Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 721–724. Pergamon Press. Printed in Great Britain

THE EFFECT OF MESTEROLONE UPON THE CONCENTRATION OF TESTOSTERONE IN RAT TESTIS

MICHELE LUISI* and CARLO FASSORRA
Endocrine Laboratory, Institute of Medical Pathology, University of Pisa, 56100 Pisa, Italy

(*Received* 8 *September* 1971)

SUMMARY

The concentration of testosterone has been determined in testicular tissue of groups of rats. The administration of 0·5–3·0 mg mesterolone/rat/day had minimal effect whereas there was a marked decrease after the administration of 0·5 mg testosterone proprionate for 30 days as compared with the concentrations in control animals.

INTRODUCTION

NUMEROUS studies have suggested that testosterone (17β-hydroxy-4-androsten-3-one) is the most potent androgenic steroid produced and secreted by the testis of many species of animals including the rat[1–5].

Mesterolone (1α-methyl-17β-hydroxy-5α-androstan-3-one) is an orally effective androgen without an alkyl group at carbon 17. In various clinical studies, this substance was found to be as androgenic as 17α-methyl-testosterone (17α-methyl-4-androsten-3-one)[6–10] and in addition showed no effect on liver function even at relatively high dose levels[11, 12]. In certain animals, mesterolone has been shown to possess high androgenic activity, while other effects appear to be minimal or absent[10].

In order to investigate the effect of mesterolone on the biosynthesis of testosterone in rats, graded doses of the drug were administered subcutaneously and the concentration of testosterone determined in samples of testicular tissue at two intervals of time after the injection.

EXPERIMENTAL

Materials

(a) *Labelled steroids and measurement of radioactivity.* [4-^{14}C]-testosterone, S.A. 58·2 mCi/mmol was obtained from the Radiochemical Centre, Amersham, England.

Radioactivity was measured with a Nuclear Chicago (Mark I) liquid scintillation counter using a toluene based scintillator.

(b) *Thin-layer and gas–liquid chromatography.* Details of the horizontal thin-layer chromatography and gas–liquid chromatography as used for the separation and determination of testosterone has been reported[13]. We point out that by using our gas chromatographic system a very good sensitivity can be obtained and exactly 0·010 μg of authentic testosterone acetate with a coefficient of variation of 3·5% (n = 15).

* Fellow of Consiglio Nazionale delle Ricerche, Roma, Italy.

(c) *Animals.* Male rats of the Wistar strain aged 60–70 days and weighing from 100–150 g were handled in groups of 5–6 animals. Graded doses of mesterolone or testosterone propionate in sesame oil were administered subcutaneously each day for 15 or 30 days. Serial dilutions of mesterolone or testosterone propionate 0·5, 1·0 and 3·0 mg/ml sesame oil were used. Two control groups of 5 rats received 1 ml/day of sesame oil solution. The animals were sacrificed by decapitation at the end of treatment on the 16th and 30th day. The testes were removed, frozen, and stored at −18°C.

Method

Extraction. The two testes from each rat were weighed and homogenized with 35 ml of ethanol in an Ultra Turrax homogenizer. A trace amount of labelled testosterone (9·000 d.p.m.) was added and the mixture centrifuged at 3·500 rev./min in conical tubes. The supernatant was decanted and the sediment resuspended in 30 ml of ethanol, centrifuged and decanted. The procedure was repeated three times. The pooled extracts were dried in a water bath at 45°C under nitrogen.

Twenty-five ml of distilled water and 1·2 ml of 6 M sodium hydroxide solution were added to the dry samples and the aqueous phase was extracted four times with 50 ml chloroform–ether (3:0·8 v/v). The pooled organic phase was dried at 40°C under nitrogen, and the samples were spotted with acetone on the thin-layer plates. Each plate was run, using B.N.-Kammer chromatographic apparatus (Desaga, Germany) for horizontal migration in a benzene–ethyl acetate (60:40, v/v) system, and dried at room temperature.

After autoradiographic detection (using Agfa-Gevaert X-ray films, with exposure in the dark for 24 or 48 h) and ultraviolet absorption, the area of silica gel containing testosterone-[4-^{14}C] was loosened with a micro-spatula, aspirated into a sintered glass disc, eluted under positive pressure with $4 \times 0{\cdot}4$ ml absolute ethanol and the eluate evaporated to dryness under nitrogen. The eluate was then subjected to a second horizontal migration, in dichloromethane–ethyl acetate (95:5, v/v). Autoradiography and elution were repeated and the dried residue was dissolved in redistilled pyridine (0·3 ml) and redistilled acetic anhydride (0·3 ml) for acetylation (time of reaction: 24 h at room temperature).

After acetylation, the reactants were removed under nitrogen at 55°C, and the residue was rechromatographed in benzene–ethyl acetate (80:20, v/v), using horizontal migration.

