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17β-Hydroxysteroid Dehydrogenase Activity in Tissues of Fetal Rhesus Macaques (41503)

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Abstract. 17β-Hydroxysteroid dehydrogenase (17β-HSDH) activity was measured in tissues (anterior pituitary, diencephalon, frontal cortex, liver, whole blood, and uterus) of fetal rhesus macaques on Days 80, 120, and 150 of gestation. The production of estrone (E_1) (quantified by radioimmunoassay) by an 800*g* supernatant incubated with excess substrate (estradiol-17β [E_2]) was used as an index of 17β-HSDH activity. Over a 30-min incubation period E_1 was produced in a linear fashion by all the tissues that we studied. The pH optimum for this activity in the pituitary gland was ~9. Boiling of the tissue for 1 min reduced its activity significantly. Significant changes in 17β-HSDH activity during development were observed for two of the six tissues studied. Activity in fetal anterior pituitary glands was greatest on Day 80 of gestation but changed to significantly lower levels by Day 120 ($P < 0.01$). This low level continued on Day 150 of gestation. The pattern of activity in the liver was different from that in the pituitary gland. High activity was found on Day 80, and this increased significantly by Day 120 ($P < 0.05$). The levels of activity observed on Day 150 were similar to those found on Day 120. Low levels of 17β-HSDH activity were found in the central nervous system, whole blood, and uterus. In rhesus adults as in fetuses we have found relatively high levels of 17β-HSDH activity in the anterior pituitary gland. The biological significance of changing levels of this activity during gestation is not known.

Many tissues of the reproductive system are estrogen sensitive, but the roles of metabolic enzymes in mediating estrogen action are not well understood. We do know, however, that estrogens such as estradiol-17β (E_2) can be converted to weaker compounds such as estrone (E_1) by microsomal enzymes, the 17β-hydroxysteroid dehydrogenases (17β-HSDHs), in reproductive tissues (1). Estrone is less active than E_2, apparently because it is less competitive for the estrogen receptor (2) and diffuses out of target cells more easily (3). Considering these two differences alone in the properties of E_1 and E_2, one can envision effective modulation of the actions of E_2 by its cellular conversion to E_1. In uterine tissue from women, the 17β-HSDHs are regulated by progesterone and the activity of these enzymes varies with the stage of the reproductive cycle (3). Similar observations have been made in uteri (4) and pituitaries (5) from rhesus macaques, but the significance of these observations is not well understood. All of the above studies involved tissues from adult animals. Recently, we had the opportunity to obtain tissues from fetal monkeys at different times in gestation, and we determined the activity of the 17β-HSDHs in these tissues.

Materials and Methods. *Animals and tissue preparations.* Tissues were obtained from 12 rhesus macaque (*Macaca mulatta*) fetuses of known gestational ages (Days 80, 120, and 150) that had been delivered by cesarean section. The three Day-80 and the three Day-150 fetuses were intact females. The six Day-120 fetuses comprised both males and females; some of these had been gonadectomized 3 weeks before and given testosterone or saline infusions for 6 hr. In this latter group we combined the data from the various treatment groups because no differences appeared evident from the following comparisons. The 17β-HSDH activity in anterior pituitary glands of two

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female fetuses spayed *in utero* on Day 100 of gestation and treated with testosterone for 21 days ranged from 220 to 238 ng E_1 mg protein^{-1} after a 30 min incubation. Pituitaries from four males similarly treated contained 207–363 (range) ng E_1 mg protein^{-1}. In a 15-min incubation a pituitary gland of a female not treated with testosterone contained 132 ng E_1 mg protein^{-1} whereas the activities of this enzyme in the pituitaries of two females treated with testosterone were 130 and 154 ng E_1 mg protein^{-1}, respectively. These preliminary observations revealed no striking differences between treatments thereby providing the rationale for combining animals between treatments. At delivery the fetuses received cold saline infusions to remove blood from tissues to be analyzed. The brains and pituitaries were quickly removed and chilled with ice. Under these conditions the various tissues were dissected, weighed, placed in ice-cold buffer, and homogenized. The following tissues were removed and analyzed for 17β-HSDH activity: liver, uterus, anterior pituitary, frontal cortex, and diencephalon (a small block of tissue between the optic chiasm and the mammillary bodies about 2 mm thick). Each tissue was homogenized in 1 ml of ice-cold tris(hydroxymethyl)aminomethane (Tris) buffer, pH 8.0, at 37°C in a 5-ml glass homogenizer. The homogenate was poured into a 13 × 100-mm glass tube and centrifuged at 800*g* in a Sorvall refrigerated centrifuge. An aliquot of the supernatant was removed for Lowry protein determinations. An aliquot of 1.5 mg of protein from the 800*g* supernatant was incubated in 0.75 ml Tris buffer with different quantities of E_2 and with a 10-fold molar excess of NAD for different periods of time. Each incubation flask contained 1.5 mg of protein, an amount we had already demonstrated to be on the linear portion of the activity versus protein concentration curve.

