Journal of Steroid Biochemistry, Vol. 11, pp. 1295 to 1298
Pergamon Press Ltd 1979. Printed in Great Britain

PROGESTERONE RECEPTORS IN BREAST FIBROADENOMAS

P. M. MARTIN*, F. KUTTENN†‡, H. SERMENT* and P. MAUVAIS-JARVIS†
*Department of Obstetrics and Gynecology, Hôpital de la Conception,
144 rue St-Pierre, 13005 Marseille
†Department of Endocrinology and Gynecology, Hôpital Necker,
149 rue de Sèvres, 75730 Paris Cedex 15, France

(*Received* 21 *November* 1978)

SUMMARY

Cytosol progesterone receptors (P-R) were measured in breast fibroadenomas from 88 women, and their levels were compared to the tumor epithelial cell density and estradiol receptor levels (E-R). Three groups of fibroadenoma were distinguished: type I with a high epithelial cell density ($n = 18$), type III ($n = 46$) with low epithelial cell density and extensive fibrosis, and type II with cell density intermediate between the two other groups ($n = 24$). Whereas E-R levels correlated well with cellular density, P-R levels were elevated in group I and absent in group III, but in contrast to E-R, the low P-R levels observed in group II could not be only explained by cellular density. Since P-R is an estrogen-dependent protein and an hormonal marker, its decrease in type II fibroadenoma might be interpreted as reflecting a rapid decrease in hormone dependence.

INTRODUCTION

An imbalance in the ovarian estradiol (E_2) and progesterone (P) secretion is generally observed in women with benign breast disease [1]. The mammary gland is a target organ for estradiol and progesterone; and an inadequate secretion of the corpus luteum reflected by low plasma P levels and normal or high plasma E_2 levels seems to be the main factor responsible for the development of benign breast diseases [2–4]. This hormone-dependence is also suggested by the presence of a relatively high concentration of estradiol receptor (E-R) in fibroadenomas with high epithelial cellularity [5] and from the effectiveness of substitutive progestin treatment in breast fibroadenomas [6].

Since the progesterone receptor (P-R) synthesis is estrogen dependent [7–9], the observation of P-R in malignant breast tumors is now considered as the best index of the hormone-dependence of these tumors [10]. Until now, there have been few studies on P-R in benign breast diseases [11]. In the present study, we have looked for P-R in breast fibroadenomas and determined appropriated assay conditions. In addition P-R levels have been compared with the epithelial cellular density and E-R levels of the tumor.

SUBJECTS AND METHODS

Fibroadenomas from 88 women 18–55 yr old were studied with parallel pathological and biochemical investigations.

E_2 = estradiol. E-R = cytosol estradiol receptor. P = progesterone. P-R = cytosol progesterone receptor. R 2858 = Moexestrol = 11 β-methoxy-17α-ethinyl-1,3,5(10)-estatriène-3,17β-diol. R 5020 = 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione.

‡ To whom reprint request should be sent.

The fibroadenomas were obtained surgically at mid-follicular phase (6th to 10th day of the cycle) and were analyzed as follows: after separation from non tumor tissue, the tumor was cut into two parts. One part was used for pathological studies, the other was frozen in liquid nitrogen until processed for biochemical investigations.

PATHOLOGICAL INVESTIGATIONS

The cellular density of the tumor was assessed by determining the relative proportion of epithelial and stromal cells. Three degrees of cellular density were distinguished: in type I, proliferation of acinar epithelial cells was predominant, and fibrosis was practically absent; in type III, the fibrosis was so important that the original lobular proliferation could scarcely be recognized as fibroadenoma. In type II, microscopic features were intermediate between type I and type III.

BIOCHEMICAL STUDY

Chemicals [1, 2, 6, 7-^{3}H]-Progesterone (90 Ci/mmol, N.E.N. Corp.) and the synthetic progestin [6, 7-^{3}H]-R 5020 (51.4 Ci/mmol, Roussel Uclaf) were used after purification. Unlabeled steroids: progesterone, R 5020, dexamethasone (DXM) were obtained from Roussel-Uclaf. Tris–HCl-EDTA buffer was routinely used (G.T.E.M. buffer = 0.01 M Tris-HCl–1 mM EDTA–12 mM monothioglycerol–glycerol 10%, pH 8.0). Acid-washed activated charcoal was obtained from Sigma and Dextran T 70 was supplied by Pharmacia. The dextran coated charcoal (DCC) suspension (0.5% activated charcoal with 0.05% Dextran 70) was stored at 0°C with constant shaking.

