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CONVERSION OF 3β,15β,16β-TRIHYDROXY-5-ANDROSTEN-17-ONE TO 1,3,5(10)-OESTRATRIENE-3,15β,16β,17β-TETROL BY PLACENTAL HOMOGENATES

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SUMMARY

Incubation of 3β,15β,16β-trihydroxy-5-androsten-17-one with a homogenate of placental tissue resulted in the formation of an oestetrol (1,3,5(10)-oestratriene-3,15β,16β,17β-tetrol), the identity of which was confirmed by gas chromatography–mass spectrometry.

This finding suggests that androstenetriolones can act as precurors of oestetrols in the human foeto-placental unit.

INTRODUCTION

Since the identification of 15α-hydroxyoestriol in pregnancy urine by Zucconi *et al.*[1] several reports have appeared which discuss the biosynthesis of this compound by the foeto-placental unit [2–4]. It has been demonstrated that 15α-hydroxyoestriol may be formed from phenolic [2, 3] or neutral precursors [4].

The recent identification in this laboratory of 15,16 dihydroxylated 5-androstenes as normal constituents of infancy urine [5] appeared to confirm the suggestion of Younglai and Solomon[4] that 15α,16α-dihydroxy DHA sulphate might be an intermediate in the biosynthesis of oestetrol from neutral precursors. Unfortunately, it has not yet been possible to establish the role of this steroid in oestetrol synthesis since an authentic standard has not been available. However, 15β,16β-dihydroxy DHA* has been synthesized and it was considered that if the conversion of this compound to an oestetrol by a human placental homogenate could be demonstrated, it would provide presumptive evidence for the similar conversion of 15α,16α-dihydroxy DHA.

EXPERIMENTAL

Tissue preparation

The method is based on that of Ryan[6]. Human placentae were dissected free of foetal membranes, the large blood vessels were teased out and the tissue was weighed.

The tissue was homogenized for one minute in precooled buffer containing 0·25 M sucrose, 0·05 M phosphate pH 7 and 0·04 M nicotinamide in the proportions 1 ml buffer to 3 g tissue. The homogenate was centrifuged at 3000 *g* for 10 min to remove cellular debris and the supernatant was stored at −20°C until use. No appreciable decrease in activity occurred during storage, as checked periodically by incubation of a portion with 16α-hydroxy DHA and measurement of the oestriol produced.

Incubations

Incubations were carried out in flasks containing 4 ml placental homogenate, 2·5 μM NAD and 10 μM ATP in a total volume of 5 ml. 15β,16β-Dihydroxy DHA (200 μg) was added dissolved in 200 μl ethanol. A control incubation was set up to which 200 μl ethanol alone was added. The flasks were capped and shaken in a water bath at 37°C for 2 h.

Extraction and purification

In order to precipitate protein, the incubation mixtures were added dropwise to flasks containing 100 ml acetone–ethanol 1:1 (v/v) held in an ultrasonic bath. The mixtures were centrifuged and the supernatants were removed and dried using a rotary evaporator. The extracts were subjected to column chromatography on Sephadex LH-20 (6 g column) using the solvent system cyclohexane–ethanol 4:1 [7]. The oestetrol fraction was obtained between elution volumes 200 and 250 ml [8].

Gas chromatography–mass spectrometry

Trimethylsilyl ether derivatives of the oestetrol fraction were prepared and gas chromatography was carried out on a 20 m OV-101 open tubular column. The retention volume of the trimethylsilyl derivatives of reference 15α-hydroxy-oestriol and the main product of placental incubation were determined relative to aliphatic hydrocarbons (methylene units, MU).

* *Trivial names used*: 15β,16β-dihydroxy DHA: 3β,15β,16β-trihydroxy-5-androsten-17-one; 15α,16α-dihydroxyandrostenedione: 15α,16α-dihydroxy-5-androstene-3,17-dione.

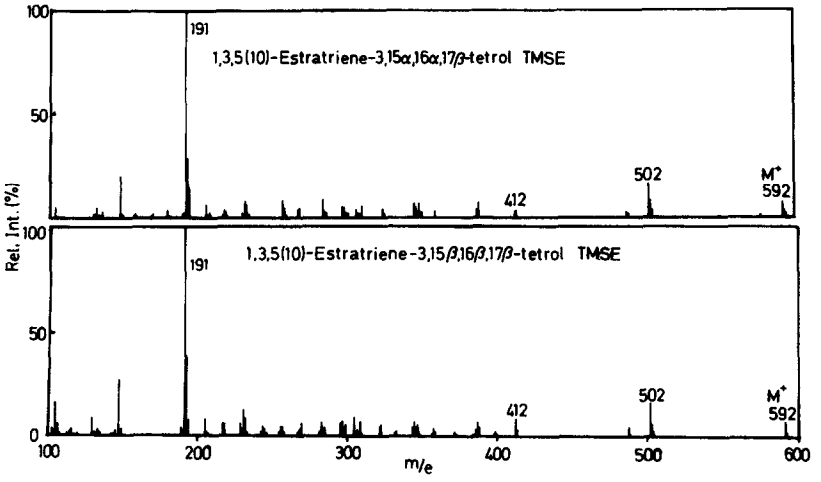

Fig. 1. A comparison of the mass spectra of the silyl ethers of reference 1,3,5(10)-oestratriene-3,15α,16α,17β-tetrol and 1,3,5(10)-oestratriene-3,15β,16β,17β-tetrol.

