Estrogen Biosynthesis in Human Liver—A Comparison of Aromatase Activity for C-19 Steroids in Fetal Liver, Adult Liver and Hepatoma Tissues of Human Subjects

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Abstract

After incubation of various tritiated C-19 steroids (androstenedione, testosterone, dehydroepiandrosterone, dehydroepiandrosterone sulfate) with human fetal liver, adult liver and hepatoma tissue homogenates, estrone, estradiol and estriol were analysed after a series of purification steps involving column chromatography, thin layer chromatography and co-crystallization. The findings indicated that the human fetal liver extensively aromatized various C-19 steroids to estrogens, whereas human adult liver and hepatoma tissues exhibited little or no aromatase activities.

The formation of estradiol from androstenedione in human fetal liver indicated the presence of 17β-hydroxysteroid dehydrogenase in this tissue.

It was therefore concluded that although the liver participated in the aromatization process during the fetal stage, extensive aromatization did not take place in the adult liver.

It is well known that the aromatization of C-19 steroid to estrogens (estrogen biosynthesis) takes place in some human tissues other than the placenta and ovary. The extragonadal aromatization of androstenedione and testosterone contributes significantly to estrogen production in normal men and serves as a major source of estrogens in postmenopausal women. The conversion of androgens to estrogens has been demonstrated in various human tissues such as adipose tissue (Schindler et al., 1972; Nimrod and Ryan, 1975; Forney et al., 1981; Yamaki et al., 1983), hair (Schweikert et al., 1975), bone marrow (Frisch et al., 1980), lung of adult subjects (Yamamoto, 1981) and some fetal organs (Mancuso et al., 1965; Slaunwhite et al., 1965; Schindler, 1975; Sakai et al., 1983; Yamamoto et al., 1983).

The authors have previously reported that the liver has the largest aromatization capacity among various fetal tissues examined at the mid-gestational stage (Yamamoto et al., 1983). The present study describes significant conversion of various labeled C-19 steroids [androstenedione (A), testosterone (T), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS)] into estrone (E1), estradiol (E2) or estriol (E3) by homogenate of human fetal liver, adult liver, hepatoma or hepatic hemangioma tissue.
Materials and Methods

1) Steroids

Labeled steroids used for this study were as follows; [7-3H]-A (SA; 8.3 Ci/mmol, Amersham), [7-3H]-T (SA; 25 Ci/mmol, NEN), [7-3H]-DHEA (SA; 25 Ci/mmol, NEN), [7-3H]-DHEAS (SA; 24 Ci/mmol, NEN), [4-14C]-E1 (SA; 55.8 mCi/mmol, Amersham), [4-14C]-E2 (SA; 59.2 mCi/mmol, NEN), [4-14C]-E3 (SA; 52 mCi/mmol, Amersham).

Non-labeled steroids (E1, E2, E3) were purchased from Sigma Chemical Co. (St. Louis, MO).

2) Clinical materials, Incubation and Isolation of Metabolites

Human fetal liver tissues (males) were obtained from artificial abortions performed for sociomedical reasons. Adult normal liver samples (males) were obtained from a 65-year-old subject with hepatic cholelithiasis, a hepatic hemangioma sample from a 58-year-old subject with hepatic hemangioma and hepatoma samples from a 60-years-old subject with hepatoma undergoing partial hepatectomy. These tissues were stored at below −60°C until sample preparation. Each tissue (0.5–1.0 g w.w) was minced and homogenized in 10 ml of 0.1 M phosphate buffer (pH 7.4) using a glass homogenizer. The shaking incubation of a mixture of labeled C-19 steroids (A, T, DHEA or DHEAS; 10 μCi), NADPH (5 mg) and the liver tissue homogenates was carried out at 37°C for 2 hrs in air. After incubation, the enzymatic reaction was terminated by adding a 3-fold volume of ethanol and then [4-14C]-E1, [4-14C]-E2 and [4-14C]-E3 (10000 dpm, 250 μg) were added as tracers. The samples were filtrated, concentrated and extracted with ethyl acetate (30 ml, 3 times). The extracts were displaced with 1 ml of methanol to chromatograph on a Bio-Rad AG1-X2 anion exchange resin column (Morreal et al., 1972). The phenolic steroids thus separated were, then, chromatographed on a thin layer chromatographic plate (developing solvent: cyclohexane-ethyl acetate, 2:1 and chloroform-ethyl ether, 4:1, v/v). The isolated E1, E2 and E3 were subjected to co-crystallization to obtain the constant specific activity and 3H/14C ratio of the crystal. The amount of estrogen transformed from C-19 steroids by the tissue was calculated from the 3H/14C ratio of the 3rd crop crystals. Detailed analysis was reported previously (Osawa et al., 1982; Yamamoto, 1982).

Results

4 of 5 human fetal liver samples investigated in this study could aromatize C-19 steroid into E1 (~820 pmol E1/hr/g), E2 (0.14–114 pmol E2/hr/g) was formed in human fetal liver from A and DHEA but not from DHEAS. E3 was also biosynthesized by human fetal liver from DHEA (0.23 pmol/hr/g) and DHEAS (0.04 pmol/hr/g) but not from A. Thus, it was demonstrated that human fetal liver exhibited vigorous aromatase, 3β-hydroxysteroid dehydrogenase (3β-HSD), 16α-hydroxylase and 17β-hydroxysteroid dehydrogenase(17β-HSD)activity.

