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PHARMACOLOGY OF STEROID RECEPTORS: FROM MOLECULAR AND CELLULAR STUDIES TO EFFICIENT ANTIHORMONAL DRUGS

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Abstract—This paper reviews the current state of steroid receptor research, mostly on the basis of the work accomplished in the author's laboratory. New results on the structure of these receptors, on their functioning and on their immunology are reported. Several aspects of antioestrogen and antiprogesterone activities and mechanism of action are also reviewed, in the view of their clinical use.

There are five distinct classes of steroid hormones: oestrogen, androgen, progestagen, glucocorticosteroid and mineralocorticosteroid. The corresponding physiological hormones are oestradiol, testosterone, progesterone, corticosterone (or cortisol) and aldosterone, respectively. Each hormone, according to the type of target cells, controls different activities which may be classified in very diverse biological categories, for instance cell growth, cell differentiation, specific protein synthesis, etc. However for each steroid hormone, whatever the type of effect, there is apparently a single and specific receptor, proper to the hormone and not to the response. The five receptors (ER, oestrogen receptor; PR, progesterone receptor; AR, androgen receptor; GR, glucocorticosteroid receptor; MR, mineralocorticosteroid receptor) are all intracellular and these soluble proteins are extractable without the use of detergent.

Autoradiography studies with radioactive steroid demonstrate accumulation of hormone quasi exclusively in the nucleus, presumably bound to its receptor (Fig. 1), as expected for the initiation of the hormonal response at the gene transcription level.

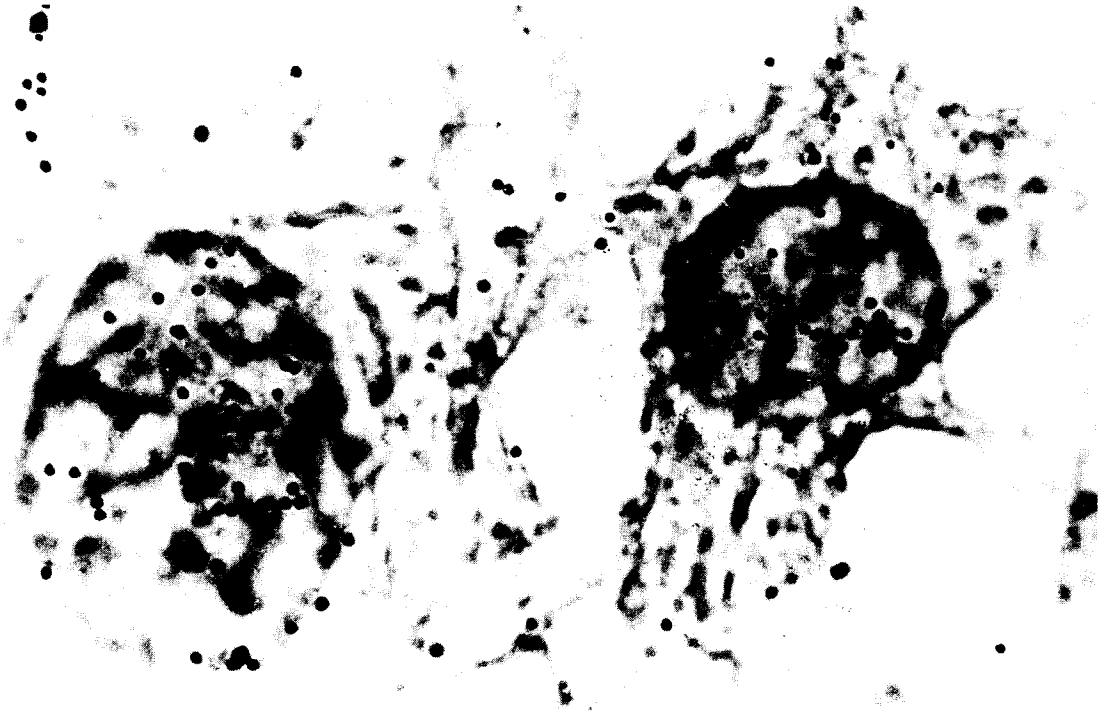

Fig. 1. Three hour incubation of Shionogi Carcinoma 115 cells at 37° with ^{3}H-dihydrotestosterone 0.5 nM followed by a 30 min chase with 200-fold non radioactive hormone. Reconstitution of several pictures at different focusing levels. The nucleus is much more labelled than the cytoplasm. S. Weiller, C. Le Goascogne and E. E. Baulieu, *Expl. Cell. Res.* **102**, 43 (1976).

