J. Steroid Biochem. Molec. Biol. Vol. 37, No. 5, pp. 741–745, 1990
Printed in Great Britain.

0960-0760/90 $3.00 + 0.00

DISTRIBUTION AND CYCLIC CHANGE OF AROMATASE CYTOCHROME *P*-450 ACTIVITY IN HUMAN UTERI

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(Received 14 April 1990)

Summary—Activities of aromatase cytochrome *P*-450 in the columnar epithelial region (CE), squamous epithelial region (SE) and connective tissue (CT) of uterine cervix, and endometrium (EM) during the menstrual cycle were determined using [4-^{14}C] and [1β-^{3}H]androstenedione. Aromatase activities in the proliferative phase ($n = 8$) were 15.0 ± 7.9, 10.9 ± 10.3, 9.4 ± 10.6 and 8.0 ± 7.3 (mean $\pm$ SD) fmol/h/mg protein in CE, SE, CT and EM, respectively, and aromatase activities in the secretory phase ($n = 6$) were 31.5 ± 7.6, 19.1 ± 7.1, 5.6 ± 4.6 and 6.3 ± 1.5 fmol/h/mg protein, respectively. The results show that the aromatase activities in these regions in the proliferative phase were not significantly different from each other. On the other hand, the aromatase activity in the secretory phase was significantly higher in CE than in any other regions ($P < 0.05$), and significantly higher in SE than in CT or EM ($P < 0.05$). There was no significant difference in aromatase activity between CT and EM. By comparison of aromatase activity between these two phases, the activity in CE was significantly higher in the secretory phase than in the proliferative phase ($P < 0.05$), but no significant difference was observed in other regions.

INTRODUCTION

Aromatase cytochrome *P*-450 enzyme complex catalyzes the conversion of androgens to estrogens [1]. In premenopausal women, this enzyme is mainly found in the ovary. The uterine cervix and endometrium change their status in a menstrual cycle, and ovarian estrogens seem to play important roles in these changes. However, West *et al.* [2] and Leach *et al.* [3] suggested that androgens could be aromatized to estrogens in peripheral, extragonadal tissues and the importance of this source of estrogens was realized by the study of MacDonald and co-workers [4, 5] and Longcope and co-workers [6], with radiolabeled techniques.

As tissue concentrations of steroids may be more important for the endocrine organ than the peripheral blood levels [7–11], locally-produced estrogens might have more influence on the changes of the uterine cervix and endometrium than ovarian estrogens. In the present study, we studied the distribution of aromatase activity in the uterus and its cyclic change.

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EXPERIMENTAL

Materials

[4-^{14}C] (27.8 Ci/mmol) and [1β-^{3}H] (52.0 mCi/mmol) androst-4-ene-3,17-dione were purchased from New England Nuclear (Boston, Mass, U.S.A.). Progesterone kit (COAT-A-COUNT) was purchased from Diagnostic Products Corporation (Los Angles, Calif., U.S.A.). NADPH was obtained from Oriental Yeast Co., Ltd (Tokyo, Japan). Activated charcoal powder was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). EDTA was purchased from Wako Pure Chem. Ind., Ltd (Tokyo, Japan). Trichloroacetic acid was obtained from Katayama Chemical Co., Ltd (Osaka, Japan) and 4-hydroxyandrostenedione was obtained from Sigma Chemical Co. (St Louis, Mo, U.S.A.). All other reagents used were the best materials commercially available.

Phase classification

Serum of the patient was obtained on the morning of hysterectomy, and serum progesterone was assayed by commercially available kit (COAT-A-COUNT). The endometrium was subjected to histological examination. The menstrual phase of each patient was determined according to these results. The serum progester-

one level was greater than 7.0 ng/ml in the secretory phase.

Enzyme preparation

Uteri from 14 premenopausal women (38–48 years of age) who had received hysterectomy because of benign uterine diseases were used in this study with informed consent. The uteri obtained were chilled in saline at 0°C immediately after removal and were washed to remove blood. Samples were obtained from the uterine cervix and endometrium. The samples from the uterine cervix were obtained from columnar epithelial regions and squamous epithelial regions as 1 mm slices, and the rest was regarded as connective tissue. All the samples obtained were stored at −80°C until assayed.

