PROGESTERONE RECEPTOR IN THE RAT ANTERIOR PITUITARY

Transformation and nuclear translocation

J. SPONA, Ch. BIEGLMAYER and R. PIRKER

Endocrine Research Unit, First Department of Obstetrics and Gynecology, University of Vienna, A 1090 Vienna, Austria

Received 12 October 1978
Revised version received 2 November 1978

1. Introduction

Cytoplasmic estrogen [1–3], glucocorticoid [3,4] and androgen receptors [3,5,6] have been detected in the rat pituitary. But, evidence for specific progesterone receptor has been conflicting [3,7]. Recently, progestin binding sites were detected in the rat anterior pituitary [8,9]. Previously, it was shown that progestins modulate according to their biological potency LH-RH-stimulated gonadotropin release in vivo [10]. In addition, progesterone was observed to influence LH-RH-stimulated LH and FSH release in vitro [11,12] and suppression of gonadotropin release is a well-acknowledged mechanism of action of hormonal contraceptives. These observations combine to suggest that progesterone acts at least partially at the pituitary level in regulating gonadotropin release.

Whether or not specific progesterone receptors are present in the hypophysis is essential for the understanding of the mechanism of action of progesterone on the brain. Since estrogen was reported to induce cytoplasmic progesterone receptors in the animal uterus [13,14] it is likely that a progesterone receptor is detectable in neural tissues containing estrogen receptors. Ontogeny of the rat anterior pituitary estrogen-binding protein was described [15] and nuclear translocation of the estrogen receptor was reported [16].

The aim of the present investigation was to study the association of a progestin with the cytosol receptor in the rat anterior hypophysis and the transformation of the receptor complex in vitro. In addition, experiments were designed to investigate the nuclear translocation of the cytosol receptor complex in vitro. We were prompted to report these data since they add further evidence that receptor translocation is a primary step in the molecular events of progesterone action.

2. Materials and methods

Female rats of the Sprague Dawley strain (Him: OFA (SD) SPF, Research Institute of Animal Breeding, University of Vienna) were used throughout this study. The animals were 60–100 days old and were bilaterally ovariectomized at least 7 days prior to each experiment unless otherwise stated. The animals were housed in a temperature-controlled room illuminated 12 h/day and unrestricted access was provided to food and water. Prior to each experiment, rats were injected subcutaneously each day with 7.5 μg estradiol-17β-benzoate dissolved in a mixture of castor oil and benzyl benzoate (95:5) for various lengths of time, and were killed 24 h after the last injection. Animals were anesthetized by ether and killed by decapitation. Pituitaries were placed in chilled TEGD buffer (10 mM Tris, 1.5 mM EDTA, 6 mM dithiothreitol, 10% glycerol, pH 7.4), the posterior lobes were removed and discarded. All further procedures were done at 0–4°C. Anterior pituitaries 12/ml TEGD buffer were homogenized by 10 strokes at 500 rev./min in a glass–Teflon Potter Elvehjem type homogenizer. The homogenate was centrifuged at 800 × g for 10 min. The pellet which contained the nuclear fraction was washed 3 times by resuspension in 3 ml

TEGD buffer and recentrifugation. Nuclei were resuspended in TEGD buffer. The yield of crude nuclei was 75% as judged by DNA [17]: protein ratio [18]. The purity of the preparation was checked by phase-contrast, fluorescence and electron microscopy, which revealed intact nuclei with only little contamination. The supernatant of the 800 × *g* centrifugation of the total homogenate was spun in a Beckmann Spinco LS2-65B preparative ultracentrifuge at 110 000 × *g* (av.) for 60 min in order to obtain the cytosol fraction.

All experiments described here were carried out with [^{3}H]R5020(17α, 21-di[6, 7-^{3}H]methyl-19-norpregna-4,9-diene-3,20-dione), obtained from New England Nuclear, Boston, at spec. act. 86 Ci/mmol. All incubations were performed in the presence and absence of unlabeled R5020 to account for unspecific binding. Results are given as specific binding, which is calculated by subtracting radioactivity data obtained from experiments in the presence of unlabeled progestin from radioactivity data recorded in the absence of unlabeled progestin.