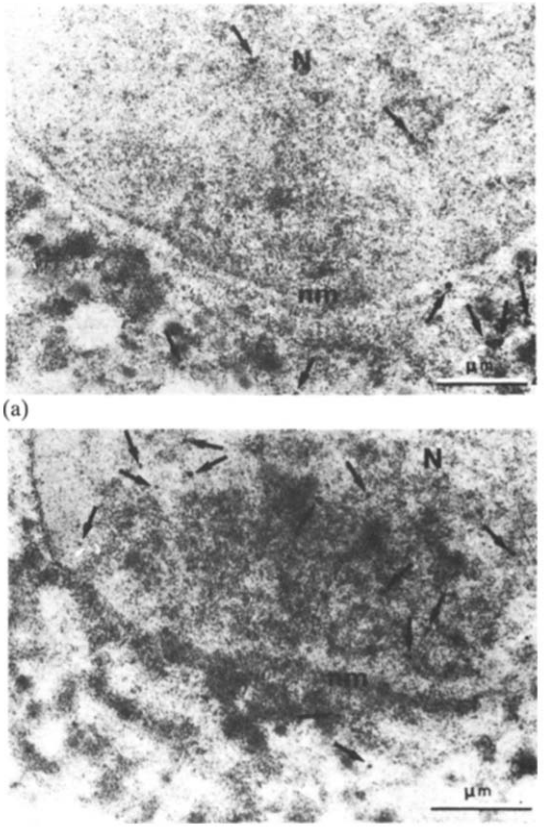

Fig. 2. Immunochemical reaction for ER in gonadotropic cells before and 2 hr after oestradiol injection in 21-day-old female rats. (a) In the untreated rat, the immunochemical reaction was observed mostly in the cytoplasm, even though there were some grains in the nucleus. (b) In the treated rat, the immunochemical reaction was again observed both in the cytoplasm and in the nucleus, but preponderantly in the nucleus. N, nucleus; nm, nuclear membrane. G. Morel, P. Dubois, C. Benassayag, E. Nunez, C. Radanyi, G. Redeuilh, H. Richard-Foy and E. E. Baulieu, *Expl Cell. Res.* **132**, 249 (1981).

In the absence of hormone, one does find more or less receptor already in the nucleus, varying with the target tissues, and we have visualized it at the electron microscopy level using anti-receptor antibodies (Fig. 2). However, there is more nuclear receptor in the presence of hormone, nuclear accumulation being favored by the steroid. That the receptor is initially synthesized in the cytoplasm, as are all proteins, is certain. That (active) hormone-receptor complexes accumulate in the nucleus is also certain. Whether or not there is a preferential distribution of the receptor within the cytoplasm in the absence of hormone seems of little importance, since hormone-receptor complexes may not act at all in the cytoplasm. Operationally, receptor in the absence of hormone is extractable by low salt containing buffer and appears in the "cytosol", high speed supernatant of target cell homogenates, while hormone-receptor complexes have a higher affinity for the nuclear fraction and high salt solution is necessary for their extraction.

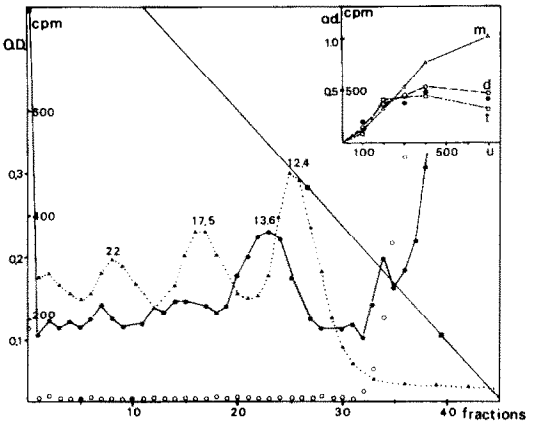

Fig. 3. Chromatin of laying hen oviduct nuclei after digestion with micrococcal nuclease and incubation with ^{3}H-oestradiol 2 nM. Centrifugation on sucrose gradient. Numbers at the top of the peaks indicate S values. ▲– – –▲ O.D.$_{260\,nm}$; O——O cpm in the absence of non radioactive oestradiol; O– – –O cpm in the presence of oestradiol 1 μM; markers: catalase (11.6 S) and bovine serum albumin (4.2 S). Inset: equal aliquots of oviduct nuclei were digested with varying concentrations nuclease and ultracentrifuged. Open symbols: O.D.$_{260\,nm}$ of monomer (m), dimer (d) and trimer (t). Closed circles: cpm in 13–14 S peak. N. Massol, M.C. Lebeau and E. E. Baulieu, *Nucl. Acids Res.* **5**, 723 (1978).

The mechanism by which hormone-receptor complexes increase specific transcription is unknown. In addition to reconstitutive experiments looking for "specific" interactions between gene fragments and purified receptors, we have developed a research strategy searching for the "acceptor" to which hormone-receptor complexes are "naturally" attached in the nucleus; we try to isolate hormone receptor-acceptor complexes and to analyse their constituents. Early results are shown in Fig. 3.

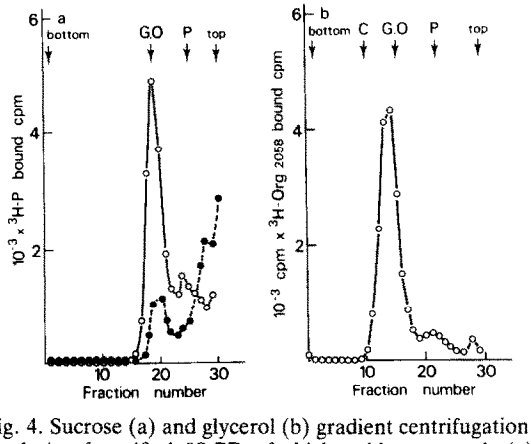

Fig. 4. Sucrose (a) and glycerol (b) gradient centrifugation analysis of purified 8S-PR of chick oviduct cytosol. (a) O——O, labelled with ^{3}H-progesterone. ●——●, i.d. after an additional incubation with 5 μM of non-radioactive progesterone for 30 min at 25°. (b) Labelled with ^{3}H-ORG 2058 (high affinity progestin). J. M. Renoir, C. R. Yang, P. Formstecher, P. Lustenberger, A. Wolfson, G. Redeuilh, J. Mester, H. Richard-Foy and E. E. Baulieu, *Eur. J. Biochem.* **127**, 71 (1982).

