J. steroid Biochem. Vol. 22, No. 4, pp. 555–557, 1985
Printed in Great Britain. All rights reserved
0022-4731/85 $3.00 + 0.00
Copyright © 1985 Pergamon Press Ltd

# SHORT COMMUNICATION

# INHIBITION OF AROMATASE AND NADPH CYTOCHROME *C* REDUCTASE ACTIVITIES IN HUMAN ENDOMETRIUM BY THE HUMAN PLACENTAL NADPH CYTOCHROME *C* REDUCTASE ANTISERUM

LINDA TSENG* and FRANCIS L. BELLINO†
*Department of Ob/Gyn, School of Medicine, SUNY at Stony Brook, Stony Brook, New York 11794 and †Department of Biological Sciences, SUNY at Buffalo, NY 14260, U.S.A.

(*Received* 2 *August* 1984)

**Summary**—The cross-reactivity of human placental microsomal NADPH-cytochrome *c* reductase antiserum, REDFBIV, against the endometrial reductase alone and as a component of the endometrial aromatase was investigated. Human endometrial particulate fractions were incubated with various amounts of REDFBIV for 1 h at 4°C and both enzyme activities were measured at the end of incubation. The extent of inhibition of these endometrial enzymes was compared with the ability of this antiserum to inhibit the placental microsomal reductase and aromatase activities. The antiserum effectively inhibited the activities of both enzymes in both tissues in a dose dependent manner with aromatase activity inhibited to a greater extent than reductase activity. These results indicate the antiserum to the placental microsomal NADPH-cytochrome *c* reductase component of aromatase recognizes the reductase component of the aromatase enzyme system in endometrium.

## INTRODUCTION

Recently we have demonstrated that human endometrium contains aromatase and the activity is regulated by estrogen and progesterone [1–4]. The characteristics of the endometrial aromatase have not been studied in detail. On the other hand, the characteristics of human term placental aromatase have been extensively studied. NADPH-cytochrome *c* reductase is a required component of human placental microsomal aromatase [5], as demonstrated in part by the ability of antibody against the 90% homogenous reductase to strongly inhibit aromatase as well as reductase activities. We have investigated the cross-reactivity of a recently obtained, highly specific antibody of this placental protein against the endometrial reductase alone and as a component of endometrial aromatase. This report summarizes our findings.

## EXPERIMENTAL

Human endometrium was obtained from a patient who had been subjected to hysterectomy for medical reasons not related to this study. The morphology of this specimen was histologically identified as normal secretory endometrium. Tissue fragments were homogenized in 0.05 M phosphate buffer (ppb, pH 7.4). Various crude particulate fractions: nuclei, mitochondria, and microsomes, were obtained by centrifuging the tissue homogenate at 800, 10,000 and 100,000 *g*, respectively. Lyophilized placental microsomes were prepared as described [5] and homogenized in 0.067 M phosphate buffer, pH 7.4.

The high molecular weight form of NADPH-cytochrome *c* reductase was purified from human term placental microsomes by modifications of the method described previously [5]. Briefly, lyophilized microsomes were homogenized in Buffer A (67 mM ppb, pH 7.3, containing 1% cholic acid-sodium salt, 20% glycerol, 1 mM EDTA, 0.1 mM DTT) with 0.002% pepstatin and 0.005% α-toluenesulfonyl fluoride at 20 mg microsomes/ml. The reductase-containing fractions which passed through an octyl-Sepharose CL-4B gel column (equilibrated with Buffer A) were pooled and applied to a 2′,5′-ADP Sepharose 4B gel column (1.6 × 20 cm) equilibrated with Buffer A. After extensive washing, the yellow-colored portion at the top of the column was transferred to a small column and the enzyme eluted with 1 mM 2′AMP in Buffer A. The preparation was dialyzed against detergent-free Buffer A, concentrated, applied to a Bio-Gel A-1.5 m column (1.6 × 68 cm) equilibrated with detergent-free Buffer A, and the high mol. wt form (73 K) of the reductase was recovered in the $A_{280nm}$ peak which trailed the void volume peak. Aliquots of the preparation, judged better than 99% homogenous by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of 6 μg protein followed by silver staining, were injected into rabbits as described [5] to obtain antiserum preparation REDFBIV. In contrast to the reductase antisera REDFBI and II described previously [5], antiserum REDFBIV gave a single precipitin band at the position of authentic reductase when analyzed by immunoelectrophoresis of the cholate-soluble fraction of human placental microsomes. The higher specificity of REDFBIV is due to the use of nearly homogeneous protein as antigen and to the apparent low immunogenicity of the trace contaminent (s).