The sample was thawed slowly on the ice, and homogenized with 2 ml of 10 mM potassium phosphate buffer, pH 7.4, containing 20% glycerol and 0.1 mM EDTA using a Polytron blender. The resulting homogenate was used as the enzyme source.

Aromatase assays

Aromatase activity was assayed by determining 1β-elimination [14–16] using a mixture of [1β-^{3}H]androstenedione and [4-^{14}C]androstenedione. A preincubation mixture was 1.8 ml in volume and contained 5 μl of propyleneglycol containing 346.4 pmol of [4-^{14}C]androstenedione, 64.8 pmol of [1β-^{3}H]androstenedione (411.2 pmol in all), 1.395 ml of 100 mM potassium phosphate buffer, pH 7.4 and 0.4 ml of homogenate. After the mixture was preincubated at 37°C for 5 min, reaction was started by addition of 1 mg of NADPH (0.2 ml in volume) at 37°C in a shaking bath under air in a total incubation volume of 2.0 ml. The final concentration of androstenedione was 205.6 nM.

For each homogenate, four reaction tubes were run in parallel, two of them were incubated for 1 h and the other two for 2 h. Reaction was stopped by adding 0.2 ml of 20% trichloroacetate. Then, 0.4 ml of 5% charcoal suspension was added and the samples were mixed. The tubes were centrifuged and 1.6 ml of the supernatant was obtained. Another 0.4 ml of 5% charcoal suspension was added and the samples were mixed and centrifuged. The supernatant was passed through a Pasteur pipette plugged with fine cotton to remove charcoal and steroids. One ml aliquots of the supernatant were counted for ^{3}H and ^{14}C by a liquid scintillation counter, Aloca LSC 702. Aromatase activity was calculated from the difference between the counts of ^{3}H at 1 and 2 h, and shown by fmol/h/mg protein. The count of ^{14}C indicated the amount of residual steroids. When the count of ^{14}C was not negligible, the datum was excluded from the analysis.

Protein determination

Protein concentrations were determined by the modified procedure [12] of the method of Lowry *et al.* [13] using bovine serum albumin as the standard.

RESULTS

Optimal measurement conditions

To determine the duration of incubation, aromatase assay was performed with incubation times of 0, 5, 15, 30, 60 and 120 min, respectively. As shown in Fig. 1, the count of ^{3}H was linear with time at least until 120 min. Effect of protein concentration on aromatase activity is shown in Fig. 2, indicating that the aromatase activity was linear at least until 4.8 mg/ml protein in the reaction mixture.

Inhibition of aromatase activity

Inhibition of aromatase activity was examined by adding various amounts of 4-hydroxyandrostenedione (4-OH-A) to the reaction mixture. A tube without 4-OH-A was referred to as the control. When 2.06, 20.6 and 206 μM of 4-OH-A were added to the substrate, aromatization was reduced to 41, 30 and 11% of the control, respectively (Fig. 3).

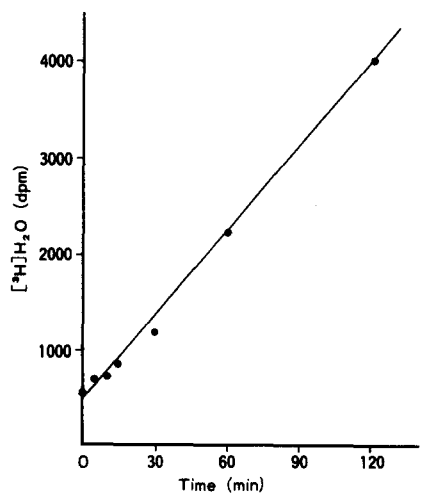

Fig. 1. Effect of time on aromatase activity. Samples were incubated for 0, 5, 15, 30, 60 and 120 min. The counts of [^{3}H]H_2O are shown by dpm.

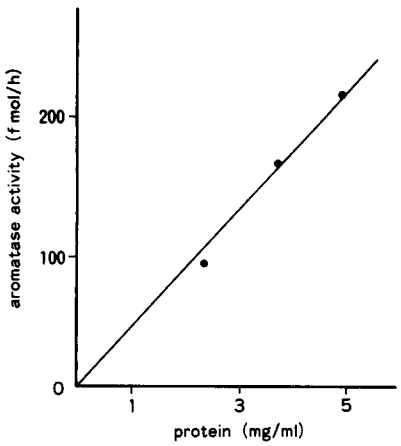

Fig. 2. Effect of protein concentration on aromatase activity. Samples were incubated with protein concentrations from 2.4 to 4.8 mg/ml.

