Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 837–842. Pergamon Press. Printed in Great Britain

# KINETICS OF [$^3$H]-TESTOSTERONE METABOLISM IN NORMAL YOUNG MEN: EFFECTS OF MEDROXYPROGESTERONE ACETATE (PROVERA)* ADMINISTRATION

A. F. CLARK, R. S. CALANDRA† and C. E. BIRD
Departments of Biochemistry and Medicine, Queen's University and Kingston General Hospital, Kingston, Ontario, Canada

(*Received* 26 *January* 1972)

## SUMMARY

We studied the effects of oral medroxyprogesterone acetate (provera) administration on the kinetics of testosterone metabolism in normal young men. We investigated changes in the metabolic clearance rate, transport and metabolic rate constants, volumes of distribution, plasma levels, blood production rates and levels of binding to plasma proteins. In all subjects the plasma level and blood production rate for testosterone decreased during provera administration. When provera was administered for 5 days the metabolic clearance rate and volumes of distribution decreased. After 10 days of administration, the changes in the metabolic clearance rate were variable. In the 5 day studies, the levels of binding of testosterone to plasma proteins increased; they showed variable changes after 10 days.

## INTRODUCTION

THE TREATMENT of children with isosexual precocity has been a challenge to physicians. The drug of choice should be an inhibitor of gonadotrophin production without side effects. Provera* has been used with some success in the treatment of the disorder[1–3]. Part of the drug's action may be through inhibition of gonadotrophin production[4]. The urine gonadotrophin levels in two of five children with isosexual precocious puberty were suppressed by provera administration[5]. Root *et al.*[6] have recently reported that provera administration decreased plasma luteinizing hormone (LH) levels in a boy with true precocious puberty. Gordon *et al.*[7] have reported that provera administration decreased plasma LH but not follicle stimulating hormone (FSH) levels in three of four normal men.

The elevated plasma and urine levels of testosterone are decreased in some but not all boys with precocious puberty by provera treatment[2, 3]. In addition to altering gonadotrophin levels, we considered that provera might affect the kinetics of testosterone metabolism. To study this possibility we used the single injection and constant infusion techniques. The techniques are based on the two-compartment model developed by Tait *et al.*[8] for studying steroid metabolism.

This paper reports the effects of provera administration on the kinetics of testosterone metabolism, as well as the plasma testosterone level, blood production rate and the level of binding of testosterone to plasma proteins in normal young men. Preliminary reports of this work have been published[9, 10].

*The following trivial names and abbreviations are used: testosterone: 17$\beta$-hydroxy-4-androsten-3-one, provera: (medroxyprogesterone acetate): 17$\alpha$-acetoxy-6$\beta$-methyl-4-pregnene-3,20-dione, epitestosterone: 17$\alpha$-hydroxy-4-androsten-3-one, etiocholanolone: 3$\alpha$-hydroxy-5$\beta$-androstan-17-one.

†Present address: Inst. de Biologia y Medicina, Exper. Obligado 2490, Buenos Aires, Argentina.

## MATERIALS AND METHODS

### *Subjects*

Six normal young men (23–30 yr) were studied prior to and during the oral administration of provera. The dosage schedules are given in Table 1. No subject was receiving any other medication and none had any indication of intercurrent disease. All were fully informed volunteers.

### *Experimental design*

The single injection technique was used for most studies. The details of [$^3$H]-testosterone injection, blood sampling and analysis of the plasma were reported previously[11].

The two-compartment model as described by Tait *et al.*[8] is the basis for analysis of the data obtained by the single injection technique. We used a computer program for this analysis which yields the following parameters:

Metabolic clearance rate (MCR)—volume of plasma totally and irreversibly cleared per unit time, l/24 h (1/24 h). The MCR is also expressed in terms of surface area as l/m$^2$ per 24 h (l/m$^2$/24 h).

$\alpha$ slope of calculated component of model[8].

$\beta$ slope of final part of disappearance curve[8].

$K_1$ rate of transfer from inner to outer pool, units/24 h (U/24 h).

$K_2$ rate of metabolism in inner pool, units/24 h (U/24 h).

$V_1$ volume of inner pool, l[1].

$V_2$ volume of outer pool, l[1].

$\bar{V}$ reciprocal of extrapolated final part of disappearance curve at time zero in l[1].

The volumes are mathematical and have no known anatomical significance. By definition, the plasma into which the injection is made is part of the inner pool.

