Biochemical Pharmacology, Vol. 24, pp. 1743–1748. Pergamon Press, 1975. Printed in Great Britain.

COMMENTARY

STEROID RECEPTORS AND HORMONE RECEPTIVITY: NEW APPROACHES IN PHARMACOLOGY AND THERAPEUTICS

ETIENNE-EMILE BAULIEU

Unité de Recherches sur le Métabolisme Moléculaire et la Physio-Pathologie des Stéroïdes de l'Institut National de la Santé et de la Recherche Médicale, Département de Chimie Biologique, Faculté de Médecine Paris-Sud, 78 rue du Général Leclerc-94270-Bicêtre, France

Hormones are informational molecules. These chemical messengers must find, at the target cell level, specific recognition mechanism(s) for selecting them appropriately from all other components of the 'milieu intérieur'. These recognition mechanisms which involve binding sites (*r* on Fig. 1.) of very high affinity (dissociation constant, K_D, between 0·1 and 10 nM adjusted to the low concentration of hormones in the plasma) and strict (stereo) specificity, constitute the primary function of receptors. It is known that the catalytic sites of enzymes also display specificity, but the affinity is 10^4 times lower than that of the hormone receptor binding sites, since substrates, such as sugars, amino acids or lipids, consumed mainly for energy purposes, are found in higher concentration and transformed into products. In contrast the interaction of hormones with their receptors does not alter their chemical composition *per se*: it is a purely 'physical' phenomenon.

RECEPTORS: DEFINITION AND STUDIES

Specific binding, even of high affinity, does not define a receptor completely. There are other proteins, in particular in the plasma, which bind hormones rather tightly and specifically (for example, in man, transcortin for cortisol and progesterone, and steroid binding plasma protein (SBP) for estradiol and testosterone), and even if the object of these 'transport' proteins is still obscure, they are certainly not receptors. Indeed, the meaning of the word receptor implicates responsibility for the interpretation of the received signal (hormone) in terms of cellular response. The binding of the hormone leads to activation of the 'executive' site (*e*) of the receptor, interacting in turn with 'acceptor' component(s) of the cellular machinery which will be set up to initiate a cascade of effects, known as the overall 'hormonal response'. At the molecular level, there will be transduction between the *r* and the *e* sites, implicating an allosteric transition of the receptor protein. At the organizational (cellular) level, the receptor is the last molecular entity with which the hormone has to interact in order to trigger the response; afterwards, it may and indeed does leave, as indicated by the permanent renewal of hormone in target cells, justifying the continuous hormonal secretion in the intact organism [1].

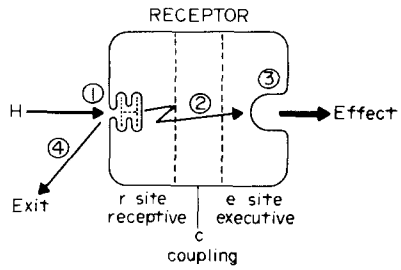

Fig. 1. Phenomenology of the steroid hormone receptor. H stands for any steroid hormone. It binds to the 'receptive' site ① with high affinity, and there is transduction ② coupling the hormone binding to the activation of the 'executive' site ③. The latter may for instance interact with another macromolecule or catalyze some reaction. When this effect is switched on, the hormone has nothing else to do than to leave ④.

Early experiments of Jensen and Jacobson [2] and of Glasscock [3] demonstrated the selective concentration and retention of radioactive estrogens in their target organs. Further studies, in the mid-sixties, by the groups of Jensen [4], Gorski [5, 6], Segal [7] and in our laboratory [8–10], have established the proteinic nature and the intracellular localization of estrogen receptors, the first hormonal receptors to be demonstrated and characterized. Thereafter, similar results have been obtained with all steroid hormones, of both sexual (progesterone and androgens) and adrenal origin [11]. The entry of steroids into cells, and then the intracellular presence in their receptors contrasts with the location of specific binding in plasma membranes found subsequently for most polypeptidic hormone receptors. A part of the 'executive' activity of these membrane receptors is to activate the membrane bound enzyme adenyl cyclase and to increase cAMP, a second messenger for the cell response according to E. Sutherland. cAMP does not play a role in the triggering of the cellular response to steroid hormones.

