3E. Steroid biosynthesis: Placenta

79. Mechanism of estrogen biosynthesis

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We have synthesized the isomeric 2β and 2α-hydroxy derivatives of 19-hydroxyandrost-4-ene-3,17-dione and of 19-oxoandrost-4-ene-3,17-dione. The role of the new compounds as possible intermediates in the biosynthesis of estrogens from androgens was investigated in incubations with the placental aromatase preparation. With the exception of the 2β-hydroxy-19-oxoandrost-4-ene-3,17-dione, none of the compounds contributed to or inhibited the biosynthesis of estrogens. The 2β-hydroxy-19-aldehyde which appeared to be an excellent enzymatic precursor of estrogen was subsequently shown to be rapidly and quantitatively converted to estrone by a nonenzymatic process in neutral and basic aqueous media. The mechanism of this process and its probable role in the biosynthesis of estrogens permits the delineation of the sequential steps of the androgen to estrogen biotransformation.

80. Inadequacy of the 19-aldehyde as an intermediate in estrogen biosynthesis

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The 19-aldehyde has not been established as an obligatory intermediate of estrogen biosynthesis. Since no equilibrium was observed during incubation between the aldehyde and 19,19-diol by use of $H_2{}^{18}O$ and mass spectrometry, we studied the degree of ^{18}O-labelling at C-19 which should reflect the difference of the pathways on the basis $\frac{1}{2}$ vs. $\frac{1}{3}$ elimination of ^{18}O-water molecule from labelled diol and triol, respectively. Androstenedione (I) incubated with human term placental microsomes in $^{18}O_2$ gave [19-^{18}O] 19-OH-I analyzed by GC–MS. 3,17-Dioxo-4-androsten-19-al (II) and [19-^2H] II (88 atom % ^2H) incubated under $^{18}O_2$ gave $HC^{18}OOH$ (m/e 48) and $^2HC^{18}OOH$ (m/e 49), respectively. 19-OH-I incubated in $^{18}O_2$ gave $HC^{18}O^{18}OH$. [19-^{18}O] 19-OH-I incubated in O_2 gave $HC^{18}OOH$. These results show that estrogen biosynthesis consists of three monoxygenations. [19-proS-^2H-19-proS-^3H-19-^{18}O] 19-OH-I (90 atom % proS-^2H, 55 atom % 19-^{18}O and tracer ^3H) was incubated to give $^2HC^{18}OOH$ (*m/e* 49) and ^2HCOOH (*m/e* 47). The ^{18}O retention in ^2HCOOH was 32%. Considering that the exchange equilibrium under these conditions can only decrease the ^{18}O-labelling, we conclude that the 19-aldehyde is not an obligatory intermediate in estrogen biosynthesis. (Research supported by USPHS Grant HD04945 and American Cancer Society Faculty Award PRA-72).

81. Inhibition of aromatization by steroidal drugs

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Some 200 steroids have been tested as potential inhibitors of the aromatization of androstenedione (A) in human placental microsomes. These studies revealed that modification at C-3, C-5, C-11, or C-17 markedly affect the ability of steroids to bind the aromatase. 5α-Reduced A was the most potent naturally occurring competitive inhibitor suggesting a possible role for this compound in the control of placental and/or ovarian estrogen biosynthesis. Among the compounds tested, 23 have been used in the treatment of breast cancer. Of these, 16 were aromatase inhibitors and 4 potentially could be converted *in vivo* to known inhibitors. Administration of the non-androgenic compound Δ^1-testololactone to males with gynecomastia inhibited peripheral aromatization of A 50–90% and resulted in clinical improvement. These results suggest (1) that physiologic control of estrogen synthesis may be achieved by aromatase inhibition and (2) that steroid induced regression of some breast cancers may result from inhibition of estrogen production rather than a direct androgenic effect. (Supported in part by NIH Grant HD 107 and the American Cancer Society Grant BC 33).

82. Inhibition of the estrogen biosynthesis by steroids

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The aim of the study is to find specific inhibitors of estrogen biosynthesis. Relationships were evaluated between structural features and inhibitory activities of the steroids investigated. Human placental microsomes were incubated with testosterone, NADPH and the inhibitor. The most potent inhibitors were found to be compounds derived from testosterone or 17α-methyltestosterone with methyl- or hydroxy groups in positions 2,4,6 or 7. 5α-and5β-metabolites of the anabolic Oral-Turinabol (4-chloro-17α-methyl-17β-hydroxy-1,4-androstadien-3-one) showed increased activities in comparison to the parent compound. Compounds derived from estradiol were also found to be potent inhibitors. The structural features for inhibitory activity are 3-methoxy, coupled with 17α-CH_2N_3- or 16α-N_3-groups, respectively. A very interesting result is that microbial degradation products with the remaining rings C and D (perhydroindane derivatives) inhibited the aromatization of testosterone too. So, not only steroid drugs, but also metabolites and degradation products may be strong inhibitors od estrogen biosynthesis. The next step is to find relationships between *in vitro* activity and activity *in vivo*.