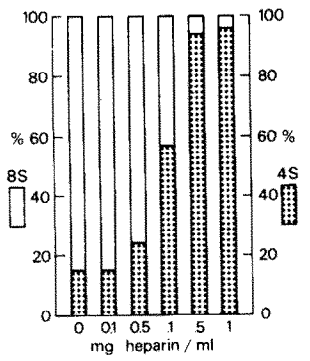

Fig. 5. Receptor transformation by heparin: concentration dependence. Cytosol labelled with ^{3}H-progesterone was incubated with various concentrations of heparin for 2 hr at 0°. Molybdate was then added to a final concentration of 20 mM, and the incubation mixtures were analyzed by sucrose-density-gradient centrifugation. The radioactivity found in the 8S (open columns) and in the 4S peaks (stippled columns) are expressed as percentages of the sum of the two peaks. C. R. Yang, J. Mester, A. Wolfson, J. M. Renoir and E. E. Baulieu, *Biochem. J.* **208**, 399 (1982).

Many experiments substantiate the notion of a 2-state system for receptors, that is "non transformed–non activated" on one hand, and "transformed–activated" on the other hand, the latter being induced by hormone binding in the physiological environment of the cell. Untransformed receptor is observed in the absence of hormone; it has a relatively low affinity for the nucleus and therefore is the "cytosol receptor". This does not mean that originally all or part of the receptor is not in the nucleus. In low salt buffer, all cytosol steroid receptors have a sedimentation coefficient ~8S, which can be stabilized by molybdate ions (Fig. 4). This stabilization includes a well defined "8S" sedimentation coefficient, resistance to the "activation" effect of heating and of increased concentration of salt, and it indicates also that the hormone binding property is more stable under denaturating conditions which would otherwise alter it (heating, proteolysis). The activated state of the receptor includes higher affinity for cell nuclei and many polyanions (f.i. DNA) and decrease of the rate of dissociation of the hormone from the receptor. It is obtained by warming the hormone-receptor complexes, for instance 25° for 1 hr in low salt