That the intracellular steroid binding proteins are 'real' receptors is likely, but this has not been formally demonstrated since the acceptor to which the receptor executive site corresponds, has not been defined in molecular terms. Three very strong but circumstantial arguments, and a fourth which is more direct have been produced. (1) There is a satisfactory parallel between the affinities of different steroids, or even of non-steroidal derivatives, of a given series for the receptor, and their biological activities, e.g. weak estrogens have low affinity. Such a correlation does not hold with transport plasma proteins. (2) The presence of the receptors in target organs, while these are undetectable in normal non-target organs, and in

B.P. 24/19—A

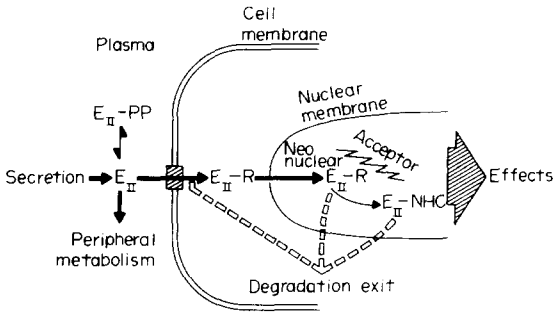

Fig. 2. Natural history of estradiol: a schematic representation. Estradiol (E_{II}), taken as a representative steroid hormone, is secreted and circulates in the plasma, mostly bound to specific plasma protein (PP). It is subjected to degradative peripheral metabolism. It is currently believed that free E_{II} enters the cell, maybe through a membrane specific step indicated on the graph. Binding to the receptor (R) leads to its transconformation (R becomes R') and translocation of the complex to a neonuclear position, with binding to an 'acceptor' still undefined. The possible role of high affinity chromatin protein (NHC) is reported in the figure and discussed elsewhere [1]. At any moment of its cellular life, estradiol may be degraded and/or released from the cell.

genetically non-responsive tissues (androgen target organs in the testicular feminizing syndrome). (3) After entry into the cell, by a process poorly understood [12], the hormone binds to the receptor which is found in the cytoplasm and provokes a change in its properties [4, 6], termed 'acidophilic activation' [13], which makes the hormone receptor complex capable of interacting with a variety of polyanions, notably DNA. This effect is probably related to the secondary location of the hormone–receptor complex in the cell nucleus [14, 4, 6] also designated 'neonuclear' receptor [15]. The final nuclear localization of the steroid hormone agrees well with different biochemical studies of the hormonal response which indicates the fundamental importance of gene transcription; again, molecular details are still unknown. (4) In a series of experiments, initially developed in our laboratory [16, 17], the nuclear gene transcribing machinery of non-stimulated tissue has been exposed to preparations containing receptor steroid complexes. There is a hormone dependent, cytosol dependent increase in RNA synthesis, a result which, whatever the limitations seen from the molecular biology point of view [1, 18], may indicate directly a function for the receptors.

These considerations are basic to the belief that the specific proteins binding selectively the corresponding hormones of a given tissue are receptors, capable of recognition, and operational in message execution. Therefore it is tempting to ask whether their qualitative and quantitative evaluation may be of practical value for a better understanding of hormone 'receptivity', and consequently if their pharmacological manipulation will contribute to better control of cellular functioning in the intact living organism.

The technicalities of these studies involve: (1) The correct delineation of specific binding from non specific binding (albumin and many other proteins bind all steroids indistinctly with an affinity corresponding to $K_D \geqslant 10^{-5}$ M). (2) The measurement of the binding affinity and the determination of the number of sites per tissular unit (for instance mg of protein or DNA). (3) The survey of binding characteristics with different hormonal derivatives, and of molecular properties, such as sensitivity to SH blocking agents, not only in order to assess the proteinic nature of the binding but also to differentiate between receptors and plasma proteins [19]. (4) An evaluation of the available binding sites, i.e. those unoccupied by hormone at the time of the study and labelled directly

