Journal of Steroid Biochemistry, 1976, Vol. 7, pp. 683–685. Pergamon Press. Printed in Great Britain

BOVINE ADRENAL 20α-HYDROXYSTEROID OXIDO-REDUCTASE AND VARIATIONS OF ACTIVITY WITH AGE

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(*Received* 5 *January* 1976)

SUMMARY

Bovine adrenal 20α-hydroxysteroid oxido-reductase has been measured in the glands from animals of various ages. The substrates 20α-hydroxyprogesterone and 17α,20α-dihydroxyprogesterone were employed under conditions favoring oxidation. The highest activity was found in the newborn and older but prepuberal glands and very little in fetal or mature tissue. This activity was notable with 20α-hydroxyprogesterone whereas 17α,20α-dihydroxyprogesterone remained unoxidized. These findings with respect to age may partly explain the adrenarche wherein the production of C-19 steroids by the adrenal rises sharply around the time of sexual maturation. Some work indicates that 20-keto and not 20-hydroxy C-21 steroids are precursors to side chain cleavage. The higher level of 20-hydroxy-steroid oxido-reductase activity in the prepuberal organs suggests that this enzyme diverts possible C-21 precursors from this cleavage reaction.

INTRODUCTION

The 20α-hydroxysteroid oxido-reductase, a soluble enzyme, has been observed in liver, muscle, placenta, adrenal, ovary and testis [1–7]. In most reports 20-ketone substrates have been used whereas we employed 20-hydroxy steroids under conditions favoring oxidation, as did Wiest [2]. We have examined this enzyme activity in beef adrenals of various ages. Differences have been noted according to age and in some details our results differ from those of other experiments.

The trivial names, abbreviations and systematic nomenclature for steroids employed herein are as follows. Progesterone (P) = 4-pregnene-3,20-dione; 20α-hydroxyprogesterone (20HP) = 20α-hydroxy-4-pregnen-3-one; 17-hydroxyprogesterone (17HP) = 17-hydroxy-4-pregnene-3,20-dione; 17,20-dihydroxyprogesterone (17,20 HP) = 17,20α-dihydroxy-4-pregnen-3-one.

MATERIALS AND METHODS

Adrenal glands were frozen with dry ice immediately upon removal and stored at −20°C. for 4–8 weeks. Adult glands were obtained at 24 months of age from males and females and were examined separately according to sex. Newborn glands were from animals 5 days old and were all male. The glands from those designated as calves were from animals 4–5 months of age and were all male. The fetal glands obtained at 33–35 weeks gestation were mixed, male and female. After stripping the fat and connective tissue they were homogenized at high speed in a Waring blender for 15 s. A portion were so homogenized in 5 vol. 0.12 M phosphate buffer, pH 7.4. This preparation was filtered through gauze, centrifuged at 10,000 *g* for 20 min and was employed in most of the studies. With tissue from calves only a portion was homogenized in 0.25 M sucrose and centrifuged at 105,000 *g* for 1 h. The supernatant was used in either instance. The enzyme activity with 20HP as substrate with the low speed and high speed centrifugation of calf adrenals were comparable on the basis of the original weight of tissue employed. For this reason the crude preparation was employed in all other studies (see Table 1).

Protein determinations on the supernatant fractions employed in incubations were performed according to the method of Lowry *et al.* [8] and the results have been expressed in terms of the total protein present in filtered homogenates.

Incubations were conducted in duplicate at 37°C. for 4 h in ambient atmosphere. A single incubation of previously boiled homogenate was included with each pair. To 50 ml Erlenmeyer flasks was first added 75 μg of 20HP or 17,20HP dissolved in 0.02 ml ethanol. To this was added 5 ml of the tissue suspension and 1.0 ml phosphate buffer containing 10 μM NADP and 1 μM $MgCl_2 \cdot 6H_2O$. At the end of the incubation 5 μg cholestane was added as an internal recovery standard for gas–liquid chromatography (g.l.c.). Four

Table 1. 20α-hydroxysteroid oxido-reductase in two fractions of calf adrenal (as nM progesterone/g protein in 4 h) duplicates

Supernatant × *g*	Per crude extract	Per protein in fraction
10,000	230–252	same
105,000	219–246	1340–1385

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vol. acetone were added and after 18 h at 4°C this was filtered through Whatman No. 1 paper and evaporated under vacuum to a vol. of 2.0 ml. Twenty-five ml water was added and this extracted twice with methylene chloride. The latter was twice washed with each 5 ml 0.1 N NaOH and water. Following evaporation and prior to g.l.c. a preliminary purification was as follows. The residue in 2.0 ml methylene chloride was applied to a column (1 × 8 cm.) of silica gel (100–200 mesh, Fischer), washed with 20 ml 0.1% ethanol in methylene chloride and finally eluted with 25 ml 10% ethanol in methylene chloride. This final eluate was evaporated and prepared for g.l.c.

