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