ORIGIN OF 5α-PREGNANE-3α,20α,21-TRIOL 3-SULPHATE IN PREGNANT WOMEN

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Received 15 September 1975

1. Introduction

In human pregnancy a number of $C_{21}O_2$ steroid mono- and disulphates appear in plasma and attain total concentrations as high as 10 μg/ml (about 30 μM) in the last trimester [1,2]. A $C_{21}O_3$ steroid monosulphate is also present and has been characterized as 5α-pregnane-3α,20α,21-triol 3-sulphate [3]. This compound seems to occur only in pregnant women and in the newborn infant [4–6]. Although the $C_{21}O_2$ steroids are almost certainly metabolites of progesterone, the precursor of 5α-pregnane-3α,20α,21-triol monosulphate is unknown. During a study of the metabolism of deuterium-labelled 3α-hydroxy-5α-pregnan-20-one sulphate in pregnant women, deuterium was found to be incorporated into this triol. Furthermore, the rate of labelling indicated that sulphated 5α-pregnane-3α,20α,21-triol in pregnancy plasma derives solely from 3α-hydroxy-5α-pregnan-20-one sulphate.

2. Materials and methods

3β-Hydroxy-5α-[3α,11,11-$^2H_3$]pregnan-20-one [7] was epimerized at C-3, without appreciable loss of deuterium [8], by the two-step sequence described by Bose et al. [9]. This gave 3α-hydroxy-5α-[3β,11,11-$^2H_3$]pregnan-20-one, whose isotopic purity was 84.5%, as determined by mass spectrometry.

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The labelled pregnanolone was transformed into its sulphate according to the method of Mumma [10] and the product purified by chromatography on a column of Sephadex LH-20, with chloroform/methanol (1:1, v/v), saturated with KCl, as the eluting solvent [11]. The resulting potassium steroid sulphate was stored at 5°C in 50% aqueous ethanol, containing a trace of KOH, at a concentration of 3.8 mM.

Aliquots of the above stock solution corresponding to about 6 μmol of the steroid were made up to 10 ml with isotonic glucose solution. Following sterilization, these solutions were injected intravenously into three healthy women in the last trimester of pregnancy. Immediately prior to the injection, and at six intervals over a period of 15–24 h, a blood sample (20 ml) was withdrawn into a heparinized tube which was immediately centrifuged. The plasma samples were stored at −18°C until analyzed.

Steroid mono- and disulphates were extracted from plasma and separated by chromatography on Sephadex LH-20 [2,12]. Following solvolysis, the steroids from the monosulphate fraction were separated by chromatography on Lipidex®-5000 (Packard International SA, Zürich) using the solvent system hexane/chloroform/methanol, 85:15:1 (by vol), into mono-, di- and trihydroxy fractions [13]. Steroids in these fractions were analyzed, as their trimethylsilyl ether derivatives, by gas chromatography-mass spectrometry. An LKB 9000 instrument was used, equipped with glass columns (3.5 m × 3 mm i.d.) packed with 1.5% HiEff 8 BP on Gas Chrom Q or 1.5% SE-30 on Chromosorb W HP. Repetitive magnetic scanning was employed [14] and the data were recorded on magnetic tape and evaluated off-line on an IBM 1800 computer [14,15]. The deuterium excess of 3α-

hydroxy-5α-pregnan-20-one, 5α-pregnane-3α,20α-diol and 5α-pregnane-3α,20α,21-triol was determined by comparison of appropriate ions (m/e 390 (M^+), m/e 269 (M-15-2X90) and m/e 449 (M-103), respectively) in averaged spectra of the trimethylsilyl ether derivatives of the unlabelled and labelled compounds in samples taken before and after injection of the deuterated pregnanolone sulphate. All samples were analyzed under identical conditions.

3. Results

Following injection of 3α-hydroxy-5α-[3β,11,11-$^2H_3$]pregnan-20-one sulphate, deuterium appeared in the monosulphates of 5α-pregnane-3α,20α-diol and 5α-pregnane-3α,20α,21-triol. Only molecules with zero or three deuterium atoms were found (after correction for isotopic impurity of the injected compound) indicating that all deuterium labels remained bound to the steroid skeleton. The rate of disappearance from plasma of the injected steroid and the rate of formation and decay of trideuterated species of the above diol and triol monosulphates in one of the subjects are shown in fig.1. Similar semi-log plots were obtained for the other subjects. The decay function for the [$^2H_3$]pregnanolone sulphate could be resolved by a curve-peeling procedure into two exponential components with half-life times of about 0.9 and 4 h, respectively. The 20-keto group was rapidly reduced and the 5α-pregnane-3α,20α-diol 3-sulphate formed also had a half-life time of 4 h. The terminal segment of the decay curve for 5α-pregnane-3α,20α,21-triol monosulphate gave a half-life time for this steroid of about 5 h. The functions for disappearance of labelled pregnanediol monosulphate and the injected precursor intersected the function for labelled 5α-pregnane-3α,20α,21-triol monosulphate close to its maximum (about 1 h after injection). This indicates that the injected compound served as the exclusive precursor of the latter steroid. Estimates of production rates of the pregnanolone/pregnanediol couple and of the pregnanetriol gave values of about 125 μmol/d and 25 μmol/d, respectively. A detailed kinetic study of these and related steroid sulphates is in progress.