2.1. *The time course of in vitro association of [^{3}H]-R5020 (6×10^{-9} M) with the pituitary progesterone receptor*

This was studied by incubation of 0.2 ml cytosol samples for various lengths of time. Steroid not bound to binding protein was removed by addition of the incubation mixture to a pellet obtained by centrifugation of 0.2 ml dextran-coated charcoal suspension (0.6% charcoal, 0.06% Dextran T-70 in TEGD buffer). After mixing the suspension was incubated for 20 min and centrifuged at 3000 × *g* for 15 min. The supernatant was added to a scintillation cocktail (Instagel, Packard) and the radioactivity was measured in a Packard liquid scintillation counter, model 2450 at ~40% efficiency.

2.2. *Analysis of cytosol receptor complexes*

This was performed on a 5–20% linear sucrose gradient prepared with TEGD buffer in polyallomer tubes and spun in a SW-65 rotor at 145 000 × g_{av} in a Beckmann ultracentrifuge for 18 h. Fractionation of density gradients was done by puncturing the bottom of the centrifuge tubes in a Beckmann Fraction Recovery System. The effluents were collected manually. Sedimentation coefficients were determined by methods in [19].

2.3. *Number of binding sites of material sedimenting in the 6–7 S and 4–5 S regions*

These were determined by Scatchard plot analysis [20] of areas under the corresponding peaks obtained by sucrose gradient analysis of cytosol samples, which were labeled with 6 increasing concentrations (0.9–14 nM) of [^{3}H]R5020.

2.4. *Transformation of the progesterone–receptor complex*

This was performed by incubation of cytosol samples with [^{3}H]R5020 (6×10^{-9} M) in the presence of 0.4 M KCl at 4°C for 2 h. Again, sedimentation patterns of transformation products were analysed by sucrose gradients in the presence and absence of 0.4 M KCl.

The specificity of the binding proteins observed in 4–5 S and 6–7 S region, respectively, was determined by sucrose gradient analysis subsequent to incubation of cytosol samples labeled with [^{3}H]R5020 in the presence of competing steroids.

2.5. *Experiments on the nuclear translocation of the cytosol receptor*

These were performed in vitro. Day 28 female rats were injected daily with 7.5 μg estradiol-17β-benzoate through 10 days and 40 anterior pituitaries of these animals were incubated in 3 ml medium 199 containing Earle's salts (Flow Lab, Bonn) with 1×10^{-8} M [^{3}H]-R5020 in the presence and absence of 1000-fold excess of unlabeled steroid under an atmosphere of 95% air and 5% CO_2. The incubations were carried out at 37°C for 15 min and at 4°C for 60 min, respectively. Crude nuclei were prepared as above, but using TGMD buffer (10 mM Tris, 3 mM $MgCl_2$, 1 mM dithiotreitol, 10% glycerol, pH 7.4) instead of TEGD buffer. The nuclear preparation was treated with 0.5 ml TGMDK buffer (TGMD buffer containing 0.4 M KCl) at 4°C for 1 h. Analysis of sedimentation behaviour of nuclear extracts was done on sucrose gradients according to the above protocol. In addition, cytosol samples prepared with TEGD buffer from pituitaries incubated at 0°C for 1 h and at 37°C for 0.5 and 1 h, respectively, were analyzed on sucrose gradients (section 2.2).

3. Results and discussion

Tritiated R5020 (17α,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione), a synthetic progestin has been found to be very useful for direction of specific progesterone-binding receptors in the presence of contaminating plasma corticosteroid binding globulin, which tightly binds progesterone [21]. In addition, the R5020–receptor complex dissociates at a slower rate than the progesterone–receptor complex [22]. The association of [^{3}H]R5020 with cytosol-binding proteins was found to be a rapid process (fig.1). Saturation was observed within 4 h and all further experiments were done by using this incubation time. It is of interest to note that no degradation of R5020–receptor complex occurred during prolonged incubation at 4°C.

