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SHORT COMMUNICATION

TESTOSTERONE METABOLISM BY PLACENTAL MICROSOMES FROM BABOONS. IDENTIFICATION OF 19-NORTESTOSTERONE AND 19-NOR-4-ANDROSTENEDIONE*

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Placental microsomes from baboon (*Papio cynocephalus*) metabolize testosterone to 4-androstenedione§, 19-hydroxytestosterone, 19-hydroxy-4-androstenedione, 19-aldotestosterone, 19-aldo-4-androstenedione, 2β-hydroxytestosterone, 4-androstene-3β,17β-diol, oestrone, oestradiol-17β, 2-hydroxyoestrone and 2-hydroxyoestradiol [1–3]. Most of these metabolites are either aromatic steroids or substrates for aromatization by human placental microsomes [4]. In the incubations of radioactive-labeled testosterone with baboon placental microsomes we were able to isolate two additional bands of radioactivity which were slightly more polar than testosterone and 4-androstenedione, respectively. Their mobilities suggested that these steroids were the corresponding 19-nor derivatives, 19-nortestosterone and 19-nor-4-androstenedione, which have been described as substrates for the aromatase system of human placenta microsomes [4–9].

Washed, lyophilized placental microsomes from baboon used in these experiments were prepared as described previously [1]. [4-^{14}C]-testosterone (50.5 mCi/mmol) and [1,2-^{3}H]-testosterone (42.4 Ci/mmol) were purified by paper chromatography before use [1].

The incubations were carried out for 1 h at 37°C using 15 mg of washed lyophilized microsomes per incubation and 2.64 μCi of [4-^{14}C]-testosterone, 58.1 μCi of [1,2-^{3}H]-testosterone, and 2.5 mg of NADPH in 4 ml of 0.05 M sodium phosphate buffer, pH 7.14, under oxygen–carbon dioxide (95:5, V/V). Incubations without tissue were used as controls. The reactions were stopped by cooling and by the addition of 15 ml acetone. Using paper chromatography [1–3] the various metabolites and substrate were separated (Fig. 1). 19-Nortestosterone was slightly more polar than testosterone on paper chromatography using the solvent system propylene glycol-saturated methylcyclohexane and paper impregnated with propylene glycol–methanol (1:1, V/V) [MeC/PG (1:1)]; the chromatogram was run for 4 days (Fig. 1). The mobility of 19-nortestosterone relative to testosterone was 0.71–0.74. The area of radioactivity corresponding to 19-nortestosterone was eluted and rechromatographed on paper for 8 days using testosterone as the reference steroid; the radioactive material corresponding to 19-nortestosterone had the same relative mobility as in the first chromatogram. 19-Nor-4-androstenedione and 4-androstenedione were collected together in the 4-day runoff (Fig. 1). Paper chromatography using MeC/PG (1:1) but running for 24 h, was found to result in a distinct separation of 4-androstenedione from the more polar 19-nor-4-androstenedione, which had a relative mobility of 0.73–0.79. The purified 19-norsteroid metabolites were crystalized to constant specific activity after dilution with the corresponding authentic steroids 19-nortestosterone and 19-nor-4-androstenedione. The solvent system used for crystallization was acetone-pentane. Specific activities of crystal aliquots obtained in three consecutive crystallizations and rates of formation of 19-nortestosterone and 19-nor-4-androstenedione are presented in Table 1. The constancy of the specific activities and the chromatographic data demonstrate the presence of 19-nortestosterone and 19-nor-4-androstenedione as metabolites of testosterone by baboon placental microsomes. The substrate used in the incubations, [4-^{14}C-1,2,^{3}H]-testosterone, and the substrate recovered in 2 experiments were crystallized 5 times to constant specific activity: the mean values and S.E. of the ^{3}H:^{14}C ratios of crystals obtained in the last three crystallizations were 21.7 ± 0.51 (n = 3) for the substrate and 19.81 ± 0.04 (n = 6) for the recovered substrate. The products, 19-nortestosterone and 19-nor-4-androstenedione, processed in the same fashion had ^{3}H:^{14}C ratios of 15.4 ± 0.19 (n = 12) (Table 1). The loss of tritium observed in the isolated 19-norsteroids is unaccounted for. We observed previously that other C_{19}-steroids obtained as products of [1,2-^{3}H-4-^{14}C]-testosterone metabolism by baboon placenta microsomes also showed some tritium loss [1]; this is probably due to spontaneous loss from the C-2 position.