Chromatography. After incubation the steroid was extracted from the incubation medium in 6 ml of ether. The ether extract was taken to dryness under a stream of air in a water bath at 37°C. The samples, dissolved in a small volume of chloroform: methanol (1:1, v/v), were applied to the origins of paper strips with capillary tubes. We used Whatman No. 1 paper strips (55 cm long and 2.5 cm wide) that had been cleaned by being boiled in methanol. The chromatograms were developed in benzene: formamide. Benzene was the mobile phase, and the paper was soaked in formamide: acetone (1:1, v/v). In this system it takes approximately 3 h for the solvent front to reach the end of the paper. E_2 and E_1 (R_fs of 0.33 and 0.71, respectively) clearly separate in this chromatography system). After chromatography, the paper strips were dried at room temperature for 16 hr. Standard solutions of E_1 and E_2 fractionated with the samples on individual paper strips were used as references for elution. The standards were visualized on paper by immersion of the paper strips in Barton's reagent, i.e., 1% $K_3Fe(Cu)_6$ and 1% $FeCl_3 \cdot 6H_2O$ (1:1, v/v) (6). An area with the mobility of E_1 was eluted with 10 ml of methanol from each paper chromatogram. The methanol extract was taken to dryness and dissolved in 1 ml of ethanol. One-tenth of the ethanol extract was dried, dissolved in 100 μl of hexane: benzene:methanol (62:20:13, v/v), applied to a Sephadex LH-20 column, and fractionated in the same solvent system mentioned above. The E_1 area was collected from the Sephadex columns and quantified by radioimmunoassay (RIA). We estimated procedural losses by adding 10,000 cpm of [^{3}H]estrone (E_1) to duplicate samples which we carried through the entire procedure. Using these independent estimates of recovery, we adjusted the final quantity of E_1 in each sample for procedural losses. Although E_1 was quantified by an antiserum that cross-reacts with E_2, we achieved specificity in our E_1 assay by chromatography on paper and Sephadex LH-20 columns. This was demonstrated by the low blanks for E_1 (<10 pg) when no tissue, only substrate, was added. These blanks were subtracted in the process of computing the E_1 values. The measurement of E_1 was used as an index of 17β-HSDH activity.

Statistics. Differences between the mean

production of E_1 among gestational ages were analyzed by *t* tests after the homogeneity of variance had been determined.

Results. In a previous publication we validated our use of RIA measurements of E_1 as an assay for 17β-HSDH activity in tissues from adult rhesus macaques (5). Similar information on fetal tissues is presented in Figures 1 through 3. Assay of the E_1 produced from E_2 by the 800*g* supernatant of fetal pituitaries and liver revealed a linear response up to minute 30 of incubation, the last time period mentioned in Fig. 1. Measurements of E_1 in the 800*g* supernatant at time 0 revealed small amounts of E_1 in the tissue before the incubation had begun. These amounts were subtracted from the 15- and 30-min values; and from all other data presented in this manuscript. Similar responses for other tissues such as uterus, cortex, and whole blood were obtained, but less E_1 was formed by these tissues *in vitro*. Figure 2 demonstrates the effect of increasing substrate concentrations on 17β-HSDH activity after a 15-min incubation. It appears that 80 μM E_2 the limit of solubility in our buffer is a near saturating quantity of substrate. The effects of pH and heat treatment (boiling for 1 min) are shown in Fig. 3. The pH maximum was about pH 9.0 since the activity declined rapidly at pH 10.0. These data generated for liver may or may not be applicable to other tissues. Boiling the 800*g* supernatant for 1 min drastically reduced the capacity of the tissue to convert $E_2 \rightarrow E_1$ but some activity remained. This activity was not subtracted from any of the values reported in this manuscript.

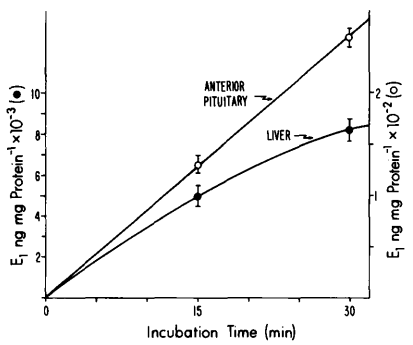

FIG. 1. Effects of incubation time on 17β-hydroxysteroid dehydrogenase (17β-HSDH) activity of liver and anterior pituitary glands from Day-120 fetuses (n = 6).

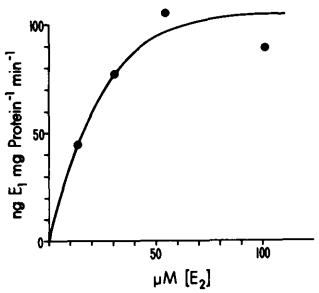

FIG. 2. Effects of substrate concentration (estradiol-17β [E_2]) on 17β-hydroxysteroid dehydrogenase (17β-HSDH) activity in Day-120 fetal pituitaries.

We used this technique to analyze the 17β-HSDH activity in various tissues from fetuses as a function of stage of gestation (Fig. 4). The greatest activities occurred in fetal livers and anterior pituitary glands. Activity levels in other tissues were much lower. Pituitary glands from Day-80 fetuses contained significantly more enzyme activity than pituitary tissues taken at later times in gestation ($P < 0.01$). Activity in liver, however, increased significantly from Day 80 to Day 120 of gestation ($P < 0.05$). Activities of all other tissues were low and did not seem to change as gestation progressed.

