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RAT BONE MARROW CELLS: ANDROGEN METABOLISM AND ACTIONS AT THE MOLECULAR LEVEL

J. MINGUELL* and J. K. GRANT
University Department of Steroid Biochemistry, Royal Infirmary, Glasgow, G4 OSF, Scotland

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SUMMARY

In vivo experiments have shown that testosterone but not dihydrotestosterone stimulates the incorporation of radioactive labelled formate into RNA-adenine in rat bone marrow cells. *In vitro* experiments support this observation in showing that testosterone is not converted to dihydrotestosterone to any significant extent by these cells.

INTRODUCTION

WHEN testosterone is injected into male animals, it is subjected to extensive metabolic transformation[1]. One of the principal metabolites, 5α-dihydrotestosterone, possesses considerable androgenic activity and is selectively and extensively retained in the cytoplasm and nuclei of androgen-dependent tissues[2, 3]. In these tissues, testosterone stimulates RNA synthesis[4]. This is thought to involve conversion of the testosterone to 5α-dihydrotestosterone and subsequent transport of a 5α-dihydrotestosterone-receptor complex to the nucleus of the affected cells[5].

Although bone marrow is not a typical androgen-dependent tissue, its metabolism, particularly that of erythroid cells is under the hormonal control of erythropoietin and androgenic steroids[6, 7]. It has been demonstrated that short pulses of testosterone stimulate RNA synthesis in rat bone marrow. This effect is due to the androgen itself and is not mediated by an increase in the production of erythropoietin[8].

The main purpose of the present work was to determine whether the effects produced in the rat bone marrow are due to testosterone itself or to some product of testosterone metabolism, such as 5α-dihydrotestosterone.

EXPERIMENTAL

Male Wistar rats (150–200 g) were each given an intraperitoneal injection of testosterone or 5α-dihydrotestosterone (200 μg/100 g rat in 0·1 ml propylene glycol). Simultaneously, [^{14}C]-formate (10 μCi/mmol, 4 μCi) in physiological saline was injected intravenously. After 2 h the rats were killed and bone marrow was removed from femurs and tibias[8]. Nucleic acids were separated as described by Smellie *et al.*[9]. For measurement of specific radioactivity, bases were eluted from chromatograms with 0·1 N HCl and counted in a liquid scintillation spectrometer. The optical density of RNA-adenine was measured at 260 and 290 nm and specific activities expressed as c.p.m./μmol of base.

*Present address: Universidad de Chile, Facultad de Ciencias Pecuarias y Medicina Veterinaria, Casilla 5539, Santiago, Chile.

In other experiments, bone marrow was removed from 3 rats injected with propylene glycol only and was pooled in 0·9% NaCl. The suspension was filtered through gauze and cells collected by centrifuging at 600 *g* for 15 min. Packed cells were resuspended in 2·5 ml Krebs–Ringer bicarbonate solution containing 5·5 mM glucose. [6,7-^{3}H] Testosterone (s.a. 41·8 Ci/mmol, 0·5 μCi in 10 μl ethanol) was added to 2·5 ml of the suspension containing 5–6 $\times$ 10^8 cells and incubated for 1 h at 37°C. Steroids were subsequently extracted from the incubated mixture and purified as described by Giorgi *et al.*[10]. Radiochemical purity was demonstrated after recrystallization to constant specific activity after addition of 5 mg carrier steroid.

RESULTS AND DISCUSSION

It will be seen from the results of the *in vivo* experiments (Table 1) that testosterone administration produces a significant increase in the incorporation of radioactivity into the nucleic acid, whereas 5α-dihydrotestosterone does not.

It is known[11, 12] that both testosterone and 5α-dihydrotestosterone are bound to specific plasma receptor proteins and may thus be transported to the bone marrow. Our results suggest that testosterone rather than its metabolite, 5α-dihydrotestosterone, is responsible for the modification of nucleic acid metabolism observed. The results of *in vitro* experiments (Table 2) support this view. Over 80% of the radioactivity incubated is recovered as unchanged testosterone and less than 3% is recovered as 5α-dihydrotestosterone. Approximately 1% of the radioactivity was present as 4-androstene-3,17-dione (androstenedione).

Table 1. *In vivo* effect of testosterone and 5α-dihydrotestosterone on the incorporation of [^{14}C]-formate into the RNA-adenine of rat bone marrow.

	specific activity mean c.p.m./ μmol adenine ± S.E.
Control (3)	97 ± 15
5α-Dihydrotestosterone (3)	120 ± 25
Testosterone (3)	227 ± 30

Figures in parentheses indicate the number of animals used in the experiment.

Table 2. Tritium labelled steroids identified in samples of bone marrow cells, after incubation with [^{3}H]-testosterone

[^{3}H]-testosterone		[^{3}H]-5α-dihydrotestosterone		[^{3}H]-androstenedione	
c.p.m./5 mg crystals	(%)	c.p.m./ 5 mg crystals	(%)	c.p.m./ 5 mg crystals	(%)
247·000	88	3000	1·1	2150	1·0
245·000	87	3370	1·2	2200	1·0

*The percentages are the fractions of radioactivity in the testosterone incubated found in the steroids isolated.

These percentage transformations are not significant compared with metabolism observed in other target tissues such as prostate[10].

The results of *in vitro* experiments presented here indicate that 5α-dihydrotestosterone is not formed in rat bone marrow cells to a significant extent. *In vivo* experiments suggest that it is without effect on nucleic acid metabolism in these cells. It thus appears likely that the effects of testosterone on these cells is due to this steroid itself and not to its metabolite, 5α-dihydrotestosterone, as reported for certain other tissues under androgenic control.

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