The identify of the product of placental incubation was established by gas chromatography–mass spectrometry using a Varian MAT 731 instrument housing a packed OV-1 column.

RESULTS

Gas chromatography of the oestetrol fraction from Sephadex chromatography of the incubation mixture containing 15β,16β-dihydroxy DHA showed the presence of a component with relative retention volume (31·35 MU), larger than that of reference 15α-hydroxyoestriol (30·90 MU). The mass spectra of trimethylsilyl derivatives of this compound and reference 15α-hydroxyoestriol are illustrated in Fig. 1.

The upper spectrum shows 15α-hydroxyoestriol trimethylsilyl ether and the lower spectrum the product of incubation of 15β,16β-dihydroxy DHA with the placental homogenate. It can be seen that these compounds are epimers. Since the placental preparation converts 16α-hydroxy DHA almost exclusively to oestriol, in the present case it is likely that the 16 hydroxyl group remains intact and that the 17 oxo group is reduced to a 17β-hydroxyl group. If it is further assumed that the 15β-hydroxyl group of the precursor remains intact the structure of its product would be 1,3,5(10)-oestratriene-3,15β.16β-17β-tetrol.

The parent ions in the mass spectra are at *m/e* 592 but the only large peaks are the base peaks at *m/e* 191, caused by a rearrangement fragment frequently found in compounds with two or more hydroxy substituents in the D-ring [9].

Semi quantitation of the incubation product was achieved by comparison of its peak area with that of the internal standard cholesterol butyrate. This showed a conversion rate from 15β,16β-dihydroxy DHA of approximately 5%.

No oestetrol was detected in the control incubation when the mass spectrometer was set in the scanning mode. The more sensitive technique of mass fragmentography [8] revealed the presence of several endogenous oestetrols but none with relative retention volume as large as that of the product.

DISCUSSION

Oestriol is formed in the placenta principally from 16α-hydroxy DHA sulphate synthesized by the foetus and transmitted *via* the umbilical circulation. Relatively little is formed from phenolic precursors such as oestrone or oestradiol. The situation regarding 15α-hydroxyoestriol synthesis is less well understood. Formation from phenolic precursors has been studied by Schwers *et al.*[3] using simultaneous intra-amniotic administration of [^{3}H] oestradiol and [^{14}C] oestriol. They conclude from the isotope ratio of the oestetrol recovered that oestradiol is the better precursor, indicating that 15α-hydroxylation probably precedes 16-hydroxylation.

In similar studies using labelled neutral precursors, Younglai and Solomon[4] found that DHA sulphate was a better precursor of 15α-hydroxyoestriol than 16α-hydroxy DHA and 15α-hydroxyandrostenedione. Thus, 15-hydroxylation of both neutral and phenolic 15α-hydroxyoestriol precursors predominantly occurs before 16-hydroxylation.

It has been impossible to evaluate the relative importance of phenolic and neutral precursors in 15α-hydroxyoestriol synthesis since the intermediates in the neutral pathway postulated by Younglai and Solomon[4] such as 15α-hydroxy DHA, 15α,16α-dihydroxy DHA and 15α,16α-dihydroxyandrostenedione have not been available.

This study has shown that 15β,16β-dihydroxy DHA can be converted to 1,3,5(10)-oestratriene-3,15β,16β,17β-tetrol by placental homogenates *in vitro*, and provides circumstantial evidence for the similar metabolism of 15α,16α-dihydroxy DHA *in vivo*. Recent work in this laboratory has shown that one androstenetriolone and two androstenetetrols

with 15 and 16 hydroxyl groups are present in infancy urine [5]. 15β,16α-Dihydroxy DHA and 5-androstene-3β,15β,16α,17β-tetrol are present in greatest amount and a third compound has provisionally been identified as 5-androstene-3β,15α,16α,17β-tetrol. Two new epimers of 15α-hydroxyoestriol have recently been identified in pregnancy urine [8] and these may well be aromatization products of the 15β-hydroxylated androstenes. Therefore, the foetus is able to produce androstenetriolones and androstenetetrols which may act as oestetrol precursors. The importance of the "neutral" pathway of oestetrol synthesis relative to the "phenolic" pathway will only be ascertained by simultaneous administration of ^{3}H neutral and ^{14}C phenolic precursors to the intact foeto-placental unit, using the methods successfully developed by the Karolinska group of Professor E. Diczfalusy.

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