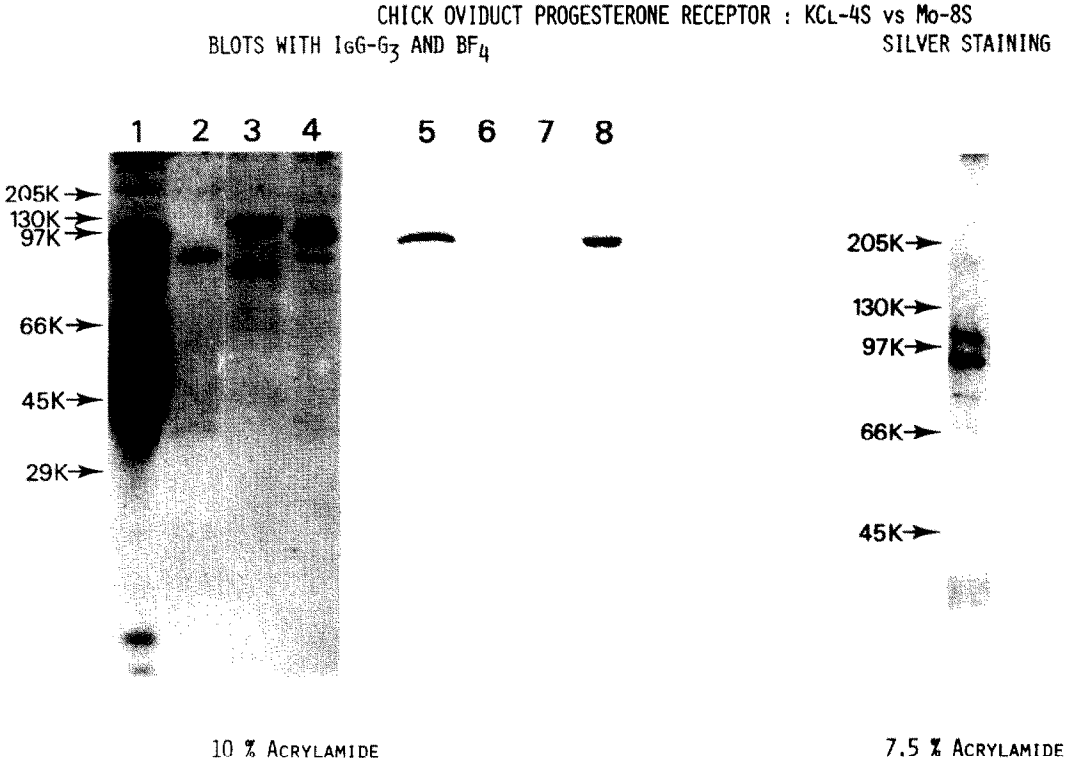

Fig. 6. Immunoblotting of chick oviduct PR. The 8S-untransformed, purified receptor prepared with molybdate (Mo-8S) is seen in lanes 4 and 8 of 10% acrylamide SDS-PAGE. It is revealed by either polyclonal anti-8S receptor antibodies IgG-G3, (J. M. Renoir, C. Radanyi, C. R. Yang and E. E. Baulieu, *Eur. J. Biochem.* **127**, 81 (1982)) or anti 90K monoclonal antibody BF4, (C. Radanyi, I. Joab, J. M. Renoir, H. Richard-Foy and E. E. Baulieu, *Proc. natn. Acad. Sci. U.S.A.* **80**, 2854 (1983)). It is also seen silver stained on 7.5% SDS-PAGE. Purified 75K-A and 110K-B subunits obtained from KCl-4S preparation of the cytosol are seen on lanes 2 and 3 revealed by IgG-G3; they are also visualized by silver staining, but not by BF4. I. Joab, J. M. Renoir, J. Mester, N. Binart, C. Radanyi, T. Buchou, R. Zoorob and M. G. Catelli. 65th Annual Meeting of the Endocrine Society, San Antonio, Texas, 8–10 June, Abstract No. 557, p. 225 (1983).

medium, or exposing them to high salt concentration, for instance for 3–4 hr, 0° in 0.3 M KCl, or by other means such as heparin (Fig. 5). In fact the binding of the hormone is not mandatory under artificial cell free conditions [5] and activation appears as an intrinsic capacity of the protein. In the case of the chick oviduct cytosol PR, we found that the non-activated 8S receptor dissociates into 4S fragments upon activation, while a slower dissociation rate of progesterone and an increased affinity for phosphocellulose columns are observed.

In order to learn more about the functioning of hormone receptor complexes in comparing the so-called activation and non activated forms of the receptor, we purified the molybdate-stabilized 8S-progesterone-receptor (8S-PR) from the chick oviduct cytosol and analyzed its constituents. The latter were also purified and obtained either from purified 8S-PR which was subsequently dissociated, or directly from cytosol in which activation had been originally provoked previously (f.i. by salt treatment), releasing the progesterone binding components.

The 8S-PR has been purified in its molybdate-stabilized form by affinity chromatography, followed by ionic exchange chromatography and gel filtration. The purified receptor has the same binding specificity as the receptor detected in the crude cytosol. SDS-PAGE analysis shows an intense 90K peptide (detected by Coomassie blue) and 2 other proteins, of mol. wt ~110 and 79 K daltons (which are better seen by silver nitrate staining). These last two proteins have been already described by Schrader, Sherman, O'Malley and their associates under the name of B and A subunits. Purification of activated PR from salt containing cytosol allowed us to obtain the 110K-B and the 79K-A proteins which, after affinity chromatography, were separated by DEAE-cellulose chromatography. We were able to raise polyclonal antibodies ("IgG-G3") against the purified 8S receptor and against the B subunit (IgG-RB), and we obtained a BF4 monoclonal antibody (after injection of 8S receptor). Immunoblotting indicated that the BF4 antibody was reacting only with the 90K protein, IgG-RB only with the B and A subunits, while IgG-G3 antibodies recognized the

