TESTOSTERONE AND ANDROSTANOLONE IN RAT PLASMA AND TISSUES

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1. Introduction

From studies of rat ventral prostate explants in culture, in which testosterone metabolism in the target organ can be correlated with testosterone and testosterone metabolite activities [1, 2], the concept has emerged that testosterone may be largely if not exclusively active through the formation of metabolic derivative(s) formed *in situ*. Parallel investigations have indicated that an active dihydro-metabolite of testosterone, androstanolone (17β-hydroxy-5α-androstan-3-one, also known as dihydro-testosterone, DHT) is accumulated in bound form in ventral prostate nuclei [3–5] whereas a prostatic cytosol *receptor* binds androstanolone with a higher affinity than testosterone [6, 7].

Whether or not androstanolone formation is an obligatory intermediary step in testosterone activity in the prostate itself and in other androgen responsive structures is still undecided, and therefore a systematic measurement of testosterone and androstanolone in selected organs of normal adult male rats has been undertaken as a first approach for understanding the physiological situation. It is believed that the local concentration of active steroids might be related to the presence and the binding characteristics of high affinity *receptors* possibly involved in hormone action.

2. Material and methods

Normal 3 month old Wistar rats, weighing 250–300 g were used throughout the experiments. They were killed by decapitation, and plasma and organ samples were collected and pooled. 10% Homogenates of the organs were made by the use of an ultra-Turrax, then 4 vol of ethanol containing tracer amounts of radioactive testosterone and androstanolone were added. After standing at −20°C overnight, the precipitate was eliminated and, after evaporation under vacuum, the extracts were taken up in water and extracted by ethyl acetate.

The gas chromatographic electron capture technique described by Van der Molen and Groen [8] for the measurement of testosterone in human plasma was modified in order to allow the measurement of testosterone and dihydro-testosterone in rat plasma and tissue samples. The ethyl acetate extract was dried, redissolved in hexane:benzene (1:1) and applied to a silica gel column [9], followed by a silica gel thin-layer chromatography in the benzene:acetone (85:15) system (2 migrations) [2]. Testosterone and androstanolone were located using a radiochromatogram scanner, and eluted. Then the heptafluorobutyrates of testosterone and androstanolone were formed, purified by thin-layer chromatography, and measured by gas liquid chromatography with electron capture detection [10]. 20β-OH-pregn-4-ene-3-one heptafluorobutyrate was used as internal standard for testosterone heptafluorobutyrate, and testosterone heptafluorobutyrate was used as internal standard for androstanolone heptafluorobutyrate. The limit of

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Table 1
Testosterone and androstanolone in rat plasma and tissues[1].

	Testosterone	Androstanolone
Plasma	2.5 ± 0.3[2]	⩽ 0.2[3]
Seminal vesicles	2.2 ± 0.3	3.0 ± 0.6[4]
Ventral prostate	2.0 ± 0.4	2.8 ± 0.5[4]
Levator ani muscle	7.9 ± 0.5[4]	⩽ 0.2
Thigh muscles	2.9 ± 0.5	⩽ 0.4
Hypothalamus	13.7 ± 2.0[4]	2.0 ± 0.4[4]
Pituitary gland	60.5 ± 5.5[4]	⩽ 6
Brain parietal cortex	1.3 ± 0.1[5]	⩽ 0.4
Kidney	12.5 ± 0.5[4]	3.0 ± 1.1[4]
Small intestine	⩽ 0.2[4]	⩽ 0.2

[1] ng/g (Organs) or ml (plasma).
[2] Mean ± SEM.
[3] ⩽ Values too low to be measured accurately. Their upper limit is given.
[4] Significantly different from corresponding plasma values $P \leqslant 0.01$.
[5] Significantly different from corresponding plasma values $P \leqslant 0.2$.

sensitivity of the method is 0.4 ng of steroid. The overall recovery of added tracers is 20–30%. Thus the initial 5 g (ml) of tissue (plasma) sample should contain at least 2 ng of steroid for processing through the entire procedure. All measurements were done in triplicate. The coefficient of variation for plasma testosterone samples was found to be 10% [10].

3. Results and discussion

The concentrations of testosterone and dihydrotestosterone were measured in rat plasma, seminal vesicles and prostate, *levator ani* and thigh muscles, hypothalamus, pituitary gland and brain parietal cortex, kidney and small intestine (table 1).

In the *plasma,* testosterone is present at a concentration similar to those previously reported [11–13], whereas androstanolone is not detected.

In *ventral prostate* and *seminal vesicles,* androstanolone is found at a slightly greater concentration than testosterone, which is itself in amount similar to that in the plasma. The fact that testosterone concentration approaches that of androstanolone is rather unexpected, although testosterone accumulation in prostate cells might be favored by the relatively high K_m of testosterone 5α-reductase [14] and the relatively high affinity of the cytosol androstanolone *receptor* for testosterone (0.2–0.5 that for androstanolone) [7]. In addition, it is not known whether both steroids are evenly distributed throughout different cell types.

In *muscles* no androstanolone is found, including in the *levator ani,* a classical androgen dependent organ. Testosterone concentration is higher than in the plasma, a finding to be correlated with the presence of a cytosol *receptor* in the *levator ani* muscle, having an affinity for testosterone greater than for androstanolone [15]. In skeletal muscles, androgens display a well known anabolic action, responsible for the sexual difference in their development even they are usually not considered as typical secondary sex organs. The concentration of testosterone in thigh muscles is of the order of that found in the plasma, whereas in control tissues as intestine or brain cortex the value is definitely lower. Therefore it might be possible that skeletal muscles contain a testosterone binding protein, possibly of feeble abundance and/or difficult to solubilize and which may be therefore very difficult to demonstrate [15]. It is noteworthy that in muscle cells which exhibit a purely hypertrophic response to testosterone, no androstanolone is found, whereas this latter compound has an hyperplastic activity previously documented in prostate experiments [1].

In several other organs, *kidney, hypothalamus* and *pituitary,* there is also testosterone accumulation, whereas androstanolone is present in a relatively small concentration. As in the muscle, testosterone itself may also be implicated in androgen action on (mice) kidney [16]. In the hypothalamus and pituitary, the nature of the steroid molecule(s) regulating the synthesis and secretion of gonadotropin releasing hormone(s) and gonadotropins is (are) still controversial. The present results suggest a role for testosterone itself, whereas arguments have been brought recently in favor of reduced [17–19] or oestrogen [20] metabolites of testosterone.

There is possibly some interest that a regulatory molecule has to proceed through several transformation steps before being active, and the formation of different active metabolite(s) in target organs may undergo a subtle regulation. This mechanism offers the possibility of selective pharmacodynamic or therapeu-

tic approaches. Testosterone might be a "hormone" in the muscles and in some cells of the kidney, the hypothalamus and the pituitary, whereas it would be a "prehormone" elsewhere, the active molecule being androstanolone in the prostate and the seminal vesicle and possibly also an oestrogen in the hypothalamus. Therefore selective analogues or antagonists could be devised in order to act upon the corresponding target tissues, thus using the metabolism of testosterone as a molecular basis to dissociate its overall effects.

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