Preparation of cytosol fractions

Because of the relative instability of P-R even in liquid nitrogen, the adenoma tissues were processed for biochemical investigation within less than one week after surgery. All processing was performed at 0–4°C. The frozen tissue was crushed with a Thermovac tissue pulverizer, and then homogenized with a Polytron PT 10 in cold G.T.E.M. Buffer in a 5:1 wt/vol ratio. This buffer was used for both homogenization and binding assay and it was preferred to the phosphate buffer usually used for P-R assay because of the concurrent E-R assay. Indeed, G.T.E.M. buffer appears to be the best compromise for multiple steroid receptor analysis [12]. The homogenate was centrifuged in a 50 Ti rotor of a Beckman $L_2$65 B centrifuge at 105,000 *g* for 1 h. The supernatant (cytosol) had a protein concentration range of 3–8 mg per ml as determined by the method of Lowry *et al.*[13].

Cytosol estradiol binding assay

E-R levels were measured by sucrose gradient analysis and dextran-coated charcoal exchange assay suitable for assaying total estradiol receptor binding sites, as previously described [5].

Cytosol progesterone binding assay

High affinity binding of progesterone was also determined by sucrose gradient analysis and dextran-coated charcoal assay:

Sucrose gradient analysis. Originally, linear 5–20% sucrose density gradient (3.6 ml) prepared in TEM–Buffer (G.T.E.M. without glycerol) were centrifuged at 320,000 *g* for 16 h at 0–4°C in a SW-60 Ti rotor (Beckman $L_2$65 B ultra-centrifuge). However it was shown that the same information could be obtained within 90 min with the vertical tube centrifugation. Since the 8 S progesterone binding protein is highly unstable, the vertical tube centrifugation was thereafter routinely used. The linear density gradient has been modified to 8.5–20% and the volume amplified to 4.5 ml. Gradients were prepared and equilibrated at 0–4°C for 2–5 h before use. 200 μl of cytosol were incubated with a 50 μl aliquot of buffer containing 10 μM [^{3}H]-P or [^{3}H]-R 5020 (final concentration 2 nM) agitated and incubated at 0°C overnight. Parallel samples were preincubated for 15 min with a 100 fold excess of unlabeled P or R 5020. Suspensions of dextran-coated charcoal (DCC, 500 μl) were centrifuged for 10 min at 2,000 *g* to sediment the DCC pellet. The incubated cytosols were then transferred into the pellets, shaken for 10 min at 0°C and centrifuged for 10 min at 2,000 *g*; 200 μl aliquots of the resulting supernatants were layered on the sucrose gradients. Centrifugation at 291,000 *g* for 90 min was performed in a VTi-65 rotor (Beckman L_2-65 centrifuge). The slow acceleration accessory is adapted to this machine and this rotor (start adjust: 5 and ramp adjust 4.1).

DCC exchange assays. Cytosol prepared as described above was diluted in G.T.E.M. buffer to approximately 2 mg protein/ml. Aliquots of 100 μl were incubated with eight different concentrations ranging from 0.1 to 1.10^{-9} M (final concentration) [^{3}H]-P or [^{3}H]-R 5020 added in a volume of 100 μl buffer and 2% ethanol. Parallel cytosol samples were incubated with a 100-fold excess of unlabeled R 5020. Incubations were carried out at 0°C overnight to allow the exchange of endogenously bound hormone with [^{3}H]-hormone and the determination of total P-R binding sites. At the end of the incubation period, 500 μl of the DCC suspension were added and bound radioactivity was counted in 500 μl aliquots of the supernatants. The data were analyzed according to the method of Scatchard after subtraction of non specific binding calculated from the preparations incubated with a 100 fold excess of non radio-active hormone.

Under optimal conditions i.e. with sufficient adenoma tissue, gradient determination and Scatchard assay were performed, but when surgical samples were too small to yield more than 1 ml of cytosol, assay was limited to sucrose gradient and the saturation assay with only four different incubations (0.1 0.5 and 1.0 nM [^{3}H]-R 5020, and 1 nM [^{3}H]-R 5020 with 100 nM unlabeled R 5020.

RESULTS

Three groups of fibroadenomas were defined according to their epithelial cellularity. In group I (n = 18) epithelial cellular density was high. In group III (n = 46) fibroadenomas exhibited extensive fibrosis and very low epithelial cellular density. The 24 other fibroadenomas (group II) had an intermediate cellular density.

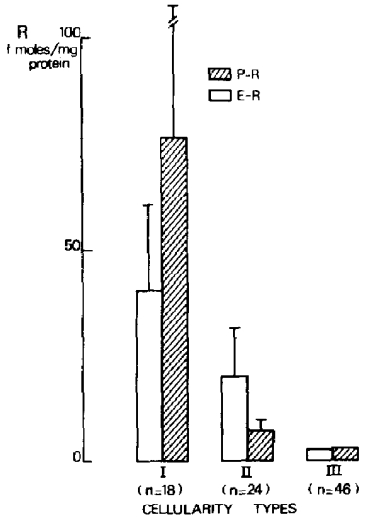

Fig. 1. Cytosol E-R and P-R levels in 3 groups of fibroadenomas according to their epithelial cell density: high in group I (n = 18), medium in group II (n = 24), low in group III (n = 46).