Discussion. An understanding of the function of metabolic enzymes for promoting hormone action in target tissues is beginning to emerge. For target tissues such

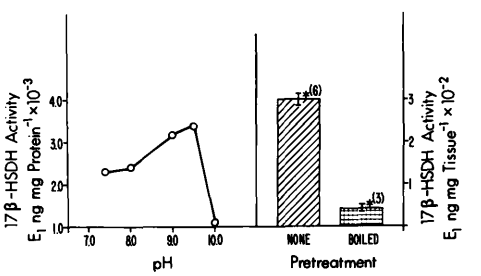

FIG. 3. Effects of pH and heat treatment on 17β-hydroxysteroid dehydrogenase (17β-HSDH) activity in liver from Day-120 fetuses.

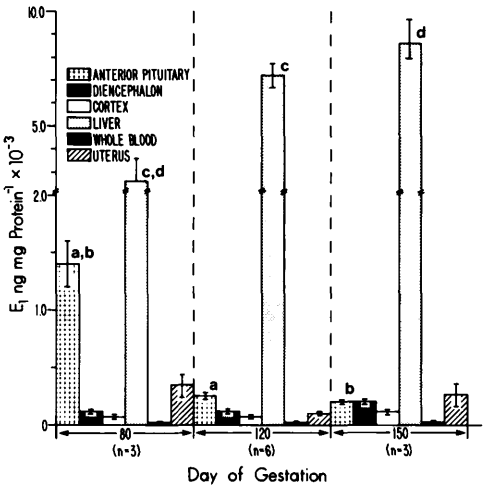

FIG. 4. Effects of age on 17β-hydroxysteroid dehydrogenase (17β-HSDH) activity in fetal tissues from rhesus macaques. Tissues were incubated with 80 μ*M* E_2 and an excess of NAD for 30 min. We used the amount of E_1 produced by this extract as an index of 17β-HSDH activity. E_1 was quantified by a specific RIA after chromatography on paper and LH-20 columns. See Methods section for more details about procedures. Bars with the same letters differed significantly ($P < 0.01$ for a, b and $P < 0.05$ for c, d).

as prostate (7) and perineal skin (8), production of dihydrotestosterone (DHT) from testosterone appears to be an essential conversion for androgen action. Likewise, the aromatization of testosterone to E_2 in neural tissue appears to be important in many species for sexual differentiation of the brain and negative feedback control of gonadotropin secretion by steroids (9, 10). The 17β-HSDHs are found in a number of mammalian tissues such as erythrocytes (11), uterine endometrium (1), liver (12), and placenta (13). The amounts of 17β-HSDH activity in human endometrium increase during the secretory phase of the cycle and in response to exogenous progesterone (14). Similar results have been obtained in uterine tissue from rhesus macaques (4). In that study both E_2 and progesterone appeared to increase the activity of these enzymes. In addition to those in the uterus, we found high levels of 17β-HSDH activity in adult anterior pituitary glands (5). Data on fetal pituitaries reported in this paper confirm this fact, but only with respect to fetuses in early gestation. Some reports link the production of DHT and E_2 from testosterone to the cellular action of this hormone in certain tissues, but the significance of the biological activity of the 17β-HSDHs is more difficult to understand. In uterine endometrium from rhesus macaques the favored conversion is $E_2 \rightarrow E_1$ rather than vice versa (4). The production of E_1 from E_2 can be viewed as simply a breakdown process or it can be construed as a more significant and dynamic way of controlling E_2 levels within the target cell. If one takes the latter point of view, the effectiveness of E_2 in initiating its biological action is proportional to the amount of active hormone that binds to the estrogen receptor. Therefore, study of the production and control of 17β-HSDH activity seems important to an understanding of the way in which E_2 acts on target cells.

The connection between the presence of significant amounts of 17β-HSDH activity and a significant biological function is the unknown factor at this time. There is good evidence in some rodent species that aromatization of androgen to estrogen is important for sexual differentiation of the fetus. Our measurements of E_2 in the serum of the fetus have shown very low or undetectable quantities of this hormone early in gestation, when differentiation of the nervous system and anlagen of the reproductive tract occurs (15). Concentrations of E_2 in the maternal serum, however, were relatively high. Apparently, the primate fetus develops a mechanism to maintain low levels of E_2 within itself and thus prevent abnormalities in sexual differentiation. The question is: Do 17β-HSDH activities in the liver and pituitary have a significant role in this process? There are analogous situations in which the metabolic conversion of steroid hormones correlates with physiological effects previously unexplained. Doves castrated several weeks earlier are insensitive to androgens that mediate perch calling (16). This insensitivity correlates with an increase in 5β-reductase activity in the preoptic region of the brain (16). There are precedents in the literature for the idea that enzymes involved in the metabolism of

steroids to inactive products serve important biological functions. The fact that their activities can be regulated by compounds such as progesterone or E_2 and the fact that they can be affected by gonadectomy indicate their importance in the physiological regulation of cells.

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