Pituitary progesterone receptor was found to be stimulated by treatment of rats with estrogen. This process was observed to be time dependent (fig.2). Sucrose gradient analysis of [^{3}H]R5020 labeled cytosol obtained from untreated animals revealed a single broad peak in the 4–5 S region. This peak increased with duration of estrogen treatment. Furthermore, a new species sedimenting in the 6–7 S region was noted. Again, this moiety increased with time (fig.2). In addition, an increase in the ratio of 6–7 S/4–5 S peak areas was noted (table 1). Recently, a specific 7 S binding component was found in the cytosol of pituitaries derived from immature estrogen-treated rats, but no binding in the 4 S region was recorded [8]. In contrast, a 4–5 S peak was noted in all present experiments (fig.2), which revealed mostly specific binding in this region as derived from competition experiments with unlabeled steroids (our own unpublished data). This discrepancy between results reported [8] and data recorded in the present experiments may be due to animals used at different ages. Similarly, Scatchard plot analysis of binding data (fig.3) revealed two populations of binding sites,

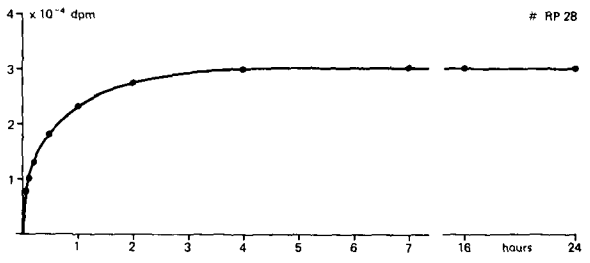

Fig.1. Association of [^{3}H]R5020 with pituitary cytosol binding proteins. Pituitaries were obtained from rats pretreated with a daily dose of 7.5 μg estradiol-17β-benzoate for 3 days. Incubation of cytosol samples was done as in section 2.1.

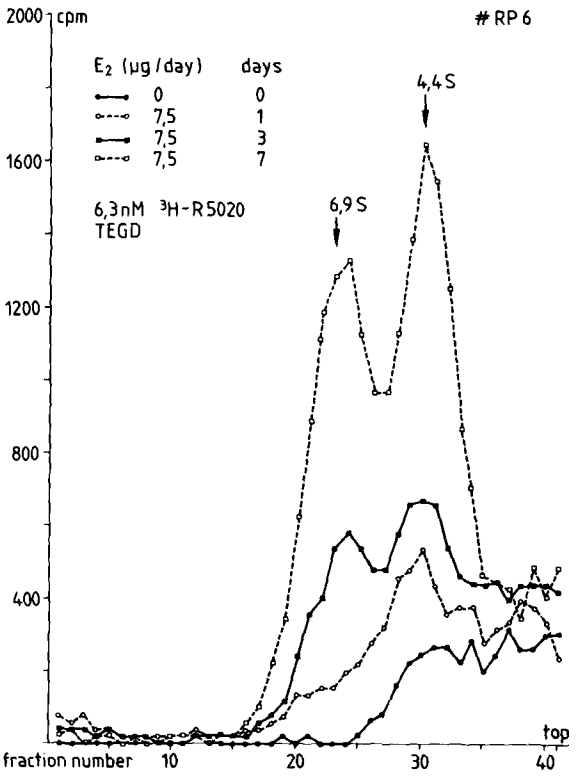

Fig.2. Sucrose gradient analysis of cytosol progesterone-binding protein in rats anterior pituitaries subsequent to estrogen treatment of animals for different periods of time. Experimental details are in section 2.2.

Table 1
Ratios of 6–7 S/4–5 S regions calculated for results of experiments shown in fig.2

Days of estrogen treatment	6–7 S/4–5 S ratio of peak areas
0	–
1	0.41
3	0.90
7	0.87