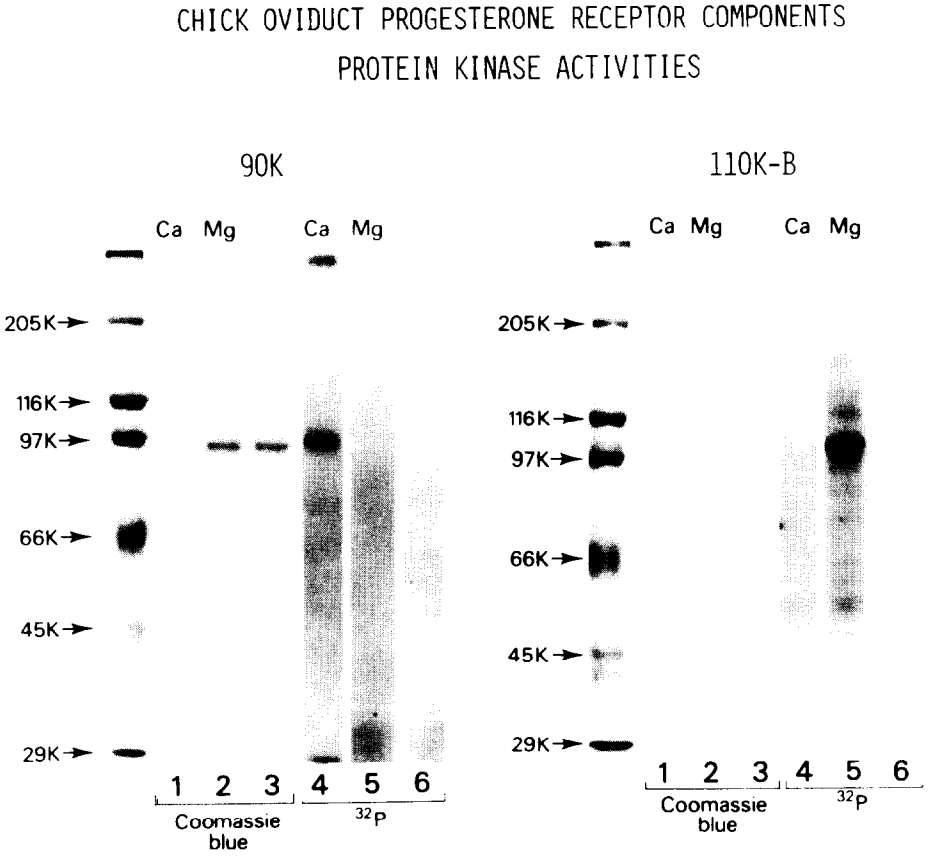

Fig. 7. Phosphorylation of 90 and 110K protein. 90K: Lanes 1–3 show the stained gel of a 4 step (affinity chromatography, DEAE-Sephacel, Ultrogel AcA 34, and removal of molybdate ions by DEAE-Sephacel) purified 90K protein rich 8S-PR after incubation 30 min at 25° with 250 μM [γ-^{32}P]-ATP (300 cpm/pmole) and with 10 mM $CaCl_2$ (lane 1), 10 mM $MgCl_2$ (lane 2) or no divalent cations (lane 3). 110K-B: Lanes 1–3 represent the stained gel of purified B subunit incubated for 30 min at 25° with 30 μM [γ-^{32}P]-ATP (3000 cpm/pmole) and with 10 mM $CaCl_2$ (lane 1), 10 mM $MgCl_2$ (lane 2) or no divalent cation (lane 3). In both cases, the corresponding autoradiographs after a 7 day exposure are shown in lanes 4–6. T. Garcia, P. Tuohimaa, J. Mester, T. Buchou, J. M. Renoir and E. E. Baulieu, *Biochem. biophys. Res. Commun.* **113**, 960 (1983).

3 proteins (Fig. 6). Recently the 90K protein has been purified by immunoadsorption.

It is remarkable (and unexpected) that we found protein-kinase activities in purified receptor fractions (Fig. 7). The 90K associated protein kinase is active in the presence of Ca^{2+}, but not Mg^{2+}, and does not phosphorylate histones. The 110K-B associated protein-kinase is active in the presence of Mg^{2+} (and not Ca^{2+}) and phosphorylates histones well. Much remains to be studied before these enzymatic activities of receptor constituents can be understood in terms of hormone action.

The 90K protein is remarkable also because it seems to be present in the 8S forms of other steroid receptors. Indeed in the chick cytosol, we could observe the interaction of the BF4 monoclonal antibody with ER, AR and GR as well as with PR. The displacement on gradient centrifugation pattern was observed only for the 8S form, while after exposure to KCl the respective steroid binding units did not react with this antibody (Fig. 8).

In conclusion, similarities in distribution, size and functioning of all steroid receptors have been already remarked, and now the 8S form of one of them displays a common non-hormone binding component with protein kinase activity of unknown significance.

PATHOPHYSIOLOGY

Steroid receptors are not at the same cellular concentration in different cells, even within the same organ, and in these different cells the hormonal control of this concentration may differ (Fig. 9). In a given cell, steroid receptors vary in concentration under physiological and pathological circumstances. For instance, variations of PR activity during the oestrus cycle in the guinea pig uterus are rather well explained by the induction of synthesis of PR by oestradiol at the proestrus level, and inactivation ("down regulation") of the binding by progesterone during the luteal phase. This regulation and these changes in concentration can be used clinically in spite of the multitude and complexity of the hormonal controls (Fig. 10). In the human endometrium (Fig. 11), there are changes of ER and PR during the cycle, and in early pregnancy, and all are "logically" explainable by the effects of oestradiol and progesterone themselves. On the contrary, in post-menopausal women with endometrial cancer, the disease apparently provides an endometrium at risk from oestrogen, with too much ER and not enough PR (Fig. 12).

During the cycle and early pregnancy, there is enough nuclear PR to be a target for antiprogesterone action, as observed with RU 486-antiprogesterone (Fig. 13). As exemplified in our studies in the chick oviduct using antioestrogens (Fig. 14), it seems that antihormone has only to "travel" by itself to the nucleus in order to exchange with the endogenous hormone, as a function of respective concentrations and affinities. The theory that antihormone should act via binding to a cytoplasmic receptor is not tenable. The antioestrogen 4-hydroxytamoxifen has a high affinity for the ER and the antiprogesterone RU 486 has a high affinity for the PR. There was a "theory" suggesting that antisteroid hormone should