4. Discussion

This study has shown that 3α-hydroxy-5α-pregnan-20-one sulphate is the sole precursor of 5α-pregnane-3α,20α,21-triol sulphate in pregnant women. It cannot be established from the available data whether the injected compound was hydroxylated at C-21 prior to reduction of the 20-keto group. However, this hydroxylation may be analogous to the 16α-hydroxylation in pregnant women of 3β-hydroxy-5α-pregnan-20-one sulphate and the corresponding 20α-hydroxy-

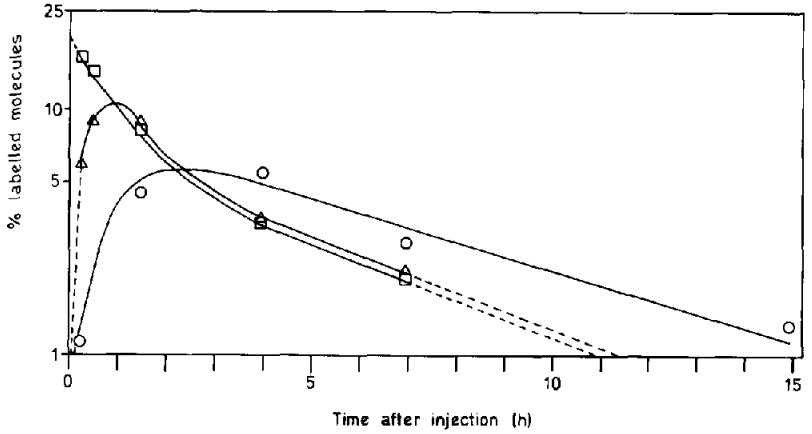

Fig.1. Semilogarithmic plots of deuterium excess vs time of steroids in plasma of a pregnant woman injected with 3α-hydroxy-5α-3β,11,11-$^2H_3$ pregnan-20-one sulphate: (□)3α-hydroxy-5α-pregnan-20-one sulphate; (△) 5α-pregnane-3α,20α-diol 3-sulphate; (○) 5α-pregnane-3α,20α,21-triol 3-sulphate.

steroid [16], where both the precursors and the 16α-hydroxylated metabolites undergo continuous oxidoreduction at C-20. Sulphates of $C_{21}O_3$ steroids possessing a 20-keto-21-hydroxy structure are present in urine during pregnancy [17,18] and oxidoreduction at C-20 of these steroids is therefore likely to occur.

Loss of deuterium from the 3β position of the injected steroid sulphate and of its metabolites was not observed. This indicates that hydrolysis, oxidoreduction and resulphation at C-3 did not occur. Indirectly, it supports the contention that the sulphate ester at C-3 remained intact since a free hydroxyl group at C-3 in all probability would undergo oxidoreduction with concomitant loss of deuterium, as is the case in the rat [19]. Thus, one may conclude that the intact steroid sulphate served as substrate for the 21-hydroxylase.

Hydroxylation at C-21 of a steroid sulphate has not been described previously. However, C_{21} steroid sulphates are known to be hydroxylated in several other positions in fetal organs, see [20,21]. More recently, microsomal preparations from liver of adults have been found to catalyze hydroxylations of 3β-hydroxy-5-pregnen-20-one sulphate [22]. Although the fetal liver has a high capacity for 21-hydroxylation of steroids [23], this organ is unlikely to be the site of formation of 5α-pregnane-3α,20α,21-triol monosulphate in maternal plasma, since transplacental passage of a 3-sulphate would probably be accompanied by hydrolysis [20] and oxidoreduction. Furthermore, 5α-pregnane-3α,20α,21-triol monosulphate has been shown to be present in maternal plasma in two cases of intrauterine fetal death [2]. This evidence, coupled with the present findings, strongly points to the maternal compartment as the site of 21-hydroxylation of sulphated, saturated progesterone metabolites. This conclusion does not exclude the formation of other isomers of pregnane-3,20,21-triol from 21-hydroxylated precursors such as 21-hydroxy-4-pregnene-3,20-dione [24].

Sulphates of pregnane-3,20,21-triols have only been found in biological fluids from pregnant women [6] and from newborn infants [6,25]. Our findings indicate that 21-hydroxylation of sulphated $C_{21}O_2$ steroids is an important metabolic pathway in pregnancy. A steroid sulphate-specific 21-hydroxylase would account for the elevated levels of 5α-pregnane-3α,20α,21-triol monosulphate in women with intrahepatic cholestasis, where the precursor is present in abnormally high concentrations [26]. Furthermore, the presence of such an enzyme system possibly located in the liver, might also explain the finding of normal amounts of 3β,21-dihydroxy-5-pregnen-20-one and 5-pregnene-3β,20α,21-triol disulphates in infants with an adrenal 21-hydroxylase deficiency [27].

Acknowledgements

The skillful technical assistance of Mrs Kerstin Robertsson is gratefully acknowledged. This work was supported by grants from the Swedish Medical Research Council (Project No. 13X-219) and from the World Health Organization. T. A. Baillie was the holder of a Postdoctoral Fellowship from the Royal Society (London), under the European Science Exchange Programme.

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