NADPH-cytochrome *c* reductase activity in mitochondrial and microsomal fractions from endometrium and in placental microsomes was measured at 23°C by the procedure of Strobel and Dignam[6]. The endometrial nuclear fraction was not included in the reductase assay due to its heavy turbidity. Endometrial aromatase activity was measured by incubating the particulate fractions (nuclei, mitochondria and microsomes) with 100 nM [7-$^3$H]testosterone (T, sp. act. 30 Ci/mmol, NEN) and 1 mM NADPH in 2 ml ppb at 37°C for 2 h. Under the assay condition, the rate of aromatization remained constant up to 4 h. (Fig. 1). The product of the aromatization, estrone

Supported by grants from the American Cancer Society PDT238 and the National Institute of Health HD16593.

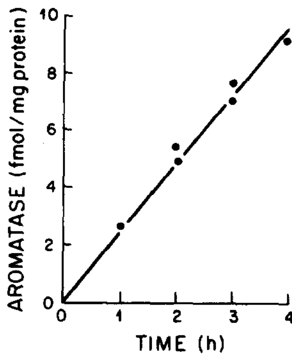


Fig. 1. Time course of the aromatization of [$^3$H]T to estrogens ($E_1 + E_2$) in the tissue homogenate (10 mg tissue protein/2 ml) of a secretory human endometrium.

($E_1$) and estradiol ($E_2$), were isolated and quantitated by the method described previously [1, 2]. Placental microsomal aromatase was measured by the $^3H_2O$ assay using [1$\beta$-$^3$H,4-$^{14}$C] androstenedione, as previously described [7].

The effect of the antiserum on the NADPH-cytochrome *c* reductase and the aromatase activities in endometrium was carried out by incubating the particulate fractions (aromatase: combined nuclear, mitochondrial and microsomal fractions; reductase: combined mitochondrial and microsomal fractions) in 2 ml ppb (1.5 mg protein/ml) with various amounts of antiserum, 2 to 25 $\mu$l, at 4°C for 1 h and then measuring the activities at the end of the incubation. Preimmune rabbit serum was added to the control and other samples to make up the difference of the antiserum volume in the reaction mixture. Homogenized placental microsomes (0.5 mg protein/ml) were preincubated with antiserum in an identical manner.

## RESULTS

*Aromatase activity in various endometrial particulate fractions*

Table 1 shows the aromatase activity in the tissue homogenate and in the fractions of various speeds of centrifugation. Aromatase activity was found to be present in the various pellet fractions similar to the subcellular distribution in placenta [8].

Table 1. Aromatase activity in endometrial homogenate and various particulate fractions

| Subcellular fractions | Relative aromatase activity (% of homogenate) |
|---|---|
| Homogenate | 100 |
| 800 *g* pellet | 31 |
| 10,000 *g* pellet | 27 |
| 100,000 *g* pellet | 30 |
| 100,000 *g* supernatant | 0 |

Human endometrial tissue fragments were homogenized in ppb (50 mg/ml). Particulate fractions obtained from various speeds of centrifugation were reconstituted with buffer to the original homogenate volume, and then used to measure the aromatase activities. The activity in the homogenate is 11 fmol/h × mg tissue protein.