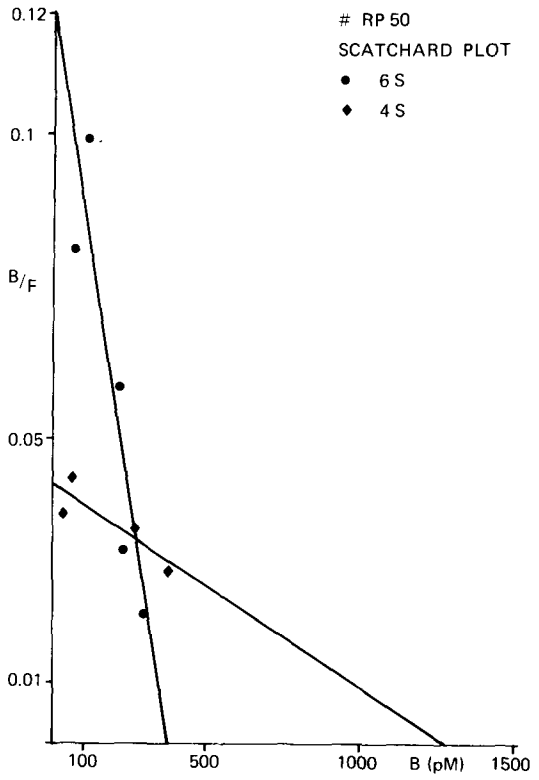

Fig.3. Scatchard plot analysis of binding data obtained by incubation of anterior pituitary cytosol samples with 0.9–14 × 10^{-9} M [^{3}H]R5020 prior to sucrose gradient analysis (section 2.3).

and K_d 3 × 10^{-8} M and 3 × 10^{-9} M was found for the progesterone binding protein in the 4–5 S and 6–7 S region, respectively. Additionally, 198 and 56 fmol progesterone-binding sites/mg cytosol protein, respectively, were calculated from Scatchard plots (fig.3). These data are in agreement with earlier observations on the binding protein sedimenting in the 6–7 S region [8].

Treatment of [^{3}H]R5020-labeled cytosol with 0.4 M KCl and analysis of sedimentation behaviour on a sucrose gradient containing 0.4 M KCl resulted in a radioactive peak in the 4–5 S region (fig.4). On the other hand, a moiety sedimenting in the 6–7 S region was observed, when the labeled cytosol treated with 0.4 M KCl was analyzed on a sucrose gradient prepared without 0.4 M KCl. These data suggest a reversible transformation of the cytosol progesterone-binding protein. Such a reversible transformation process of an 8 S species into an 4–5 S moiety was reported for the cytosol estrogen receptor of the uterus [23] and the anterior pituitary [24]. Transformation of cytosol receptors is an acknowledged process prior to nuclear translocation [25], but no definite mechanism of the transformation of the cytosol progesterone receptor may be derived from the present experiment.

In vivo translocation of the progesterone–receptor was reported [9]. Maximal nuclear uptake was found 30 min after injection of R5020 in estrogen-primed rats. Analysis by sucrose gradients of nuclear extracts obtained subsequent to incubation of whole pituitaries in vitro revealed a nuclear receptor sedimenting in the 3 S region (fig.5). Similarly, translocation of the cytosol receptor was achieved by incubation of [^{3}H]-R5020-labeled cytosol with crude nuclei (data not

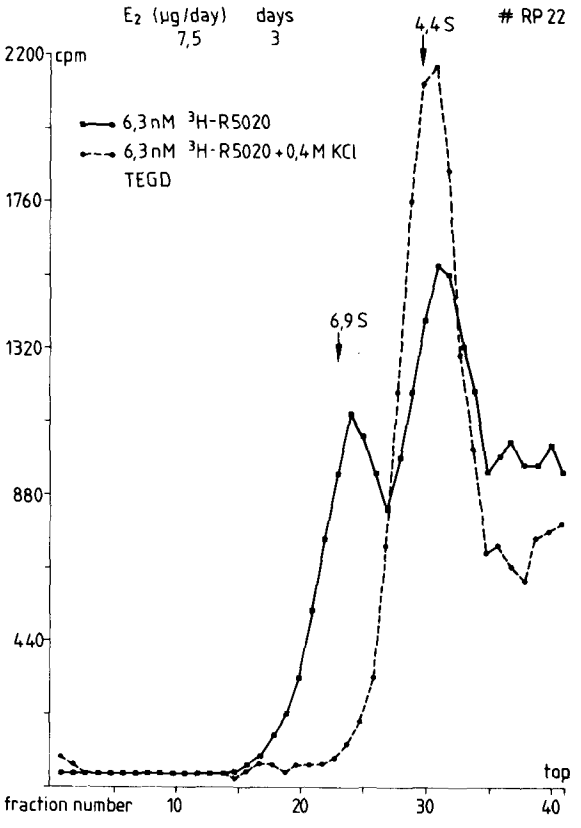

Fig.4. Transformation by 0.4 M KCl of the 6–7 S progesterone receptor into a 4–5 S species (section 2.4).