Estradiol receptors in fibroadenomas (Fig. 1)

In this series, the highest E-R levels were found in fibroadenomas with the highest cellular density. In group I, the mean E-R level was 41.2 ± 24.3 fmol/mg protein. It was 18.7 ± 11.4 fmol/mg protein in group II and lower than 5 fmol/mg protein in group III.

Progesterone receptors in fibroadenomas (Fig. 1)

The concentration of binding sites, P-R levels were constantly elevated when the cellular density was high: 76.9 ± 54.2 fmoles/mg protein in the 18 fibroadenomas of group I. They were low in the two other groups: 6.0 ± 3.7 and < 5 fmol/mg protein (sensitivity limit of the assay) in groups II and III respectively.

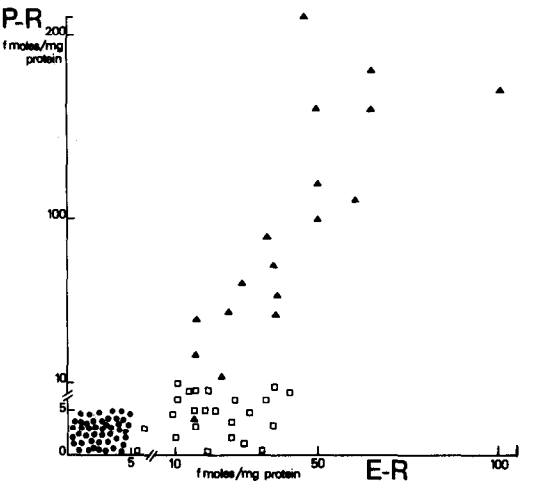

Fig. 2. Correlation between cytosol P-R and E-R levels in the 3 groups of fibroadenomas (▲ type I with high epithelial cellular density, □ type II with mid-epithelial cellular density, ● type III with low epithelial cellular density).

DISCUSSION

The presence of P-R has been exceptionally reported in breast fibroadenomas [14]. This is probably due to the fact that very careful conditions are required for studying this receptor. The first condition is the time of surgery: there is indeed a great variability in P-R synthesis throughout the menstrual cycle [15]. In order to obtain an optimal level of P-R, tumor may be removed in the mid follicular phase when estradiol production is elevated and progesterone not yet secreted [16]. In addition, at this period of the menstrual cycle, there is no translocation of progesterone cytoplasmic receptor into the nucleus [9, 15, 17, 18].

On a technical point of view, the routinely use of [^{3}H]-R 5020 gives better results than those obtained with [^{3}H]-P. R 5020, indeed, does not bind CBG, and binds P-R with high affinity and better stability than progesterone itself. However R 5020 is known to bind glucucorticoid receptor. Also, in order to eliminate the interference of this receptor in this assay, incubations were performed with a 250 fold excess of dexamethasone. In these conditions no displacement of [^{3}H]-R 5020 binding was observed.

On a pathological point of view, it seems likely that P-R levels essentially depend on cellular density of the tumor. These levels are particularly elevated in fibroadenomas with high cellular density (group I) whereas they are low or absent in fibroadenomas exhibiting medium or low cellular density (group II and III). This observation differs from what is observed with E-R levels in the same fibroadenomas. E-R levels, indeed, are perfectly correlated to the epithelial density of the tumor and might reflect its "growing capacity" [5]. As shown in Fig. 2, there is no parallelism between P-R and E-R levels. When P-R levels are plotted as a function of E-R levels, three different areas can be distinguished that closely correspond to the three cellular types of tumor: (i) fibroadenomas with elevated levels of both E-R and P-R correspond to type I cellularity; (ii) fibroadenomas with medium E-R levels and low P-R levels correspond to cellularity type II; (iii) fibroadenomas with both E-R and P-R levels under 5 fmoles/mg protein correspond to type III cellularity ($P < 0.1$).

The observation in 24 fibroadenomas of group II of a persistent estrogen dependence without the presence of progesterone receptors is interesting to consider, since P-R is an index of estrogen sensitivity of target cells and reflects their differentiated and functional state. It appears likely that in breast fibroadenomas, the hormone dependence is rapidly decreasing, indicating a loss of cellular differentiation. Such a "dedifferentiation" might explain the rapid insensitivity of fibroadenomas to progesterone both secreted by corpus luteum or administered as exogenous treatment [6].