Studies in one subject were performed with the constant infusion technique [12, 13]. The plasma samples were processed as for the single injection procedure. Only a value for the MCR is obtained with this procedure.

Urine was collected for 24 h prior to the single injection or constant infusion studies. Excretion values for creatinine, 17-ketosteroids, 17-ketosteroid fractions, 17-ketogenic steroids, testosterone and epitestosterone were obtained for these urines. Urine was collected for 48 h following the injection of [$^3$H]-testosterone for the single injection studies. The specific activities of the testosterone metabolites in these urines were determined. The results of all these urine measurements are reported separately[14].

Just prior to the injection of [$^3$H]-testosterone for both the single injection and constant infusion studies, blood was taken for the determination of plasma testosterone and the level of binding of testosterone to plasma proteins. Plasma testosterone was measured by a modification of the method of Mayes and Nugent[15]. In our laboratory the plasma extracts were chromatographed on paper in the system: cyclohexane: *p*-dioxane: methanol: water(100:25:100:10 by vol.). The testosterone areas on the chromatograms were eluted overnight in ethanol at 4°C for assay as originally described. Levels of binding of testosterone to plasma proteins were measured by a slight modification[16] of the method of Forest *et al.*[17].

Radioactive compounds were purchased, checked for purity and counted as previously described[18]. Chromatography for the kinetic studies was performed as described[18]. Provera (medroxyprogesterone acetate) was obtained from the Upjohn Co. of Canada, Don Mills, Ontario, Canada.

Table 1. The subjects studied, with ages (parenthesis) and their calculated metabolic clearance rates, transport and metabolic rate constants and volumes of distribution; plasma testosterone levels, blood production rates (PR) and level of binding to plasma proteins

| Subject (Age, yr) | Provera Dose (days) | MCR* (l/24 h) | MCR* (l/m²/24 h) | $\alpha$ | $\beta$ | $K_1$ | $K_2$ | $V_1$ | $V_2$ | $\overline{V}$ | Plasma T (ng/100 ml) | PR (mg/24 h) | Binding (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | U/24 h | | | | | (l) | | | | |
| MMcG (23) | Nil | 1084 | 524 | 108·3 | 34·6 | 19·5 | 59·1 | 18·3 | 5·9 | 47·6 | 552 | 6·0 | 92·9 |
| | 20 mg (5) | 949 | 458 | 116·6 | 36·4 | 22·7 | 63·9 | 14·9 | 5·1 | 40·0 | 209 | 2·0 | 93·7 |
| AM (30) | Nil | 940 | 531 | 106·3 | 29·7 | 23·2 | 59·9 | 15·6 | 6·9 | 52·6 | 995 | 9·4 | 91·1 |
| | 20 mg (5) | 873 | 494 | 132·9 | 36·0 | 23·0 | 72·8 | 12·0 | 5·5 | 38·5 | 757 | 6·6 | 93·4 |
| GP (29) | Nil | — | — | — | — | — | — | — | — | — | 847 | — | 93·1 |
| | 20 mg (5) | 1028 | 544 | 127·9 | 39·7 | 24·8 | 71·2 | 14·4 | 5·1 | 20·0 | 560 | 5·8 | 94·4 |
| RC (29) | Nil | 750 | 407 | 102·6 | 21·5 | 30·0 | 48·9 | 15·3 | 10·2 | 52·6 | 434 | 3·3 | 92·0 |
| | 20 mg (11) | 807 | 437 | 129·3 | 25·5 | 39·7 | 63·6 | 12·7 | 9·7 | 50·0 | 116 | 0·9 | 92·0 |
| MK (24) | Nil | 1065 | 512 | 117·2 | 33·7 | 25·3 | 61·8 | 17·2 | 6·8 | 47·6 | 496 | 5·3 | 94·0 |
| | 40 mg (10) | 940 | 452 | 110·2 | 31·1 | 24·1 | 58·6 | 16·1 | 6·6 | 45·5 | 247 | 2·3 | 92·5 |
| DD (28) | Nil | 963 | 510 | — | — | — | — | — | — | — | 634 | 6·1 | 91·9 |
| | 40 mg (13) | 1143 | 605 | — | — | — | — | — | — | — | 74 | 0·9 | 91·9 |
| Mean ± SEM | 14 normal young men (20–40 yr.) | 1099 ±47 | 599 ±23 | 115·7 ±4·9 | 28·6 ±1·9 | 28·6 ±2·1 | 56·0 ±3·0 | 20·2 ±1·3 | 9·8 ±0·8 | 60·6 ±4·3 | — | — | — |

