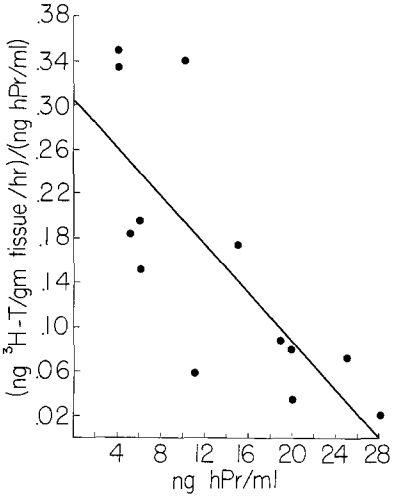

Fig. 5. Uptake sensitivity (defined as in Fig. 4) as a function of hPr, ng/ml. Coef. of correlation, r = -0.75 (P<0.01). Sensitivity = -0.011 hPr + 0.306

statistically significant in the stimulated BPH group (Fig. 4; r = -0.83, p<0.01) but is also apparent in the control group (r = 0.47) and the stimulated cancer group (r = -0.67). The responsiveness, or "sensitivity", decreases with rise in T. Fig. 5 shows this same decline in unit responsiveness as hPr is progressively elevated. As in the in vitro experiment, despite the great increases in androgen uptake in the stimulated groups, the proportion of T reduced to (DHT + DIOL) is not significantly different from that in the control group.

DISCUSSION

The infusion experiments are interpreted to show that, in five of the seven patients, raising the serum prolactin concentration increases the conversion of T to DHT as well as the rate of clearance of DHT. These results do not prove that a

rise in serum hPr increases T uptake into the prostate, but they are consistent with the hypothesis that it does. Reports of others are also compatible with this hypothesis. Ishimaru and Horton (18), who incidentally obtained conversion rates in BPH patients comparable to those found here, observed that over 50% of DHT arises extrasplanchnically, mainly from prostate and testis, in aged males. Also, plasma DHT was higher in prostatic venous blood than in peripheral venous blood in 8/9 patients with BPH (26). Furthermore activities of the three obvious alternative sites of steroid metabolism, liver, adrenal and testis, seem unlikely to account for the accelerated clearance seen here. Animal studies (4, 13, 22) show that ectopic pituitary implants or direct administration of prolactin do not increase hepatic 5α-reductase significantly and do significantly inhibit adrenal 5α-reductase (4). Prolactin probably blocks testicular 5α-reductase as well for Magrini et al. (25) found the block of testicular reductase activity by hyperprolactinemia was relieved by treatment with bromocriptine and Jacobi et al. (19) showed that depletion of hPr with this drug increased 5α-reductase activity of the inner pool.

Two patients with initially high hPr failed to respond to the TRH-induced increase in serum hPr. One may speculate that, if receptors are involved, these may have been essentially saturated by the high basal hPr so that they were incapable of greater response. The prolonged occupancy of prolactin on prolactin receptors, shown by Brooks and Welsch (3) with rat liver preparations, might well produce a saturated receptor pool too stable to be affected by acute changes in serum hPr. Such a mechanism might explain the decline in response of metabolic clearances to increased hPr seen in Fig. 1a, b. An alternative cause of the insensitivity of the high hPr patients to further stimulation might be that these two subjects have a lower concentration of prolactin receptors. Prolactin receptor assays would have been helpful here. Djiane et al. (5) showed how prolactin injections deplete receptors in the rabbit mammary gland.

Results obtained in vitro (Table 2) strongly suggest that hPr has an androgen-enhancing effect on the prostate. This result may indicate that the prolactin was tightly bound to the tissue and remained with it in spite of washing (cf. Brooks and Welsch, 3). In favor of this idea is Farnsworth's finding (6) that the ability of androgen to activate acid phosphatase of minced prostatic tissue is significantly reduced by treatment of the tissue with anti-placental lactogen anti-serum (cf. 1 and 16).

The bolus experiment (Table 3) appears to both complement and extend the findings and interpretations of the in vitro experiments. Uptake of androgen into the tissues of the stimulated patients is greater and occurs more rapidly (Fig. 2). The extent of incorporation is dose-related (Fig. 3). As in the infusion study, high levels of circulating prolactin appear to attenuate uptake (Fig. 5). When this is recognized, the deleted data of Fig. 3 are seen to conform. The relationship is seen to be more complex, however, since Fig. 4 suggests that rising serum testosterone levels also impede binding. This is reminiscent of earlier findings (6) that, while androgen binding to prostatic microsomes rises with lactogen binding, there is a tendency for the increases in both to plateau, perhaps indicating the saturation of binding sites for the two species of ligand.

It seems evident, then, that in man prolactin does increase prostatic uptake of androgen. This effect appears to be blunted by pre-existing full occupancy of responsive sites with their specific ligands (androgen and/or prolactin). The experiments of Jacobi et al. (19) show that treatment of prostatic cancer patients with bromocriptine, a prolactin-depleting drug, reduces androgen uptake into the prostate. The ineffective or inhibitory action of elevated prolactin concentrations found here was not unexpected in view of Lasnitzki's studies of prostate growth (21) and the findings of Lloyd's group with regard to androgen uptake (24) that high levels of prolactin are inhibitory although lower levels stimulate.

A direct effect of prolactin on 5α-reductase of testosterone seems unlikely in view of no significant change in the ratio of (DHT + DIOL)/(DHT + DIOL + T) with gross changes in testosterone uptake in both in vitro and bolus experiments. It appears that an adequate quantity of enzyme activity is available to metabolize a constant fraction of the entering testosterone. It is true that both the present study (Table 3) and that of Boyns et al. (2) revealed a small, insignificant decrease in the percentage reduction in the presence of higher prolactin levels.

These experiments lead to the conclusion that availability of androgen to prostatic tissue may be increased by rising hPr. It follows that means to deplete hPr might, by reducing the uptake of testicular and adrenal androgens by prostatic tumors, permit more effective management of prostatic cancer. Indeed, Jacobi et al. (20), using bromocriptine, and Farnsworth and Gonder (7), using L-Dopa to inhibit prolactin secretion in their prostatic cancer patients, have recorded significant palliation and some stabilization of the disease. Studies are in progress to determine if effectiveness of these depletion measures is related to the density of prolactin receptors in the patients under study. Recent reports show a high correlation between clinical response to hormone therapy and steroid receptor content in prostatic cancer (14) and that the concentrations of nuclear androgen receptors are significantly higher in well differentiated prostatic carcinomas than in normal or benign hyperplastic glands (23).

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