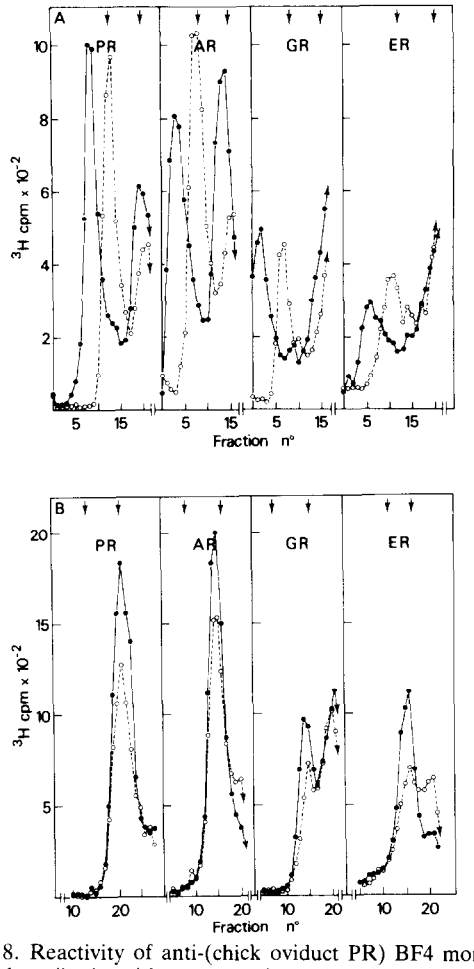

Fig. 8. Reactivity of anti-(chick oviduct PR) BF4 monoclonal antibody with non-transformed and transformed forms of progesterone (PR), androgen (AR), glucocorticosteroid (GR) and oestrogen (ER) receptors from chick oviduct. Cytosol containing molybdate-stabilized, non-transformed form of receptors were prepared with ^{3}H-P, ^{3}H-DHT, ^{3}H-dexamethasone or ^{3}H-oestradiol. ^{3}H-P (0.2 pmole), ^{3}H-DHT (0.3 pmole) and ^{3}H-dexamethasone (0.3 pmole) labelled receptors were incubated with partially purified BF4 monoclonal antibody from ascitic fluid or control myeloma cell culture medium (300 μl) for 5 hr at 4° in a final volume of 500 μl. Non-transformed and transformed forms of ^{3}H-ER (0.07 pmole) were incubated with BF4 or control medium (150 μl) in a final volume of 250 μl. Two hundred microliters were layered on top of 10–35% glycerol (PR) or 5–20% sucrose (other receptors) gradients in homogenization buffer and ultracentrifugation. Sedimentation profiles of ^{3}H-steroid labelled receptor in the presence of BF4 (●——●) or control medium (○– – –○) in low salt medium containing molybdate (part A) or high salt medium containing 0.5 M KCl (part B). Another series of controls (not shown) was carried out with total non-immune rat IgGs in control culture medium and with unrelated monoclonal antibody; the data were similar to those seen when using myeloma cell culture medium. Bound tritiated ligand sedimenting in the 4S region of gradients (part A) can be explained either by non-specific interaction (partially decreased when cytosol was incubated with ^{3}H-ligand in the presence of unlabelled hormone) or partial transformation of the receptors upon incubation with antibodies or during the centrifugation. I. Joab, C. Radanyi, J. M. Renoir, T. Buchou, M. G. Catelli, N. Binart, J. Mester, and E. E. Baulieu, *Nature*, in press (1984).

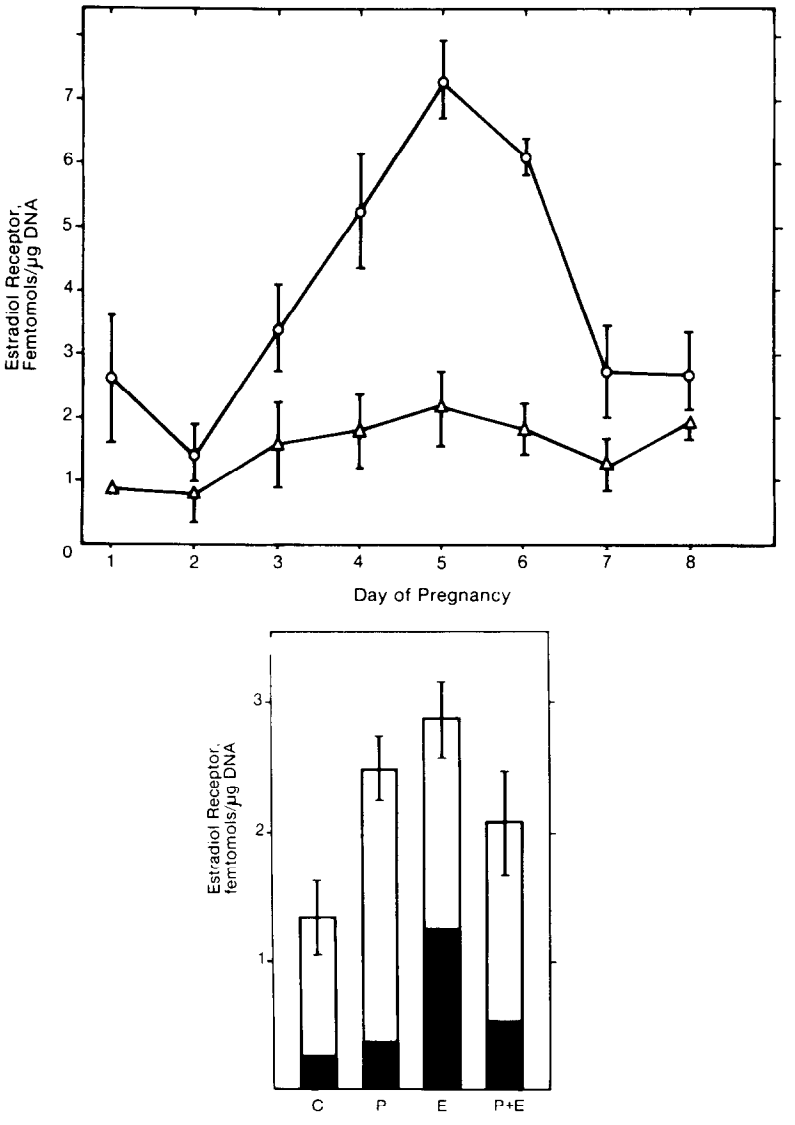

Fig. 9. Changes and hormonal control of ER in rat uterus during early pregnancy. Top, concentration of endometrium (top line) and myometrium (bottom line) ER during early pregnancy. Bottom, ER in castrated rats endometrium (open bars) and myometrium (shadowed) in control (C) animals and after various hormonal regimens. Oestradiol (E) increases receptor in both endometrium and myometrium. Progesterone (P) increases oestradiol in endometrium but not in myometrium. Progesterone abolishes oestradiol induced increase of myometrium receptor. Therefore, simultaneous administration of P + E simulates what is observed during early pregnancy (3–6 days after fertilization) when both hormones are increased in plasma. E. E. Baulieu, *J. Am. med. Ass.* **234**, 404 (1975).