Table 1. Estrogen formation from C19-steroids by human fetal liver homogenates

<table>
<thead>
<tr>
<th>Gestational stage</th>
<th>Substrate</th>
<th>Estrogen formation (pmol/hr/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E1</td>
</tr>
<tr>
<td>20 W</td>
<td>A</td>
<td>17.5</td>
</tr>
<tr>
<td>23 W</td>
<td>A</td>
<td>N.D</td>
</tr>
<tr>
<td>20 W</td>
<td>A</td>
<td>820</td>
</tr>
<tr>
<td>20 W</td>
<td>DHEA</td>
<td>0.78</td>
</tr>
<tr>
<td>23 W</td>
<td>DHEAS</td>
<td>0.04</td>
</tr>
</tbody>
</table>

(N.D: under 0.01 pmol/hr/g)

The tissue homogenate prepared in 10 ml phosphate buffer (0.1 M, pH 7.4) was mixed with 10 μCi of 7-3H-substrate and NADPH (5 mg). The mixture was incubated at 37°C for 2 hrs in air. Estrogen formation by human fetal liver homogenate was determined after purifications involving Bio-Rad AG1-X2 column chromatography, and T.L.C. followed by cocrystallization.
The levels of $E_1$ and $E_2$ formed in the 20-week fetal liver tissue were remarkably high compared to those formed in fetal liver samples of other developmental stages, as shown in Table 1.

On the other hand, while normal liver and hepatoma tissues of adult subjects also transformed A to $E_1$, the conversion rates were extremely low compared to those in fetal liver specimens. The hepatoma tissue biosynthesized $E_1$ at a slightly higher rate than the normal liver tissue. No estrogen, however, was transformed from A by the hepatic hemangioma tissue. $E_2$ and $E_3$ were not formed at all by human adult liver samples from any C-19 steroid examined. The control samples (a sample terminated at preincubation [10 sec., 20-week fetal liver] and a blank sample [2 h, no liver tissue]) exhibited $E_1$, $E_2$, and $E_3$ levels below 0.01 pmol/hr/g, irrespective of the C-19 steroid examined. The results of incubation with adult liver samples are shown in Table 2.

In summary, it was found that the fetal livers exhibited $10^3$-$10^4$ fold aromatase activity for C-19 steroid compared to the adult liver samples.

**Discussion**

Extensive *in vitro* aromatase activity for C-19 steroids was observed with human fetal liver at the mid-gestational stage of development. $E_1$ and $E_2$ were formed from A by human fetal liver samples. Amongst the fetal liver samples at various stages of development the 20-week fetal liver sample aromatized A at a remarkably high level into $E_1$ and $E_2$ (820 pmol/$E_1$/hr/g and 114 pmol $E_2$/hr/g). However, the fetal liver tissues could not form $E_3$ from A. On the other hand, $E_3$ was transformed from DHEA or DHEAS in human fetal liver samples. Takayama (1975) indicated that human fetal liver at mid-term of gestation was possessed of high activities of 16α-hydroxylase, 17β-HSD and sulfokinase for DHEA. Furthermore, it has been reported that a low activity of 3β-HSD is present in human fetal adrenal tissue (Hirato et al., 1982). So, DHEA or DHEAS would become a preferred precursor rather than A for $E_3$ formation in human fetal liver. Our data were assessed in the light of previously published reports. Mancuso et al. (1965) reported for the first time the aromatization of A and T with previable human fetuses under perfusion using labeled C-19 steroids. Isolated estrogens accounted for 0.7 to 6.2% of the radioactive material recovered from the liver, but no aromatization of DHEA by the fetuses was observed. *In vitro* incubation of T with fetal liver homogenates also resulted in an extensive aromatization of T into $E_2$ (10%) but little aromatization of DHEA (Slaunwhite et al., 1965; Jungmann...
et al., 1966; Jungmann and Schweppe, 1967).

On the other hand, the aromatization was also observed in the liver of infant but its extent was small compared to that in the fetal liver (Frost et al., 1980). The rate of conversion of A to E1 by a 4-year-old girl's liver was reported as 0.18%. Frost et al. (1980) showed that this conversion rate in an infant girl (0.18%, corrected for losses) was close to the value reported by Smuck and Schwers (1977, 0.09%, uncorrected).

Human adult liver homogenates investigated in our study were capable of transforming C-19 steroids to E1 but not to E2 or E3. It was found that the human adult liver, hepatoma and hepatic hemangioma tissues could produce E1 from A in some cases, and the amount of E1 produced was remarkably low in comparison with that in the fetal liver. Smuck and Schwers (1977) have also reported the conversion of A into E1 (0.09%) on incubation of tritiated A with human adult liver homogenates. There was a low aromatization of A to E2, the average conversion rate being 0.013% (Mocikat and Engelhardt, 1982) or 0.13% (Smuck and Schwers, 1977). No formation of E2 or E3 from T, however, was noted with human adult liver. These in vitro findings of ours and other investigators demonstrate that the conversion of androgen to estrogens in the adult liver is low in comparison with that in the fetal liver. Therefore, it appears likely that extrahepatic tissues are mainly responsible for the aromatization of androgens observed in vivo in the adult.

Acknowledgement

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Systematic names of steroids used in this study are:

1. A; androstenedione = androst-4-ene-3,17-dione.
2. T; testosterone = 17β-hydroxyandrost-4-en-3-one.
3. DHEA; dehydroepiandrosterone = 3β-hydroxy-androst-5-en-17-one
4. DHEAS; dehydroepiandrosterone sulfate = 3β-hydroxy-androst-5-en-17-one-3β-yl-sulfate.
5. E1; estrone = 3β-hydroxy-estra-1,3,5 (10)-tri-en-17-one.
7. E3; estriol = estradiol-17β = 3β-hydroxy-estra-1,3,5 (10)-tri-en-3,16α, 17β-triol.

References

Schweikert, H. U., L. Milewich and J. D.