*Effect of the antiserum on endometrial and placental NADPH-cytochrome* c *reductase and aromatase activities*

The inhibition of NADPH-cytochrome *c* reductase and aromatase activities by the antiserum are shown in the combined endometrial particulate fractions (Fig. 2A) and in placental microsomes (Fig. 2B). The antiserum effectively inhibited the activities of both enzymes in both tissues in a dose dependent manner with aromatase activity inhibited to a greater extent than reductase activity. The 50% inhibition levels are for the endometrial particulate fractions: 2.5 $\mu$l for reductase and 1.5 $\mu$l for aromatase; and for placental microsomes: 10 $\mu$l for reductase and 4.5 $\mu$l for aromatase.

## DISCUSSION

These results indicate (a) the highly specific antiserum against the placental microsomal NADPH-cytochrome *c* reductase component of aromatase recognizes the same component of the aromatase enzyme system in endometrium, (b) in both cases, the reductase is probably exposed on the membrane surface since the antibody–antigen interaction does not require detergent solubilization, and (c) NADPH-cytochrome *c* reductase is required for aromatization in endometrium as well as placenta. The

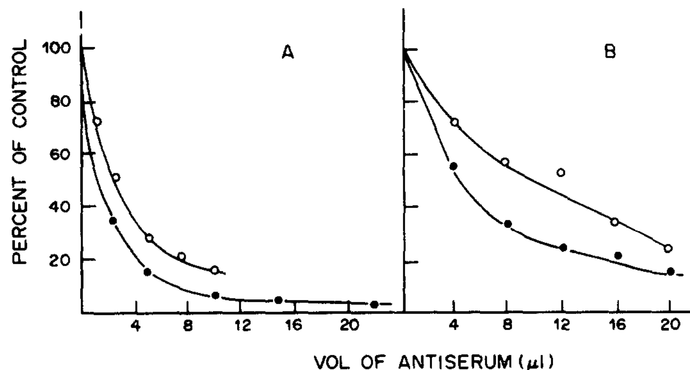


Fig. 2. Antiserum inhibition of aromatase and NADPH-cytochrome *c* reductase in human endometrium and placenta. Antiserum REDFBIV, prepared against homogenous NADPH-cytochrome *c* reductase from human term placental microsomes, was incubated at the indicated volumes with either the particulate fractions of human endometrium (A), or human placental microsomes (B) at 4°C for 1 h. Aliquots were then taken for measurements of aromatase, in solid circle (●), and NADPH-cytochrome *c* reductase, in open circle (○), activities, as described in the Experimental section. The data are presented as % of the control (pre-immune antiserum) activities. The control activities are 3.1 nmol/min × mg protein and 27 fmol/h × mg protein for endometrial reductase and aromatase, respectively, and 16 nmol/min × mg protein and 52 pmol/min × mg protein for placental microsomal reductase and aromatase, respectively.

requirement for smaller amounts of antiserum for 50% inhibition of both enzymes in endometrium compared with placental microsomes is probably due to the 5-fold lower reductase specific activity and much lower aromatase activity in endometrium.

In the only other instance, to the best of our knowledge, where an antiserum to a steroid metabolizing enzyme (17$\beta$ hydroxyl steroid dehydrogenase) from human term placenta was tested in human endometrium, no cross-reactivity was found either by immunohistochemistry [9] or by enzyme inhibition [10] despite the apparent physical similarity of the two enzymes [11]. This indicated that the enzymes in the different human tissues are immunologically distinct. Thompson and Siiteri[12] previously described the ability of antiserum against purified rat liver NADPH-cytochrome *c* reductase to inhibit the reductase and aromatase in human placental microsomes, but they provided no comparative information regarding the potency of the antiserum in both species. The amino acid composition of purified placental reductase is similar to, but not identical with, the amino acid composition of the rat and rabbit liver reductase (F.B., unpublished observations). Osawa and co-workers showed that antiserum against human placental NADPH-cytochrome *c* reductase had varying degrees of potency against that enzyme when tested in human breast tumors [13], horse and baboon placental microsomes and rat liver microsomes [14]. The results reported here demonstrate for the first time the immunologic similarity of reductase alone and as a component of aromatase in human endometrial and placental tissues which will facilitate further characterization and elucidation of the hormonal regulation of endometrial aromatase.