19-Nortestosterone and 19-nor-4-androstenedione are presumably derived from the 19-hydroxy- and/or 19-aldo steroids which have been identified as metabolites of isotope-labeled testosterone by placental microsomes [1–3]. These C-19 oxygenated metabolites, which are also

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§ Abbreviations, trivial names and systematic equivalents used in this paper are: Test, testosterone; 4-and, 4-androstenedione, 4-androstene-3,17-dione; 19-OH-Test, 19-hydroxytestosterone, 17β,19-dihydroxy-4-androsten-3-one; 19-OH-4-And, 19-hydroxy-4-androstenedione, 19-hydroxy-4-androstene-3,17-dione; 19-aldo-Test, 19-aldotestosterone, 17β-hydroxy-3-oxo-4-androsten-19-al; 19-aldo-4-And, 19-aldo-4-androstenedione, 3,17-dioxo-4-androsten-19-al,; 2β-OH-Test, 2β-hydroxytestosterone, 2β,17β-dihydroxy-4-androsten-3-one; E_1, oestrone; E_2, oestradiol-17β; 2-OH-E_1, 2-hydroxy-oestrone, 2,3-dihydroxy-1,3,5(10)-oestratrien-17-one; 2-OH-E_2, 2-hydroxy-oestradiol, 1,3,5(10)-oestratriene-2,3,17β-triol; 19-nor-Test, 19-nortestosterone, 17β-hydroxy-4-oestren-3-one; 19-nor-4-And, 19-nor-4-androstenedione, 4-oestrene-3,17-dione.

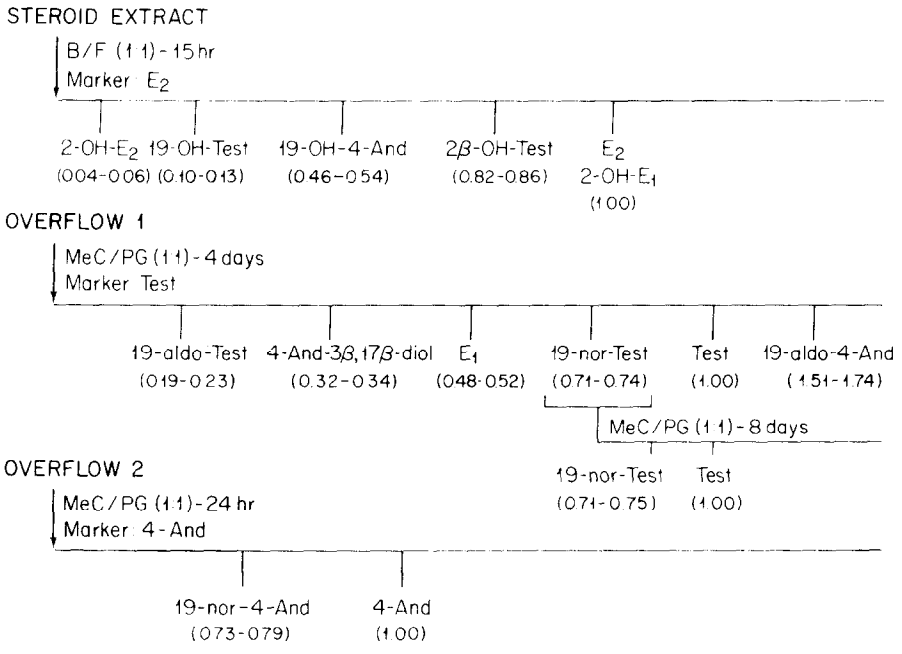

Fig. 1. Flow diagram depicting the separation of steroid metabolites isolated from incubation of washed, lyophilized baboon placental microsomes with [4-^{14}C-1,2-^{3}H]-testosterone and NADPH by paper chromatography. Relative mobilities towards marker steroids are indicated in parenthesis. Consecutive chromatograms were carried out. The abbreviations used are: B/F (1:1), benzene–formamide (diluted 1:1, V/V, with methanol; MeC/PG (1:1), methylcyclolexane–propylene glycol (diluted 1:1, V/V with methanol). The remaining abbreviations are explained in the text.

intermediates in the synthesis of oestrogens [8, 10, 12], may collapse to the 19-nor steroids enzymatically or by general acid-base catalysis. It is suggested that this event occurs before 2β-hydroxylation of 19-aldotestosterone or 19-aldo-4-androstenedione, since it it is known that this last step leads to the spontaneous synthesis of oestrogens [11]. Moreover, the synthesis of oestrogens from C_{19}-steroids *via* 2β-hydroxy-19-aldo-4-androstenedione leads to the simultaneous formation of formic acid derived from the C-19-aldo-group [13]. It has been observed, however, that in addition to formic acid, formaldehyde derived from the C-19 position of the substrate is also formed [14, 15]. The presence of formaldehyde arising from the C-19 position can be explained, at least in part, as a product of collapse of 19-hydroxysteroid intermediates in the formation of the 19-norsteroids.