DECREASE OF RECEPTOR SITES/CELL

BY HETEROLOGOUS HORMONE
Progesterone → Oestrogen receptor ↓

BY HOMOLOGOUS HORMONE
Progesterone → Progesterone receptor ↓

INCREASE OF RECEPTOR SITES/CELL

BY HETEROLOGOUS HORMONE
Oestradiol → Progesterone receptor ↑

BY HOMOLOGOUS HORMONE
Oestradiol → Oestradiol receptor ↑

Fig. 10. Changes of PR and ER concentrations under progesterone and oestradiol actions. The data have been obtained in different model systems.

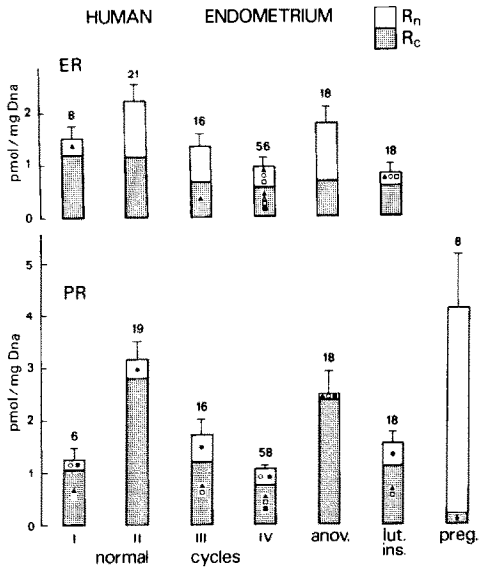

Fig. 11. Cytoplasmic and nuclear oestradiol (upper panel) and progesterone (lower panel) receptors in normal and abnormal menstrual cycles and in early pregnancy. The rectangles represent the sum of cytoplasmic and nuclear receptor sites given ± S.E.M. Shaded areas, cytoplasmic receptors; clear areas, nuclear receptors. Abbreviations: I, early proliferative phase; II, late proliferative phase; III, early secretory phase; IV, late secretory phase; an, anovulatory cycles; li, luteal insufficiency; ep, early pregnancy. The numbers of samples investigated are indicated above each column. Symbols indicate the probability of the considered concentration being significantly lower (at the 5% level) than the corresponding concentration of: I, △; II, ▲; III, ○; □, an; ■, li; ●, ep; *, I, II, III, an, and li. C. Levy, P. Robel, J. P. Gautray, J. De Brux, U. Verma, B. Descomps and E. E. Baulieu, *Am. J. Obstet. Gynecol.* **136**, 646 (1980)

Oestradiol and progesterone receptors in human endometrium of normal and cancerous women (number of sites per cell)

	ER	PR
Normal women, proliferative phase	8800	12400
Normal women, luteal phase	3600	4400
Post-menopausal women, cancer	9000	4000

Fig. 12. The numbers are the mean of results obtained in several studies. Note that in endometrial cancer ER is relatively high and PR relatively low, defining endometrium at oestrogen risk. C. Levy, P. Robel, J. P. Gautray, J. De Brux, U. Verma, B. Descomps and E. E. Baulieu, *Am. J. Obstet. Gynecol.* **136**, 646 (1980). R. Mortel, C. Levy, J. P. Wolff, J. C. Nicolas, P. Robel and E. E. Baulieu, *Cancer Res.* **41**, 1140 (1981).

show a rapid dissociation from the receptor; this does not hold, and pharmacological screening for antihormone should not be only based on affinity to receptor if one wants to detect antihormones. RU 486 combines the feature of a 19-nor testosterone progestin derivative, and an extra ring attached to the 11β-carbon position. This substituent seems responsible for the non-agonist characteristics of the molecule, while the compound keeps a high affinity for the receptor and therefore is a good antagonist. It is remarkable that this antiprogesterone (and other antisteroid hormones synthetized by Roussel-Uclaf) shares, with antioestrogens of the triphenylethylene series (such as tamoxifen), an additional cyclic structure which is just out of the portion of the steroid moiety in front of the C1-C9-C10-C11 regions. This may indicate analogies between neighbouring portions of the steroid binding site of the receptors, where there would be room for a substitution; at the same time the interaction would convey a conformational change which abolishes hormone action.