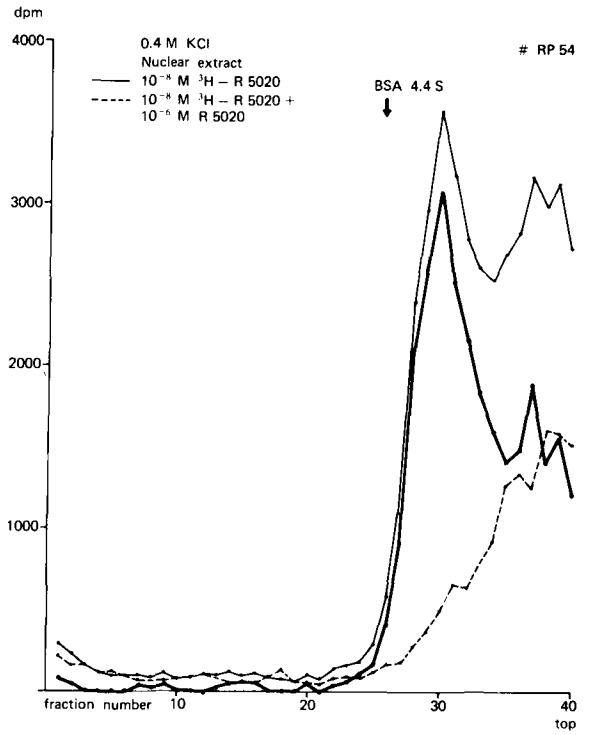

Fig.5. Nuclear translocation of the progesterone receptor in vitro as done by the methods in section 2.4. Nuclear extracts (TGMDK buffer) were analyzed on sucrose gradients (—— total bindings, – – – – – unspecific binding, ▬▬ specific binding).

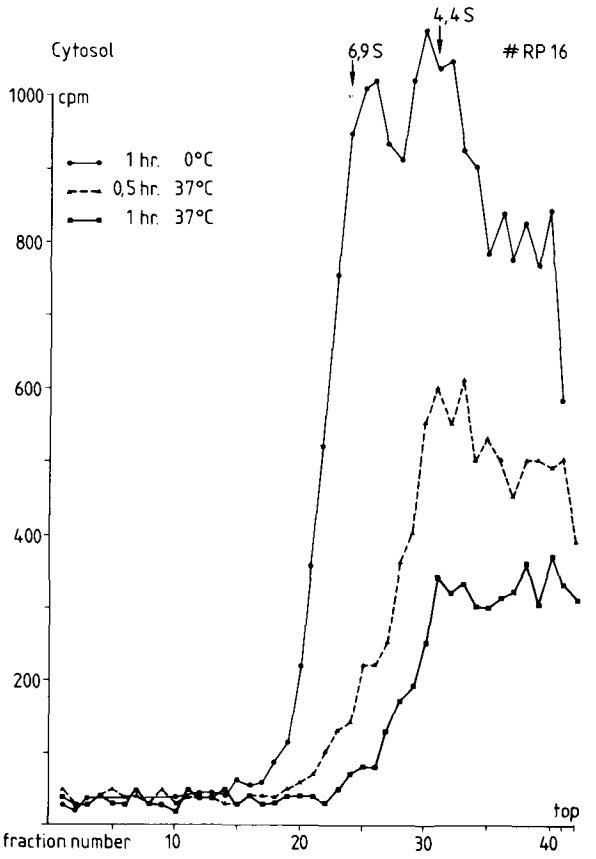

Fig.6. Analysis of cytosol samples on sucrose gradients (section 2.2.) obtained from pituitaries incubated in vitro (section 2.5) at 0°C and 37°C for various lengths of time.

shown). In addition, translocation was found to be a temperature-dependent process, since no nuclear receptor complex could be extracted when the in vitro experiment was performed at 4°C. Furthermore, analysis on sucrose gradients of cytosol samples obtained from pituitaries incubated in vitro at 37°C for various lengths of time shows a rapid decrease of cytosol progesterone receptor sedimenting in the 6–7 S region and of 4–5 S material (fig.6). These data and those shown in fig.5 combine to suggest nuclear translocation of the pituitary cytosol receptor of progesterone. The present results are similar to those for the pituitary cytosol estrogen receptor [16].