Autoradiography and elution were repeated and the dried residue was dissolved in acetone. 1/10 of the solution was removed for liquid scintillation counting and appropriate aliquots were taken for gas chromatography using liquid injection (10 μl Hamilton syringe). The gas chromatograph was a Fractovap D (Carlo Erba, Milan) with dual ionisation detectors and the analyses were performed using 80 cm × 2 mm (internal diameter) columns containing 3% SE-30 on 80–100 mesh acid-washed silanised Chromosorb W.

The peak areas were measured by triangulation and by an automatic electronic decade counting system (maximum count rate: 1·500 pulses/sec) with a Keinzle digital printer (D-11 E). The testosterone was expressed as testosterone-acetate per 100 g of testicular tissue.

RESULTS

The results in treated animals and appropriate controls are shown in Table 1.

There is evidence for a fall in the mean testicular concentrations of testosterone after the administration of testosterone propionate for 15 and 30 days treatment as compared with the tissue concentrations in control animals. After 30 days of administration, this difference is highly significant ($P > 0{\cdot}001$; t test).

Following the administration of mesterolone, there is no significant difference in the testosterone response to 0·5 mg/rat/day (15 and 30 days of treatment) and 1·0 mg/rat/day (15 days of treatment) when compared with untreated and sesame oil solution treated controls. However, this difference is moderately significant ($P > 0{\cdot}01$) when the results in controls are compared with those of animals receiving 1·0 mg/day (30 days of treatment) and 3·0 mg/day (15 and 30 days of treatment). With increasing doses of mesterolone, there was a fall in the concentration of testicular testosterone which was, however, much less significant than that obtained in the rats after administration of testosterone propionate.

Table 1. Concentration of testosterone in testicular tissue in groups of treated and untreated rats. In parenthesis the weight of the testicles in mg ± S.D.

Compounds	Dose (mg/rat/day)	No. of rats	Testosterone μg ± S.D./100 g testicular tissue 15 days of treatment	30 days of treatment
Mesterolone	0·5	6+6	13·0 ± 0·74 (1588 ± 76)	12·5 ± 1·58 (1520 ± 68)
	1·0	6+6	12·6 ± 1·16 (1616 ± 64)	11·8 ± 1·36 (1426 ± 59)
	3·0	5+5	11·6 ± 1·60 (1717 ± 57)	11·0 ± 1·23 (1912 ± 62)
Testosterone propionate	0·5	5+5	10·1 ± 1·82 (1281 ± 67)	5·5 ± 1·65 (1016 ± 80)
Sesame oil solution	1 ml	5+5	13·7 ± 0·73 (1670 ± 96)	14·3 ± 1·54 (1694 ± 81)
Untreated control	—	10	14·5 ± 1·19 (1620 ± 79)	

DISCUSSION

The concentration of testosterone in the testicular tissue from normal, untreated or sesame oil treated rats is consistent with previous studies[1, 4]. The present work indicates that mesterolone even in high doses for 30 days does not significantly influence the concentration of testosterone in the testes whereas there is a marked depletion of testicular androgen after administration of a lower dose of testosterone propionate for the same period of time. Both mesterolone and testosterone propionate exhibit androgenic and anabolic effects when administered subcutaneously to rats[10] but the anabolic effect of mesterolone appears to be dissociated from the androgenic effect in comparison with testosterone propionate. The present results suggest that this may reflect differences in central inhibitory activity associated with major changes in endogenous androgen secretion.

ACKNOWLEDGEMENTS

The authors are grateful to the "Consiglio Nazionale delle Ricerche, Roma (Italy), for financial support, and to the Schering A. G. Berlin for the supply of mesterolone.

REFERENCES

1. Hashimoto I. and Suzuki Y.: *Endocrinol. Japan* **13** (1966) 326.
2. Lindner H. R.: *J. Endocrinol.* **23** (1961) 161.

3. Eik-Nes K. B.: *Endocrinology* **71** (1962) 101.
4. Bardin C. W. and Peterson R. E.: *Endocrinology* **80** (1967) 38.
5. Resko J. A., Feder H. H. and Goy R. W.: *J. Endocrinol.* **40** (1968) 485.
6. Schirren C.: *Z. Urol. Nephrol.* **58** (1965) 541.
7. Schirren C.: *Neue Ergebnisse der Endocrinologie*. Springer, Berlin (1965), p. 69.
8. Schirren C.: *Arzneim.-Forsch.* **4** (1966) 463.
9. Weller O.: *Arzneim.-Forsch.* **4** (1966) 465.
10. Neumann F., Wiechert R., Kramer M. and Raspé G.: *Arzneim.-Forsch.* **16** (1966) 455.
11. Pohle H. D.: *Arzneim.-Forsch.* **4** (1966) 473.
12. Schnack H. and Wewalka F.: *Arzneim.-Forsch.* **4** (1966) 471.
13. Luisi M., Fassorra C., Levanti C. and Franchi F.: *J. Chromatog.* **58** (1971) 213.