*The symbols used in this table are as follows: MCR = metabolic clearance rate or volume of plasma totally and irreversibly cleared per unit time; $\alpha$ = slope of calculated component of model (8); $\beta$ = slope of final part of disappearance curve; $K_1$ = rate of transfer from inner to outer pool; $K_2$ = rate of metabolism in inner pool; $V_1$ = volume of inner pool; $V_2$ = volume of outer pool; $\overline{V}$ = reciprocal extrapolated final part of disappearance curve at time zero; PR = production rate.

## RESULTS

All six subjects were studied immediately prior to the administration of provera. Control kinetic data was not obtained for subject GP due to technical problems. Subject DD was studied with the constant infusion technique, the others by the single injection method. The control kinetic data for all subjects was within our range for normal young men[18]. The control values for plasma testosterone, blood production rate of testosterone and the level of binding to plasma proteins are also within normal limits[16, 19].

As shown in Table 1 the first three subjects received 20 mg of oral provera daily for five days. The plasma kinetics for subjects MMcG and AM show slight decreases in the MCR, $V_1$, $V_2$ and $\bar{V}$ and small increases in $\alpha$, $\beta$ and $K_2$. Only the changes in $\alpha$, $V_1$ and $\bar{V}$ are statistically almost significant ($P < 0{\cdot}1$). While the changes in the kinetic parameters were slight, there were major reductions in the plasma testosterone levels for all these subjects and the testosterone blood production rates for the first two subjects. There were increases in the levels of binding for the three subjects. The mean ± standard error of the mean for the kinetic data on 14 normal young men are given at the bottom of Table 1.

The last three subjects received 20–40 mg provera for 10–13 days as shown in Table 1. In the kinetic data the only consistent changes were the small decreases in $V_1$, $V_2$ and $\bar{V}$. However there were again large decreases in the plasma levels and the blood production rates for testosterone. The level of binding decreased in one and remained the same in two of the volunteers.

## DISCUSSION

Rivarola *et al.*[2] and Gordon *et al.*[7] have looked at the effects of provera administration on some of the parameters of testosterone metabolism. In their studies the provera was given at higher dosages and for longer time periods. In our studies with smaller doses given for a shorter period of time we found similar changes in the plasma level and blood production rate of testosterone.

Many investigators feel that the MCR is the best overall measure of steroid metabolism. The MCR is influenced by a number of factors including the plasma concentration, and extent of binding to plasma proteins, (reflected in changes in the volumes of distribution) and rate of metabolism in the inner pool. Southren and Gordon[19] have indicated that the plasma concentration of testosterone has to be greatly altered in order to significantly affect its MCR. Vermeulen *et al.*[20] have postulated that the MCR of testosterone is inversely related to the fraction of the hormone in plasma not bound to the specific binding $\beta$-globulin. This fraction will be reflected by our protein binding determination.

Long term studies (several months) with provera administration[21] show that the level of protein binding is decreased. Using Norit treated plasma this change was found to be independent of the concentration of steroid, therefore, the change must be due to a decrease in the capacity (concentration) of the specific $\beta$-globulin. Our ten day studies gave inconsistent results, similar to the findings of Gordon *et al.*[7] and Rivarola *et al.*[2]. In contrast our five day studies show a rise in the level of protein binding of testosterone. If this increase is independent of the plasma testosterone level[21] then the provera must initially increase the quantity of the $\beta$-globulin. The important end result of the increased binding (whether due to increased $\beta$-globulin or decreased steroid levels) is a decreased unbound testosterone fraction resulting in a decreased MCR.

Gordon *et al.*[22] have shown that provera administration to humans increases hepatic testosterone 4-en-reductase activity; this was coupled with an increase in the MCR. In our kinetic studies, the increase in $K_2$ in three of four subjects may indicate that 4-en-reductase activity was also being changed in our short-term experiments. In the 5 day studies, the increase in $K_2$ is overridden by the increase in binding to plasma proteins, resulting in a decrease in the MCR. In subject RC where provera was given 10 days, the level of binding was the same as the control value and the small increase in the MCR can be related to the increase in $K_2$. The results in subject MK are difficult to explain.

