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END ORGAN METABOLISM OF TESTOSTERONE
I—EFFECTS OF HYPOPHYSECTOMY AND GROWTH HORMONE

THOMAS MOSHANG, Jr. and ALFRED M. BONGIOVANNI
The Children's Hospital of Philadelphia, The University of Pennsylvania School of Medicine, Philadelphia, Pa., U.S.A.

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SUMMARY

The effects of hypophysectomy and growth hormone (GH) replacement therapy on the metabolism of testosterone by the rat prostate *in vitro* were studied. Forty-one Sprague–Dawley rats were hypophysectomized and twenty-one of these animals were treated with rat GH until 60 days of age. Intact animals were sacrificed at 25, 35 and 60 days of age. The ventral prostates from these animals were incubated with increasing concentrations of radio-active testosterone. The disappearance rate of testosterone and the rate of formation of 5 α-dihydrotestosterone (DHT) were determined for each level of substrate concentration for each group of animals. In all animals, the disappearance rate of testosterone increased progressively with increasing concentrations of testosterone. In intact animals, the rate of formation of DHT increased progressively and leveled off at a maximum rate with higher concentrations of testosterone. In contrast, the rate of formation of DHT by the prostrates from hypophysectomized rats appeared to be inhibited by higher substrate concentration. Although GH increased the protein content of the prostates from hypophysectomized rats, GH therapy did not correct the inhibition of the conversion of testosterone to DHT by high concentration of substrate.

INTRODUCTION

AN INTERRELATIONSHIP between pituitary hormones and the biologic effectiveness of testosterone on male accessory organs has been suggested in both human and animal studies[1–3]. The exact nature of this relationship or the specific pituitary hormones involved are not clear. Recent data indicate that the biologic action of testosterone (T), in certain target tissues, may require conversion of T to 5 α-dihydrotestosterone (DHT), and require as well cytosol and nuclear receptors for T and DHT[4–7]. This preliminary report presents data indicating that hypophysectomy modifies the ability of the rat ventral prostate to convert T to DHT with increasing concentrations of T. Furthermore, although growth hormone (GH) increases the protein content of the rat prostate, GH does not correct the metabolic changes in the prostate from the hypophysectomized rat.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats, obtained from Charles–Rivers laboratories, were hypophysectomized or sham-hypophysectomized at 21 days of age. Twenty-one of the hypophysectomized rats were treated with 0·1 mg of rat GH subcutaneously 5 days per week until 60 days of age. The sham hypophysectomized rats were sacrificed at 25, 35 and 60 days of age. The various experimental groups are summarized in Table 1.

Experimental procedure. The ventral prostate from each animal was dissected free of all fat and then minced into small pieces with a scalpel. The minced prostates from each experimental group of animals were combined and divided

Table 1. Summary of the various experimental groups of animals

Animal groups	No.	Age at sacrifice (days)	Mean weight (g)	Mean length (cm)	Mean mg protein per prostrate
Prepubertal	15	25	56·1 ± 3·6	12·7 ± 0·4	10·1
Prepubertal	10	35	108·5 ± 7·9	15·2 ± 0·4	16·9
Post Pubertal	8	60	300·3 ± 55·5	21·1 ± 1·6	86·0
Hypophysectomized	20	60	99·7 ± 15·9	14·6 ± 0·6	1·9
GH Treated	21	60	122·5 ± 10·5	16·3 ± 0·6	5·26

into aliquots equalling approximately 5 mg of protein. The prostatic tissue was incubated with varying amounts (25–600 nmol) of [1, 2-^{3}H]-testosterone in 3 ml of Krebs–Ringer bicarbonate buffer. Incubations at each level of substrate were carried out in triplicate or quadruplicate. The incubation procedure and the extraction of steroids were carried out as described by Northcutt *et al.*[8]. Testosterone and the metabolites formed were separated by paper chromatography using a hexane–propylene glycol system[9]. Reference standards for T and DHT were detected by ultraviolet absorption and alkaline m-dinitrobenzene[10] respectively and the appropriate areas for T and DHT eluted. The amount of T metabolized and the amount of DHT formed were quantitated based on radioactive recovery and corrected for protein content in each incubation. Protein concentration was determined by the method of Lowry *et al.*[11]. The radiochemical purity of the T and DHT eluted was ascertained in one experiment, after dilution of the radioactive material eluted with nonradioactive T and DHT, by chromatographic mobility and by constant specific activity after chromatography in three paper chromatographic systems and one thin layer system (Table 2).

RESULTS AND COMMENTS

The effect of increasing concentrations of substrate on the disappearance rate of T (testosterone metabolized) by each of the experimental groups is depicted in Fig. 1. The effect of increasing substrate concentration on the rate of formation

Table 2. Radiochemical purity of testosterone and 5 α-dihydrotestosterone

Type	Chromatography systems Solvents	Ratio	Time (h)	Specific activity T (cpm/10 μg steroid)	DHT
I. PC	Cyclohexane–nitromethane–water	100:100:10	48	4695	1006
II. PC	Heptane–methanol–water	5:4:1	8	5006	1030
III. PC	Hexane–propylene glycol		18	4220	1104
IV. TLC	a. Toluene–ethylacetate	4:1	1		
	b. Toluene–methanol	97:3	1	4720	1063

500 μg of nonradioactive T or DHT was added to the radioactive material eluted from the areas corresponding to T or DHT after the initial separation. After each chromatographic separation, the material eluted from the T and DHT areas was assayed for specific activity. Steroids were quantitated by m-dinitrobenzene reaction after chromium trioxide oxidation[12].