Acknowledgements—We would thank J. P. Raynaud (Lab. Roussel, France) for R 5020 gift.

REFERENCES

1. Sitruk-Ware L. R., Sterkers N., Mowszowicz I. and Mauvais-Jarvis P.: Inadequate corpus luteal function in women with benign breast diseases. *J. clin. Endocr. Metab.* **44** (1977) 771–774.
2. Mauvais-Jarvis P. and Kuttenn F.: L'insuffisance en progestérone est-elle cancérigène. *Nouv. Presse Med.* **4** (1975) 323–326.
3. Mauvais-Jarvis P., Kuttenn F., Mowszowicz I. and Sitruk-Ware R.: Mastopathies bénignes: étude hormonale chez 125 malades. *Nouv. Presse Med.* **6** (1977) 4115–4118.
4. Mauvais-Jarvis P., Sitruk-Ware R., Kuttenn F. and Sterkers N.: Luteal phase insufficiency = a common pathophysiological factor for development of benign and malignant breast diseases. In: *Commentaries on Research in Breast Cancer* (Edited by R. D. Bulbrook and D. J. Taylor). A. R. Liss Inc., New York (1978) In press.
5. Martin P. M., Kuttenn F., Serment H. and Mauvais-Jarvis P.: Studies on clinical, hormonal and pathological correlations in breast fibroadenomas. *J. steroid Biochem.* **9** (1978) 1251–1255.

6. Mauvais-Jarvis P., Sterkers N., Kuttenn F. and Beauvais J.: Traitement des mastopathies bénignes par la progestérone et les progestatifs. Résultat en fonction du type de la mastopathie (à propos de 260 observations). *J. Gyn. Obst. Biol. Repr.* **7** (1978) 477–484.
7. Rao B. R., Wiest N. G. and Allen W. M.: Progesterone "receptor" in rabbit uterus. I. Characterization and estradiol-17β augmentation. *Endocrinology* **92** (1973) 1229–1240.
8. Leavitt W. W., Toft D. O., Strott C. A. and O'Malley B. W.: A specific progesterone receptor in the hamster uterus. Physiological properties and regulation during the estrous cycle. *Endocrinology* **94** (1974) 1041–1053.
9. Milgrom E., Luu Thi M. and Baulieu E. E.: Control mechanisms of steroid hormone receptors in the reproductive tract *Acta Endocr. Copenh.* (suppl.) **180** (1973) 380–397.
10. Horwitz K. B. and McGuire W. L.: Estrogen and progesterone: their relationship in hormone-dependant breast cancer. In: Progesterone receptors in normal and neoplastic tissues (Edited by W. L. McGuire, J. P. Raynaud and E. E. Baulieu) Raven Press, New York (1977) pp. 103–124.
11. Raynaud J. P., Ojasoo T., Delarue J. C., Magdelenat H., Martin P. and Philibert D.: Estrogen and progestin receptors in normal and neoplastic tissues (Edited by W. L. McGuire, J. P. Raynaud and E. E. Baulieu) Raven Press, New York (1977) pp. 171–191.
12. Martin P. M., Rolland P. H., Jacquemier J., Rolland A. M., and Toga M.: Multiple steroid receptors in human breast cancer, I. Technological features. *Biomedicines* (1978) **28** 278–287.
13. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.
14. Raynaud J. P.: R 5020, a tag for the progestin receptor. In: Progesterone receptors in normal and neoplastic tissues (Edited by W. L. McGuire, J. P. Raynaud and E. E. Baulieu) Raven Press, New York (1977) pp. 9–21.
15. Bayard F., Damilano S., Robel P. and Baulieu E. E.: Cytoplasmic and nuclear estradiol and progesterone receptors in human endometrium. *J. clin. Endocr. Metab.* **46** (1978) 635–648.
16. Saez S., Martin P. M. and Chouvet C. D.: Estradiol and progesterone receptor levels in human breast adenocarcinoma. In relation to plasma estrogen and progesterone levels. *Cancer Res.* **38** (1978) 3468–3473.
17. Walters M. R. and Clark J. H.: Nuclear and cytoplasmic progesterone receptors in the rat uterus: effect of R 5020 and progesterone on receptor binding and subcellular compartmentalization. In *Progesterone Receptors in Normal and Neoplastic Tissues* (Edited by W. L. McGuire, J. P. Raynaud and E. E. Baulieu) Raven Press, New York (1977) pp. 271–285.
18. Flickinger G. L., Elsner C., Illingworth D. V., Muechler E. K. and Mikhail G.: Estrogen and progesterone receptors in the female genital tract of humans and monkeys. *N.Y. Acad. Sci.* **286** (1977) 180–189.