The baboon and human placentas have similar morphological characteristics [16], and steroid metabolizing enzymes [1, 4]. Bolton[17] reported the isolation of 19-nor-4-androstenedione from incubations of isotope-

Table 1. Criteria for radiochemical homogeneity in the characterization of 19-norsteroid metabolites isolated from incubations of [4-^{14}C-1,2-^{3}H]-testosterone with baboon placental microsomes. Crystallization to constant specific activity*, and quantification†

Metabolite	Crystallization number	Experiment 1: Crystals d.p.m./mg ^{3}H	^{14}C	Experiment 1: Rates of formation fmol/mg protein/h	Experiment 2: Crystals d.p.m./mg ^{3}H	^{14}C	Experiment 2: Rates of formation fmol/mg protein/h
19-Norstestosterone	1	10,700	697		3,960	256	
	2	10,400	642		3,920	264	
	3	9,790	625	5.2	3,920	249	2.0
19-Nor-4-androstenedione	1	22,500	1,370		7,010	497	
	2	19,900	1,270		8,070	520	
	3	21,400	1,380	10.6	7,940	548	4.1

* The bands of radioactivity corresponding to 19-nortestosterone and 19-nor-4-androstenedione, purified by paper chromatography, were diluted with authentic carriers (5 mg) and crystallized 5 times. The specific activities of the crystals obtained in the last three crystallizations are reported. † The formation rates reported are minimal, since losses throughout the extraction and purification steps were not accounted for.

The mean value is reported.

labeled 4-androstenedione with human placental microsomes in the presence of NADPH. To our knowledge, however, this is the first report of the isolation of 19-nortestosterone and 19-nor-4-androstenedione as metabolites of isotope-labeled testosterone by baboon placental microsomes.

REFERENCES

1. Milewich L. and Axelrod L. R.: Oestrogen synthesis from [4-^{14}C-1,2-^{3}H]-testosterone by placental microsomes from baboons. *J. Endocr.* **52** (1972) 137–145.
2. Milewich L. and Axelrod L. R.: Metabolism of [4-^{14}C-1,2-^{3}H]-testosterone by placental microsomes from baboon: identity of new metabolites in studies of aromatization. *Endocrinology* **91** (1972) 1101–1105.
3. Milewich L. and Axelrod L. R.: Metabolism of [4-^{14}C]-testosterone by lyophilized baboon placental microsomes. *Endocrinology* **88** (1971) 589–595.
4. Ryan K. J.: Biological aromitization of steroids. *J. biol. Chem.* **234** (1959) 268–272.
5. Ryan K. J.: Biogenesis of estrogens. In *Biosynthesis of Lipids* (Edited by Popjak, G.) Vol. 7, MacMillan, New York (1963) p. 381.
6. Gual C., Morato T., Hayano M., Gut M. and Dorfman R. I.: Biosynthesis of estrogens. *Endocrinology* **71** (1962) 920–925.
7. Townsley J. D. and Brodie H. J.: Studies on the mechanism of estrogen biosynthesis–III. The stereochemistry of aromatization of C_{19} and C_{18} steroids. *Biochemistry* **7** (1968) 33–40.
8. Meigs R. A. and Ryan K. J.: Enzymatic aromatization of steroids—I. Effects of oxygen and carbon monoxide on the intermediate steps of estrogen biosynthesis. *J. biol. Chem.* **246** (1971) 83–87.
9. Nambara T., Anjyo T. and Hosoda H.: Stereochemistry of enzymic aromatization of 19-norsteroids. *Chem. Pharm. Bull.* **20** (1972) 853–854.
10. Thompson E. A. and Siiteri P. K.: Utilization of oxygen and reduced nicotinamide adenine dinucleotide phosphate by human placental microsomes during aromatization of androstenedione. *J. biol. Chem.* **249** (1974) 5364–5372.
11. Goto J. and Fishman J.: Participation of nonenzymatic transformation in the biosynthesis of estrogens from androgens. *Science* **195** (1977) 80–81.
12. Wilcox R. B. and Engel L. L.: Kinetic studies on the role of 19-hydroxy-androst-4-ene-3,17-dione in estrogen biosynthesis. *Steroids* **5** (Suppl I) (1965) 49–57.
13. Akhtar M. and Skinner S. J. M.: The intermediary role of a 19-oxoandrogen in the biosynthesis of oestrogen. *Biochem. J.* **109** (1968) 318–321.
14. Axelrod L. R., Matthijssen C., Rao P. N. and Goldzieher J. W.: The fate of carbon-19 in the course of estrogen biosynthesis from testosterone-19-^{14}C. *Acta endocr. Copenh.* **48** (1965) 383–391.
15. Breuer H. and Grill P.: Bildung von Formaldehyd bei der Aromatisierung neutraler Steroide zu phenolischen Steroiden. *Z. physiol. Chem.* **234** (1961) 254–261.
16. Wynn R. M., Panigel M. and MacLennan A. H.: Fine structure of the placenta and fetal membranes of the baboon. *Am. J. Obstet. Gynec.* **109** (1971) 638–648.
17. Bolton S. K. M.: Studies on the aromatization of androgens to estrogens in human placental microsomes. M.A. thesis, University of Texas, 1969.