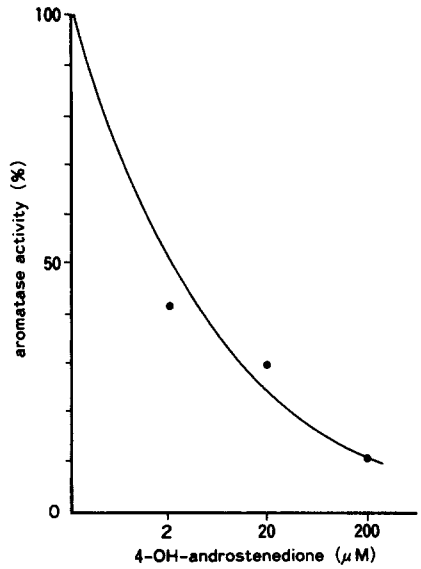

Fig. 3. Inhibition of aromatase activity by 4-hydroxyandrostenedione. Samples were incubated with 0, 2, 20 and 200 μM of 4-hydroxyandrostenedione. The activity of control without 4-hydroxyandrostenedione is shown as 100%.

Assay of aromatase activity

Among the fourteen uteri studied, 8 were in the proliferative phase and 6 were in the secretory phase.

Aromatase activities in the four regions were compared within each phase (Table 1). In the proliferative phase, aromatase activities (mean ± SD) were 15.0 ± 7.9, 10.9 ± 10.3, 9.4 ± 10.6 and 8.0 ± 7.3 fmol/h/mg protein in the columnar epithelial region, squamous epithelial region, connective tissue and endometrium, respectively (Fig. 4). There was a tendency for aromatase activity to be higher in the columnar epithelial region than in others, although no significant difference was seen between any two of the four regions.

In the secretory phase, aromatase activities (mean ± SD) were 31.5 ± 7.6, 19.1 ± 7.1, 5.6 ± 4.6, and 6.3 ± 1.5 fmol/h/mg protein in the columnar epithelial region, squamous epithelial region, connective tissue and endometrium, respectively. Aromatase activity was significantly higher in the columnar epithelial region than in any other region, and significantly higher in the squamous epithelial region than in connective tissue or endometrium. There was no significant difference between connective tissue and endometrium.

Comparison of aromatase activities between the two phases revealed that aromatase activity in the columnar epithelial region in the secretory phase was significantly higher than that in the proliferative phase, but there was no significant inter-phase difference in any other region.

Statistical analysis was first done by F-test, and where appropriate, Student's t-test or Welch's method was employed.

DISCUSSION

Aromatase cytochrome P-450 catalyzes the conversion of androgens to estrogens. The aromatization reaction is supposed to involve three sequential dioxygen- and NADPH-dependent oxygenations. It is suggested that a single enzyme species carries out all three steps of the reaction and that androgens differing in D-ring substitution (androstenedione,

Table 1. Aromatase activity (fmol/h/mg protein)

	Columnar epithelial region	Squamous epithelial region	Connective tissue	Endometrium
Proliferative phase	15.0 ± 7.9 (n = 8)	10.9 ± 10.3 (n = 8)	9.4 ± 10.6 (n = 8)	8.0 ± 7.3 (n = 6)
Secretory phase	31.5 ± 7.6 (n = 5)	9.1 ± 7.1 (n = 6)	5.6 ± 4.6 (n = 6)	6.3 ± 1.5 (n = 3)

*P < 0.05 (by Student's t-test); **P < 0.05 (by Welch's method). Means ± SD.