Our studies on the urine metabolites of testosterone in these subjects support the idea that testosterone metabolism was altered by only five days of provera administration[14]. The urine excretion of testosterone, epitestosterone, androsterone and etiocholanolone decreased significantly. Labelled metabolites were isolated from the urine following the injection of [$^3$H]-testosterone. During provera administration the fall in testosterone production was reflected in increases in the specific activities of those metabolites excreted as glucuronic acid conjugates. Etiocholanolone glucuronide showed an increase in its specific activity during provera administration, but etiocholanolone sulphate did not.

The changes in volumes of distribution during provera administration do not correlate with the levels of binding which is in contrast to our studies when oestrogen is administered[18].

Alterations in the gonadotrophin levels are probably the major cause of the reduction in the plasma level and blood production rate of testosterone when provera is administered. There is good evidence however that provera alters the peripheral metabolism of testosterone resulting first in a decrease and then an increase in the MCR.

## ACKNOWLEDGMENTS

This work was supported in part by grants MA1852 and MA2338 from the Medical Research Council of Canada. The technical assistance of Miss Bernice Delory, Mrs. Barbara Burns and Mrs. Elizabeth Clemow is greatly appreciated. The assistance of Miss McCleary, Mrs. Gonu and the nursing staff of the Special Investigation Unit was essential to the success of these studies.

## REFERENCES

1. Collip P. J., Kaplan S. A., Boyle D. C., Plachte R. and Kogut M. D.: *Am. J. Dis. Child.* **108** (1964) 399.
2. Rivarola M. A., Camacho A. M. and Migeon C. J.: *J. clin. Endocr.* **28** (1968) 679.
3. Schoen E. J.: *J. clin. Endocr.* **26** (1966) 363.
4. Kupperman H. S. and Epstein J. A.: *J. clin. Endocr.* **22** (1962) 456.
5. Rifkind A. B., Kulin H. E., Cargille C. M., Rayford P. C. and Ross G. T.: *J. clin. Endocr.* **29** (1969) 506.
6. Root A. W., Moshang T., Bongiovanni A. M. and Eberlein W. R.: *Pediat. Res.* **4** (1970) 175.
7. Gordon G. G., Southren A. L., Tochimoto S., Olivo J., Altman K., Rand J. and Lemberger L.: *J. clin. Endocr.* **30** (1970) 449.
8. Tait J. F., Tait S. A. S., Little B. and Laumas K. R.: *J. clin. Invest.* **40** (1961) 72.
9. Clark A. F., Calandra R. and Bird C. E.: *Excerpta Med. (Amst.) Int. Cong. Ser.* **210** (1970) 137.
10. Clark A. F., Calandra R. S. and Bird C. E.: *Clin. Res.* **18** (1970) 718.
11. Bird C. E., Green R. N. and Clark A. F.: *J. clin. Endocr.* **29** (1969) 123.
12. Tait J. F., Little B., Tait S. A. S., Black W. P., Riondel A. and Gut M.: In *Hormonal Steroids*, Vol. 1. (Edited by Martini L. and Pecile A.). Academic Press, New York (1964) p. 81.
13. Tait J. F. and Burstein S.: In *The Hormones*, Vol 5, (Edited by Pincus G., Thimann K. V. and Astwood E. B.). Academic Press, New York (1964) p. 441.
14. Calandra R. S., Clark A. F. and Bird C. E.: *Acta endocr. Panam.* **2** (1971) 135.

15. Mayes D. and Nugent C. A.: *J. clin. Endocr.* **28** (1968) 1169.
16. Clark A. F., Calandra R. S. and Bird C. E.: *Clin. Biochem.* **4** (1971) 89.
17 Forest M. G., Rivarola M. A. and Migeon C. J.: *Steroids* **12** (1968) 323.
18. Bird C. E., Green R. N., Calandra R. S., Connolly J. G. and Clark A. F.: *Acta endocr. (Kbh.)* **67** (1971) 733.
19. Southren A. L. and Gordon G. G.: *Mt. Sinai J. Med.* **37** (1970) 516.
20. Vermeulen A., Verdonck L., Van der Straeten M. and Orie N.: *J. clin. Endocr.* **29** (1969) 1470.
21. Forest M. G. and Bertrand J.: *Steroids* **19** (1972) 197.
22. Gordon G. G., Altman K., Southren A. L. and Olivo J.: *J. clin. Endocr.* **32** (1971) 457.