

Short Research Article

Preparation of ^3H -labelled testosterone metabolites[†]

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Introduction

Androgen dependant maladies can appear due to blocks in the biosynthesis of various sex hormones¹ (testosterone, dihydrotestosterone) or to some modification in signaling pathways through androgens receptors². Those maladies appear both in men: male pseudo hermaphroditic, androgenic alopecia, prostate cancer, and in women: polycystic ovaries, hirsutism³. In the diagnosis of such maladies an important role is played by the following approaches and determinations: plasma hormone dosing, free testosterone and SHBG binding, dihydrotestosterone dihydroepiandrosterone and urine hormone dosing; free 3α - and 3β -androstane diols and/or glucuronates⁴. Also important is the enzyme-activity determinations of some key enzymes in steroid biogenesis: steroid 5α -reductase, 3α and 3β -hydroxy-steroid-dehydrogenase, 17β -hydroxysteroid-dehydrogenase.

In this work, we describe the biosynthesis of ^3H -labelled dihydrotestosterone.

Results and discussion

Synthesis of (1,2- $^3\text{H}_2$)testosterone and (1,2,4,5- $^3\text{H}_4$)dihydrotestosterone diastereoisomers

After TLC purification, the determined radiochemical purity of [1,2- $^3\text{H}_2$]testosterone substrate was >98% and the material proved suitable for use in dihydrotestos-

terone biosynthesis. From this material we obtained a mixture of [$^3\text{H}_4$]dihydrotestosterone diastereoisomers.

Biosynthetic preparation of tritiated testosterone metabolites

Biosynthetic products were separated by column chromatography (Figures 1 and 2) and consisted of the following compounds: Δ^4 -androstenedione, dihydrotestosterone, testosterone, 3α -androstane diol and 3β -androstane diol. In case of prostate tissue homogenates (Figure 1, Table 1) we identified low conversion of testosterone into dihydrotestosterone and Δ^4 -androstane diol, whilst the 3α and 3β -diols were also obtained in lower yields than was case with the reference. In case of the fibroblast culture testosterone conversion into metabolites was almost total, particularly in respect of dihydrotestosterone biosynthesis (Figure 2 and Table 1). We obtained conversions higher than 10% in case of Δ^4 -androstane diol and 3α -androstane diol. In the case of dermal fibroblast

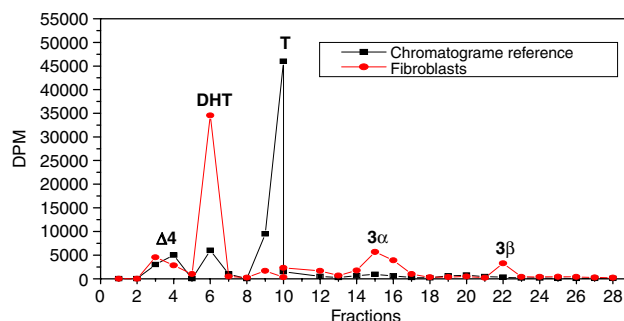


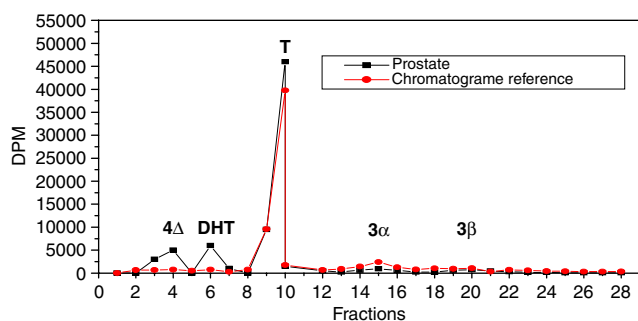
Figure 1 Chromatographic profile obtained at separation of testosterone metabolites from prostate homogenate media.

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Table 1 Testosterone conversion in tissue homogenates

Labeled compound	Conversion (%)		
	Reference sample	Fibroblast culture	Prostate homogenates
[1,2- $^3\text{H}_2$]testosterone	65.7	7.1	73.8
[1,2- $^3\text{H}_2$]dihydrotestosterone	2.5	51.5	10.3
[1,2- $^3\text{H}_2$] Δ^4 -androstanedione	1.7	10.5	9.0
[1,2- $^3\text{H}_2$]3 α -androstanediol	12.4	19.6	2.5
[1,2- $^3\text{H}_2$]3 β -androstanediol	4.4	8	2.3
Impurities	13.3	3.2	2.1

**Figure 2** Chromatographic profile obtained at separation of testosterone metabolites from fibroblast culture.

tissue homogenates the enzymatic activity of 5 α -reductase was 967 fmol/ $\mu\text{g}/\text{h}$.

The experiment confirms that tritium labeled testosterone metabolites may be obtained by biosynthesis. In case of dermal fibroblasts from inguinal zone cultures the conversion of tritiated testosterone into

dihydrotestosterone was higher than 50%. Δ^4 -androstanedione and 3 α -androstanediol can also be prepared, though at lower conversions. We do not recommend the use of prostate tissue homogenates for this application. In our case, the low conversion may be explained by the fact that the prostate tissues were obtained from prostates-affected patients, possibly showing some enzyme inhibition.

REFERENCES

1. Azziz R, Carmina E, Sawaya ME. *Endocrine Rev* 2000; **21**(4): 347–362.
2. Hiipakka RA, Liao S. *Trends Edocrinol Metab* 1998; **9**: 317–324.
3. Khoury MY, Baracat EC, Pardini DP, Vieira JGH. *Fertility and Sterility* 1994; **62**(1): 76–80.
4. Wilson DJ, Foster DW, Kronenberg M, Larsen PR. *Williams Textbook of Endocrinology* (9th edn). WB saunders Co.: Philadelphia, 1998; 751–927.