PC = Paper chromatography (Whatman No. 1 paper).

TLC = Thin layer chromatography (Aluminum oxide).

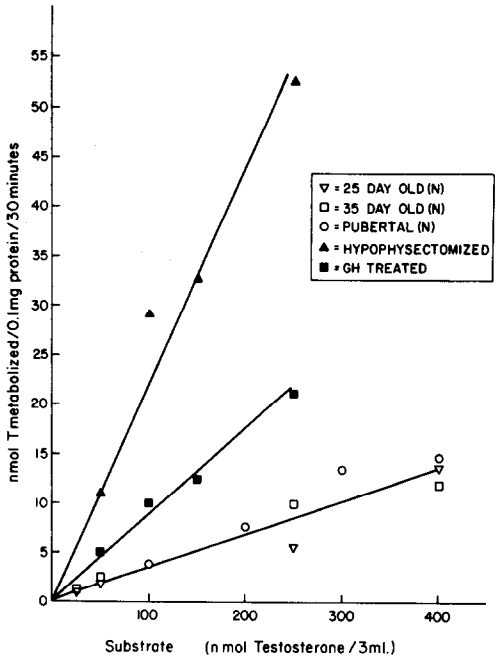

Fig. 1. The effect of substrate concentration on the disappearance rate of testosterone. Each symbol is the mean of three or four incubations.

of DHT is depicted in Fig. 2. The disappearance rate of T and the rate of formation of DHT are expressed as nmol T metabolized or nmol DHT formed per 0·1 mg protein in 30 min.

Intact animals. In the intact animals, regardless of age, the disappearance rate of T progressively increased with increasing substrate concentrations. The rate of formation of DHT increased progressively until the substrate concentra-

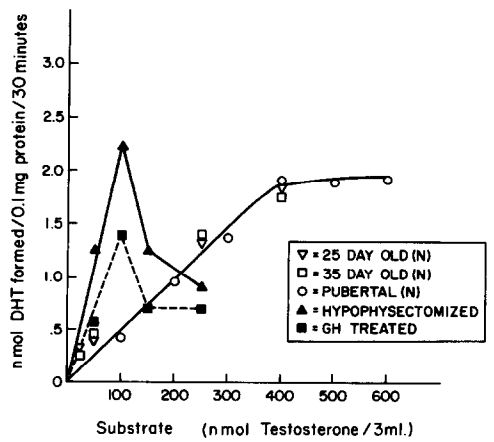

Fig. 2. The effect of substrate concentration on the rate of formation of dihydrotestosterone. Each symbol is the mean of three or four incubations.

tion exceeded 400 nmol T per 3 ml of buffer. At higher substrate concentrations, the rate of formation of DHT remained level at 1·8 nmol DHT formed per 0·1 mg protein in 30 min. The rates for T metabolized and DHT formed were comparable between pubertal and prepubertal intact animals at each concentration of substrate tested. These results indicate that the enzymes and other factors (such as cytosol and nuclear receptors) involved in the metabolism of T and formation of DHT in the prostate of the intact rat remain constant at all ages, indicative of a parallel increase in these factors with increasing weight and protein content of the prostate with increasing age. There is apparently a species variation since the rate of formation of DHT by human prepuce has been demonstrated to decrease with age[5] as also demonstrated in the bull prostrate[13]. Similar to the data presented for the rat in this paper, the rate of formation of DHT has been shown to remain fairly constant through all ages for the dog and cat[13].

Hypophysectomized animals. The actual rates of metabolism of T by the prostates of the hypophysectomized rats are greater than the intact animals at every substrate concentration tested. The rates of formation of DHT by the prostates of the hypophysectomized animals at low substrate concentrations are also greater than normals. However, these increased rates are probably a reflection of relatively decreased protein content in these prostates as recorded in the last column of Table 1 rather than an actual increase in the enzymes and other factors involved in the metabolism of T. GH therapy increased the protein content of the prostates (Table 1) as shown by others[2] and this is reflected in lower rates of metabolism of T and formation of DHT.

In a fashion comparable to the intact animals, the disappearance rate of T progressively increased with increasing substrate concentrations in both the non-treated and GH treated hypophysectomized rats. However, hypophysectomy modified the effect of increasing substrate concentration on the rate of formation of DHT. In normal animals, the rate of formation of DHT progressively increased and then plateaued with increasing concentrations of substrate. In the hypophysectomized animals, the rate of formation of DHT was inhibited by higher substrate concentrations and the rate of formation did not level off but decreased sharply. This inhibition of the conversion of T to DHT by high concentrations of T persisted in the GH treated rats.

The metabolism of T by the rat ventral prostate has been demonstrated to utilize both the 17 hydroxyl pathway, T → DHT → 5 α-androstane-3 α and 3β, 17 β-diol (androstanediols), and the 17-oxo pathway, T → androstenedione → 5 α-androstane-3, 17-dione → androsterone → androstanediols[4]. The data presented suggest that the formation of DHT by prostates from hypophysectomized rats is inhibited by high substrate concentrations. Since the disappearance of T was not inhibited but continued to progressively increase, these data suggest that prostates from hypophysectomized rats either preferentially utilized the 17-oxo pathway at higher concentrations of substrate or, alternatively, high concentrations of substrate induced a more rapid conversion of DHT to the androstanediols. It appears that deficiency of a pituitary hormone caused a deficiency of a factor in the rat ventral prostate important in the formation of DHT at high substrate concentrations. This factor (or factors) might be either the cytosol or nuclear receptors for T or DHT. The results suggest that the pituitary hormone involved is not GH.

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