*Acknowledgements*—The authors wish to thank Mr James Mazella and Ms Boling Sun for technical assistance, and Vivian Kulaya for typing the manuscript.

## REFERENCES

1. Tseng L., Mazella J., Mann W. J. and Chumas J.: Estrogen synthesis in normal and malignant human endometrium. *J. clin. Endocr. Metab.* **55** (1982) 1029–1031.
2. Tseng L., Mazella J., Funt M. I., Mann W. J. and Stone M. L.: Preliminary studies of aromatase in. human endometrial cancer. *Obstet. Gynec.* **63** (1984) 150–154.
3. Tseng L.: Estrogen synthesis in human endometrial epithelial glands and stromal cells. *J. steroid Biochem.* **17** (1982) 361–370.
4. Tseng L.: Effect of estradiol and progesterone on human endometrial aromatase activity in primary cell culture. *Endocrinology* **115** (1984) 833–835.
5. Bellino F.: Estrogen synthetase. Demonstration that the high molecular weight form of NADPH-cytochrome *c* reductase from human placental microsomes is required for androgen aromatization. *J. steroid Biochem.* **17** (1982) 361–370.
6. Strobel H. W. and Dignam J. D.: Purification and properties of NADPH-cytochrome P-450 reductase. In *Methods in Enzymology* (Edited by S. Fleischer and L. Packer). Academic Press, New York, Vol. L11, Part C (1980) p. 91.
7. Bellino F. L., Gilani S. S. H., Eng S. S., Osawa Y. and Duax W. L.: Active site-directed inactivation of aromatase from human placental microsomes by brominated androgen derivatives. *Biochemistry* **15** (1976) 4730–4736.
8. Bellino F. L. and Osawa Y.: Localization of estrogen synthetase in the chorionic villus fraction after homogenization of human term placenta. *J. clin. Endocr. Metab.* **44** (1977) 699–707.
9. Marovitz W., Loucopoulos A., Satyaswaroop P. G., Gurpide E., Todd R. and Naftolin F.: Apparent immunologic nonidentity of human placental and endometrial 17$\beta$-estradiol dehydrogenase. *Am. J. Obstet. Gynec.* **138** (1980) 643–647.
10. Tseng L., Gusberg S. B. and Gurpide E.: Estradiol receptor and 17$\beta$-dehydrogenase in normal and abnormal human endometrium. *Ann. N.Y. Acad. Sci.* **286** (1977) 190–198.
11. Tseng L., Mazella J. and Tseng L.: Kinetic studies of human endometrial hydroxysteroid dehydrogenase. *J. steroid Biochem.* **14** (1981) 437–442.
12. Thompson E. A. Jr and Siiteri P. K.: The involvement of human placental microsomal cytochrome P-450 in aromatization. *J. biol. Chem.* **249** (1974) 5373–5378.
13. Osawa Y., Tochigi B., Higashiyama T., Yarborough C., Nakamura T. and Yamamoto T.: Multiple forms of aromatase and response of breast cancer aromatase to antiplacental aromatase II antibodies. *Cancer Res.* **42** (suppl) (1982) 3299–3306.
14. Osawa Y., Higashiyama T. and Nakamura T.: Species specificity of estrogen biosynthesis in pregnancy. Immunochemical difference of placental NADPH-cytochrome c (P-450) reductase in human, baboon, and horse. *J. steroid Biochem.* **15** (1981) 449–452.