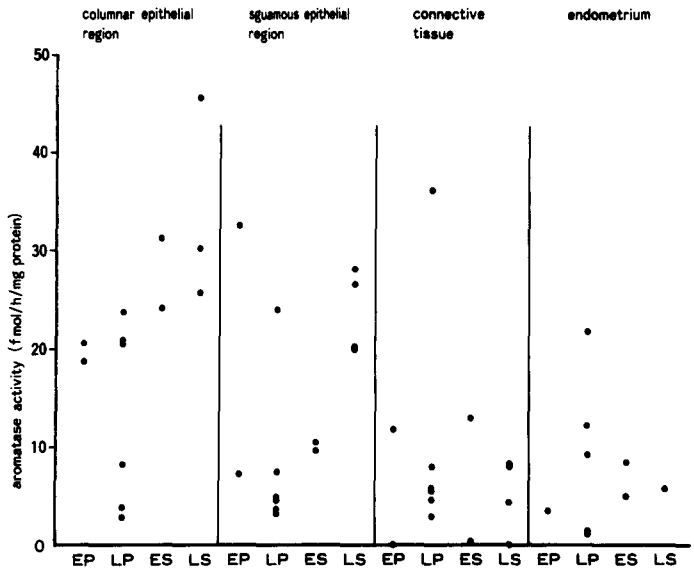

Fig. 4. Aromatase activity in the menstrual cycle. Aromatase activities in columnar epithelial region, squamous epithelial region, connective tissue and endometrium are divided into four phases and shown. EP: early proliferative phase. LP: late proliferative phase. ES: early secretory phase. LS: late secretory phase.

testosterone, 16α-hydroxyandrostenedione and 16α-hydroxytestosterone) can each serve as substrate [1, 17].

This enzyme plays important roles in endocrine physiology and pathology, especially in the menstrual cycle and pregnancy. Aromatization can be stimulated by follicle stimulating hormone (FSH) in ovarian granulosa cells [18], but the gonadotropins do not affect peripheral aromatization and control of peripheral aromatization remains uncertain [19].

In the normal menstrual cycle, the uterine cervix and endometrium change their states depending on the stimulation by sex steroids. Ovarian secretion by the granulosa or corpus luteal cells provides the major source of estrogen [20] and only a minor fraction of the circulating estrogen pool is synthesized via the extraglandular aromatization of ovarian and adrenal androgens such as androstenedione and testosterone [5]. However, there is a possibility that local production of estrogens has a significant influence on the cyclic change of the uterine cervix and endometrium, because tissue concentration of steroid hormones may be more important with respect to the endocrine organ than the peripheral blood levels of these hormones [7–11].

In the present study, samples from three regions of uterine cervix and endometrium were incubated with [1β-^{3}H, 4-^{14}C]androstenedione. In this assay, when we removed steroids with charcoal only once, the background counts of ^{14}C were high enough to indicate that a significant amount of residual steroids was still present and that we had to remove steroids with charcoal twice.

Concerning aromatase, various K_m values for androstenedione have been reported by several authors. Yoshida and Osawa have shown K_m of aromatase for androstenedione to be 10–12 nM, using human placental microsomes and purified aromatase [17]. Depending upon this value, in the present study, we regarded 205.6 nM of androstenedione as a large enough amount of substrate.

4-Hydroxyandrostenedione (4-OH-A) has been shown to decrease the peripheral aromatization of androgens [21–23]. In the present study, the count of [^{3}H]H_2O was decreased according to the dose of 4-OH-A added to the sample.

As a result, the columnar epithelial region showed the highest activity in both phases, although it was not statistically significant in the follicular phase. As this region is abundant in glands, it was supposed that aromatase in uterine cervix is localized in the cervical glands.

By comparison between the two phases, the columnar epithelial region showed significantly higher activity in the secretory phase. The reason for the difference observed in the columnar epithelial region remains uncertain, but it is possible that an inhibitor is secreted from the ovary in the follicular phase. Campeau *et al.* reported a factor from ovarian follicular fluid

which inhibits aromatization locally [24]. Also Longcope *et al.* [19] indicated that menopause is associated with a relatively abrupt increase in peripheral aromatization, using constant infusions of [^{3}H]androgens.

Concerning the endometrium, although it undergoes drastic change in the menstrual cycle, aromatase activity was lower than expected in this region. Also no significant difference was observed between the two phases. However, aromatase activity was shown per mg protein in the present study. Therefore, taking account of the fact that the endometrium is thicker in the secretory phase than in the follicular phase, the endometrium is supposed to form more estrogens in the secretory phase than in the proliferative phase.

In the present study, the columnar epithelial region of the uterine cervix had significantly higher aromatase activity than the squamous epithelial region, connective tissue or endometrium in the secretory phase. There was a tendency that the columnar epithelial region had higher aromatase activity than the other three regions in the proliferative phase, and aromatase activity in columnar epithelial region was significantly higher in the secretory phase than in the proliferative phase.

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