Since estrogen priming of animals is a prerequisite for the stimulation of the progestin receptor its presence can be closely related to the estrogen receptor. In addition, a concomitant increase of DNA-dependent RNA polymerase was recorded (our unpublished data) during the rise of progesterone receptor levels depicted in fig.2.

Data of the present experiment combine to suggest that translocation of the progesterone–receptor complex from the cytosol to the nucleus may be one of the primary steps of progesterone action at the pituitary level.

Acknowledgements

Secretarial work provided by Mrs E. Friedel and Mrs. E. Maly is greatly appreciated. Research Carreer Award of the City of Vienna given to one of us (J.S.) is gratefully acknowledged. This work is part of the International Atomic Energy Agency, research agreement no. 1357/CF.

References

[1] Kato, J. (1975) J. Steroid Biochem. 6, 979–987.
[2] Raynaud, J. P. and Moguilewsky, M. (1977) in: Progress in Reproductive Biology (Hubinont, P. O. et al. eds) vol. 2, pp. 78–87, Karger, Basel.
[3] Kato, J. (1977) in: Receptors and Mechanism of Action of Steroid Hormones (Pasqualini, J. R. ed) part 2, pp. 603–671, Marcel Dekker, Basel.
[4] Olpe, H. R. and McEwen, B. S. (1976) Brain Res. 105, 121–128.
[5] Jouan, P., Samperez, S., Thieulant, M. L. and Mercier, L. (1971) J. Steroid Biochem. 2, 223–236.
[6] Gustafsson, J. A., Pousette, A. and Svensson, E. (1976) J. Biol. Chem. 251, 4047–4054.
[7] Seiki, K. and Hattori, M. (1973) Endocrinol. Japon. 20, 111–119.
[8] Kato, J. and Onouchi, T. (1977) Endocrinology 101, 920–928.
[9] Moguilewsky, M. and Raynaud, J.-P. (1977) Steroids 30, 99–110.
[10] Spona, J., Bieglmayer, Ch., Schroeder, R. and Pöckl, E. (1978) in: Radioimmunoassay and Related Procedures in Medicine 1977, vol. 2, pp. 245–256, International Atomic Energy Agency, Vienna.
[11] Spona, J. (1974) Endocrinol. Exp. 8, 19–29.
[12] Schally, A. V., Redding, T. W. and Arimura, A. (1973) Endocrinology 93, 893–904.
[13] Freifeld, M. L., Feil, P. D. and Bardin, C. W. (1974) Steroids 23, 93–103.
[14] Philibert, D. and Raynaud, J. P. (1973) Steroids 22, 89–98.
[15] Spona, J., Bieglmayer, Ch., Adamiker, D. and Jettmar, W. (1977) FEBS Lett. 76, 306–310.
[16] Bieglmayer, Ch., Pöckl, E., Spona, J., Adamiker, D. and Jettmar, W. (1977) FEBS Lett. 81, 342–346.
[17] Burton, K. (1956) Biochem. J. 62, 315–323.
[18] Lowry, O. H., Rosebrough, N. J., Farr, A. F. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[19] Martin, R. G. and Ames, B. N. (1961) J. Biol. Chem. 236, 1372–1379.
[20] Scatchard, G. (1949) Ann. NY Acad. Sci. 51, 660–671.
[21] Westphal, U. (1971) in: Steroid–Protein Interactions. Monographs in Endocrinology, vol. 4, p. 232, Springer-Verlag, Berlin.
[22] Feil, P. D., Glasser, S. R., Toft, D. O. and O'Malley, B. W. (1972) Endocrinology 91, 738–746.
[23] Alberga, A., Jung, I., Massol, N., Raynaud, J. P., Raynaud-Jammet, C., Rochefort, H., Truong, H. and Baulieu, E.-E. (1970) Adv. Biosci. 7, 45–74.
[24] Spona, J., Bieglmayer, Ch. and Pöckl, E. (1979) submitted.
[25] Jensen, E. V. and DeSombre, E. R. (1977) in: Receptors and Mechanism of Action of Steroid Hormones (Pasqualini, J. R. ed) part 2, pp. 569–602, Marcel Dekker, Basel.