The last point in this short review of some present interests in my laboratory again concerns the antihormonal field. It was observed with the antioestrogen tamoxifen that it is a very good "pure", non agonist, antagonist compound in the chick oviduct system (Fig. 15). We have observed that, in presence of progesterone, tamoxifen acquires some oestrogen like properties. The mechanism of such a "potentiation" is unknown, but we like to see it as an invitation to caution against the use of antihormones, even

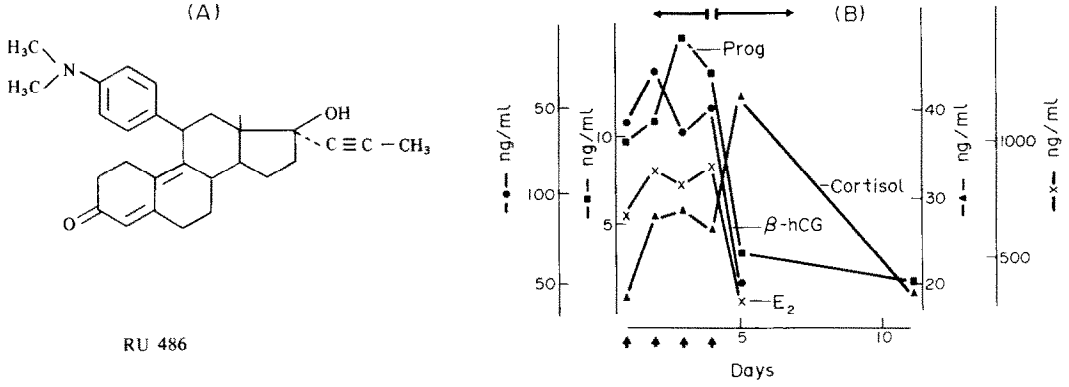

Fig. 13. Seven week pregnancy. Administration of 4 times 200 mg of RU-486 (↑). The horizontal line indicates the length of the bleeding (saignement), and the double vertical signals the abortion itself. Progesterone, ■; E_2 (oestradiol), ×; cortisol, ▲; βhCG, ● in the plasma. W. Herrmann, R. Wyss, A. Riondel, D. Philibert, G. Teutsch, E. Sakiz and E. E. Baulieu, *C.r. Acad. Sci. Paris* **294**, 933 (1982).

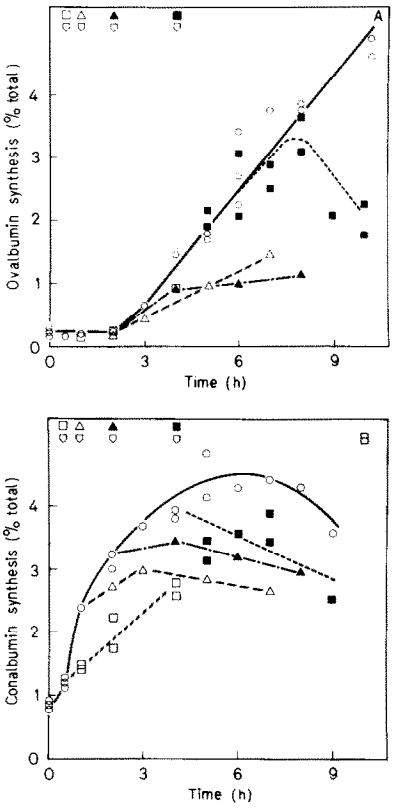

Fig. 14. Effect of delayed administration of tamoxifen on the early induction of ovalbumin and conalbumin synthesis by oestradiol benzoate. All chickens received 1 mg oestradiol benzoate as secondary stimulation. Tamoxifen (10 mg/kg) was injected at times indicated by arrows, and the relative rate of ovalbumin (A) and conalbumin (B) synthesis was measured. Treatment by oestradiol benzoate alone (○), or by tamoxifen injected 1/2 hr (□), 1 hr (△), 2 hr (▲) or 4 hr (■) after the oestradiol benzoate. M. G. Catelli, N. Binart, F. Elkik and E. E. Baulieu, *Eur. J. Biochem.* **107**, 165 (1980).

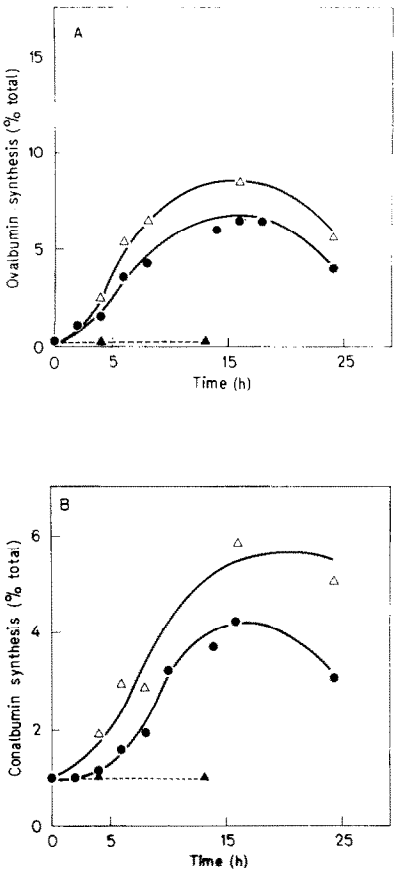

Fig. 15. Comparison of a single injection of progesterone, tamoxifen and progesterone plus tamoxifen. Withdrawn chickens received 3 mg/kg of progesterone (●), 10 mg/kg of tamoxifen (▲), or progesterone and tamoxifen simultaneously (△). At the times indicated, the chicks were killed and the relative rate of ovalbumin and conalbumin measured. N. Binart, J. Mester, E. E. Baulieu and M. G. Catelli, *Endocrinology* **111**, 7 (1982).

those carefully tested in experimental model systems. We make progress when using molecular and cellular approaches, and we certainly like to apply them straight to human pharmacology. But we should remain very prudent, and try to create as many perturbations in our laboratory experiments as possible, in order to predict as much as we can in the best interest of patients.

Note: We have not presented here a systematic review of the field. References to other laboratory researchers is found in the few references cited in figure legends.