Each specimen was examined by g.l.c. in four ways. As the "free" steroid, as the methoxyamine (MOX), the bis-trimethylsilyl-acetamide (BSA) and the combined methoxyamine-TBT (a 3:2:2 mixture of trimethylsilylimidazole/N,O-bis-trimethylsilylacetamide/trimethylchlorosilane). These derivatives were prepared according to Chambaz and Horning [9]. Residues and their derivatives were applied to g.l.c. in benzene. A Barber–Coleman gas chromatograph, model 5000 with a hydrogen flame ionization detector was employed. The column was of glass, 6 feet length and 3 mm i.d. packed with 100–200 mesh Gas-Chrom Q (Applied Science Labs., State College, Penna.) coated with QF 1. The quantitation was by planimetry and there was a correction for recovery based upon the cholestane added to the samples Since the combined methoxyamine-TBT (MOX-TBT) derivatives yielded the sharpest peaks for all steroids, these were employed to quantitate the steroids as described in the results. However agreement of quantitation among the several derivatives was within 5% in all cases.

RESULTS

Table 2 gives the relative retention times to etiocholane for those steroids identified and measured in this study. It is notable that in all experiments with 17,20 HP as substrate, there were no conversions noted and only the added substrate was found in quantities equal to those added at the start of the experiment. Thus derivatives relative to this substrate are not included.

Table 3 shows the enzymatic activities of the adrenal glands from various ages. The results are shown in duplicate for two individual animals, or in the case of fetal glands for different pools. As has been noted above (Table 1) since there was equivalent activity per unit original protein in the supernatant of the 10,000 and 105,000 *g* of calf adrenals, for simplicity the former was employed in most of these studies. The addition of the supernatant of fetal or adult male to the calf preparations showed no inhibition. The results in Table 3, expressed entirely on the nM progesterone produced from 20HP, show the highest activity in calf adrenal, with moderate activity in the newborn and little or none in fetal or adult tissues. We observed no other significant products such as might be expected from 17-hydroxylation or side-chain cleavage and the recognition of such products have been well standardized on our equipment. There were no significant unidentified peaks on g.l.c. Finally the quantity of product plus that of the substrate itself measured upon g.l.c. accounted for 91–96% of the total steroid added at the beginning. The boiled tissue preparations revealed only the substrate.

Table 2. g.l.c. Retention times relative to etiocholanolone

Steroid	Derivative	RT
Progesterone	free and BSA	4.45
Progesterone	MOX and MOX-TBT	0.824
20HP	free	2.95
20HP	BSA	2.50
20HP	MOX	1.11
20HP	MOX-TBT	1.00

Table 3. Activity of 20α-hydroxysteroid oxido-reductase in bovine adrenals of various age (as nM progesterone from 20HP/g protein in 4 h)

Adrenals	Results in duplicate from two specimens	
Fetus	0–0	0–0
Newborn	68.4–73.0	48.2–56.1
Calf	228.5–249.5	251.0–269.2
Adult (male)	3.9–6.9	4.1–10.1
Adult (female)	2.2–4.8	3.8–6.2

DISCUSSION

Most of the available information concerning 20α-hydroxysteroid oxido-reductase pertains to gonadal tissues. Usually the reductive reaction has been examined. Little has been written about this enzyme in the adrenal. In brief report Matthijssen *et al.* [6] found reduction of 17HP but not of progesterone. This is different from our results and of those employing ovarian tissue. Matthijssen used tissue fractions obtained by ammonium sulfate precipitation. Their substrates were radioactive and products were detected and measured by scanning paper chromatograms.

Our laboratory has been interested in the phenomenon of adrenarche with its attendant rise of adrenal C-19 steroids at puberty. This may be related to an increase in the side-chain cleaving enzymatic reaction. Although Sweat *et al.* [10] and Axelrod and Goldzieher [11] found that both 17-hydroxylation and 20-keto reduction were needed for cleavage, others do not agree. Lynn and Brown [12] and Shikita and Tamaoki [7] find that 20-keto steroids are more efficiently cleaved by testis and that 20-reduction precludes removal of the side chain. The latter also find 17HP a better substrate for 20-reduction

than progesterone. Although our results differ in that progesterone appears more efficient, it must be stressed that we employed adrenal rather than testicular tissue and examined the reverse reaction.

Our results do not finally resolve the question of the changes in adrenal activity with maturation but rather raise further questions. Perhaps a high level of 20α-hydroxysteroid oxido-reductase prior to puberty (calf adrenals) renders possible precursors unavailable for cleavage. Might the adrenarche then be related to a diminution of this enzyme?

Acknowledgements—Supported by grants HD 00371 and AM 05197, National Institutes of Health, USPHS.

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