83. Preferential utilization of unconjugated 3β-hydroxy-androst-5-en-17-one (D) for placental estrogen synthesis by pregnant baboons (*P. papio*)

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Studies with trace doses of radioactive D and its sulfate (DS) indicate preferential conversion of D to estrogens by pregnant baboons, suggesting limitation of estrogen synthesis from conjugated precursors by placental steroid sulfatase. To determine whether differences in pool sizes of endogenous D and DS might alternatively explain this effect, changes in urinary total estrogen (E) were estimated after i.v. administration of 100 mg D or DS to normal or betamethazone treated (2 weeks) pregnant animals. The animals (70–170 days gestational age) normally excreted 150–1800 μg E/24 h. Betamethazone (3 mg bi-daily) reduced

E > 90% within 3 days, presumably by reducing endogenous D/DS. E (mainly estrone) was determined by RIA after enzyme hydrolysis of conjugates. Increments in E, estimated from the difference in E during 5 days pre- and post-treatment, were [μg mean (range), number of expt.]: Control A (no treatment), −47 (−462/+472), 8; Control B (5 mg estradiol−17β), +2465 (+1358/+3537), 7; 100 mg D, +3206 (+1610/+5321), 7; 100 mg D + betamethazone, +2244 (+1655/+2824), 2; 100 mg DS (≡74 mg D), +35 (−1587/+659), 7; 100 mg DS + betamethazone, +107 (+46/+169), 2. Thus, preferential use of tracer D is unrelated to pool size, and variations in D availability and placental sulfatase activity may both contribute to regulation of estrogen production.

84. The biochemical basis of placental sulfatase deficiency

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The human placenta is normally a rich source of the enzymes 3β-steroid sulfatase and arylsulfatase but pregnancies have been recognized recently with deficiency in the activity of these enzymes. Fetal growth and development are normal but estrogen levels are extremely low since the placenta is unable to metabolise the estrogen precursors androst-5-en-17-one-3β-yl sulfate (DHEAS) and 16α-hydroxyandrost-5-en-17-one-3β-yl sulfate. In attempting to establish the biochemical mechanism of the enzyme defect we have investigated the possibility that lack of enzyme activity was due to the presence of a sulfatase inhibitor. Sulfatase activity was determined kinetically from the hydrolysis of DHEAS-7α^3H by microsomal preparations under optimum conditions. Microsomal preparations from affected placentas exhibited sulfatase activity. Ultrasonic (100 W, 24 k Hz) treatment of the microsomes directed at disrupting a possible enzyme-inhibitor complex failed to increase the sulfatase activity of the preparation. Addition of 105,000 μg supernatant from affected placental tissue to the incubate did not inhibit sulfatase activity of normal placental microsomes. It is concluded that placental sulfatase deficiency is likely to be due to deficiency of enzyme rather than the presence of a sulfatase inhibitor.

85. Human placental Δ_5-3β-hydroxysteroid dehydrogenase: intracellular distribution, substrate specificity and inhibition

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Δ_5-3β-hydroxysteroid dehydrogenase (Δ_5-3β HSD) activity has been assayed either by spectrophotometric method or by use of radioactive substrates. The enzymatic activity is equally distributed between mitochondrial and microsomal fractions verified by electronic microscopy. The specific activity is similar in both fractions, as well as the K_m for NAD (5×10^{-5}M), for the substrate, and the optimal pH. But the enzymatic activity is different with C_{21} or C_{19} natural or synthetic substrates; the optimal pH is 7·4–8 for C_{21} steroids and 10–10·2 for C_{19} steroids. The Δ_5-3β HSD is strongly inhibited by Δ_4-3-oxosteroids (progesterone and 4-androstenedione) and the inhibition seems to be of the competitive type. Oestrogens and cyclic AMP have also an inhibitory action. These results suggest that human placental Δ_5-3β HSD which has identical characteristics in mitochondrial and microsomal fractions has different properties with C_{21} or C_{19} steroid substrates, and that endogenous steroids play a physiological role in the regulation of the enzymatic activity.

3F. Steroid biosynthesis: Non endocrine tissues

86. Testosterone biosynthesis by human skin

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Steroid metabolism in the human skin was studied using purified progesterone-4-^{14}C as a testosterone and 17α-hydroxyprogesterone precursor. Skin minces were placed in Eagle's medium, with penicillin and streptomycin, and thereafter incubated for a five days period at 37 C. Neither PPLO or other bacteria were detected as contributing factors to steroid metabolism. Organic extracts were paper chromatographed and labelled steroid detected by a gas-flow strip scanner. Separation, elution and radioactivity quantification was performed until constant specific activities of the compounds were obtained. 17α-Hydroxy-4-pregnene-3,2 - dione and 17α-hydroxy-4-androsten-3-one yields accounted for less than 1% of the incubated precursor. The remaining metabolites have been previously described. Skin steroid metabolic pathways are becoming established. 17α-Hydroxy-4-pregnene-3, 20-dione might constitute an intermediate step to testosterone; both may be formed either as a compensatory route or just as a tissue response to form adaptive enzymes.

87. *In vitro* synthesis of active sex steroids in normal and inflamed human gingiva

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This study employed 4 groups (each of 12 samples) of normal and inflamed human gingival tissue of male and female subjects. 300 mg of tissue slices were incubated, separately, in 3 ml of 0·1 M potassium phosphate buffer (pH 7·4) with 50 μCi of androstenedione-1,2-^{3}H (Δ^4A) or estrone-2,4,6,7-^{3}H (E_1) in the presence of $NADPH_2$ at 37 for 3 h. Organic solvent extracts of the incubated tissue slices were separated by paper and silica gel thin layer chromatography. Radioactive testosterone (T) and estradiol-17β (E_2) were identified as the only conversion products of (Δ^4A) and (E_1). The mean rates of conversion of (Δ^4A) to (T) and (E_1) to (E_2) in normal and inflamed tissue samples were 3·5 and $7{\cdot}3 \times 10^{-7}$ μM/g/min in the males and 6·8 and $19{\cdot}4 \times 10^{-7}$ μM/g/min in the females. These results reveal the presence of 17β-ol-dehydrogenase in human gingival tissue. The significant increase in the biosynthesis of active sex steroids, (T) and (E_2), in inflamed as compared to normal gingiva of both sexes might be a factor in the aggravation of gingival inflammation due to the known hyperemic effects of these hormones. (Supported by USPH DE-03031-03.)