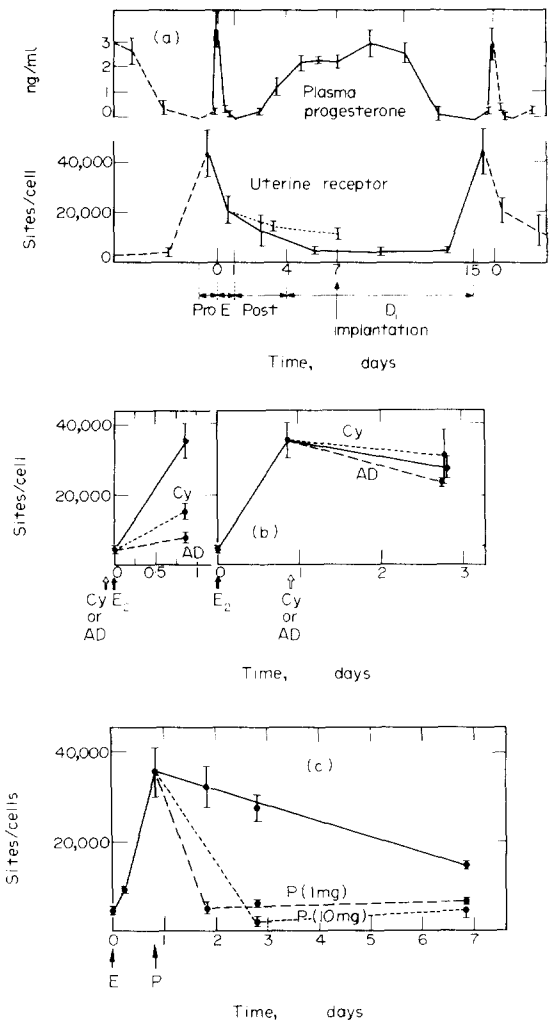

Fig. 3. Changes and hormonal control of progesterone receptor in guinea-pig uterus. (a) Plasma levels of progesterone over the cycle, and concentration per cell of its uterine receptor. Pro, proestrus; E, estrus; Post, postestrus; Di, diestrus. The case of pregnancy is indicated (...). (b) Estrogen induction of progesterone receptor in castrated guinea-pig uterus. Maximum approx. 1 day after injection of estradiol (E_{II}). The negative effects of protein and RNA synthesis inhibitors, Cy and Ad, are shown. The prolonged apparent disappearance of the receptor (half-life 5 days) is shown on the right panel and would not account for the physiological decrease of the receptor observed during the cycle between day 0 and day 4. (c) Progesterone injected when the R is maximum as in (b) accelerates the decay of the receptor and therefore may be implicated in its physiological control.

by radioactive hormone added to the extract, and/or, often better, total binding sites taking into consideration the endogenous hormone occupying part of them and using an 'exchange technique' for this purpose [20]. (5) Eventually, an estimation of the receptor content of the soluble part (cytosol) and of the nuclear (KCl-extractable and 'insoluble) fractions of tissue homogenates.

All these requirements necessitate precise and rather complex analyses, and warn against simplified methods which may be very seriously misleading.

PHYSIOLOGICAL CHANGES OF RECEPTOR CONTENT AND RECEPTIVITY

Recent studies on the guinea-pig demonstrate that the content of progesterone receptor of the uterus varies during the estrus cycle, depending on complex hormonal control [21, 22]. Progesterone levels in the plasma and concentration per uterine cell of the progesterone receptor, whether it is free or occupied by the endogenous hormone, show cyclic variations which are not coincidental. Plasma progesterone is increased at ovulation and is at a prolonged high level during diestrus (luteal phase). On the other hand, a peak of uterine receptor is rapidly developed at proestrus. However the maximum value is not maintained very long and a decrease follows, so that the receptor level is very low during the luteal phase, even though this is also the period when implantation eventually takes place. Incidentally, in pregnancy, the receptor concentration is similar to that found in the absence of fertilization, up to implantation.

These observations suggest that, if the progesterone receptors have an obligatory involvement in egg implantation, progesterone available when its receptors are high, around ovulation, may be physiologically important. There is circumstantial evidence that this might be the case, since Deanesly [23] obtained a number of successful implantations in the guinea pig even after ovariectomy, provided the latter was performed after the 3rd day following ovulation (implantation takes place on the 7th day after ovulation).

The increase of progesterone receptor during proestrus is probably attributable to estrogens, and in castrated animals, estradiol provokes an important augmentation of binding sites. Such an increase of receptors, suppressible by protein and RNA synthesis inhibitors (Fig. 3), may be the molecular mechanism of the classical priming of progesterone action by previously administered estradiol [24]. The apparent decay of the progesterone receptor induced by estradiol in non-cycled (castrated) animals corresponds to a half-life of at least 5 days, and does not explain the rapid decrease during the cycle. However, in this model situation where hormonal manipulations are performed easily, progesterone can be injected when receptor is maximum, and indeed it accelerates the decay of binding sites, only 20% remaining measurable after 1 day [22]. Therefore, during the guinea-pig estrus cycle, it is logical to attribute the rapid decrease of the progesterone receptors after proestrus to the progesterone of the first and possibly of the early part of the second luteal peaks (see Fig. 3). In the diestrus period, progesterone receptor level is even lower than that measured in castrated animals untreated by hormone, a fact still poorly understood that some 'negative' effect of progesterone might explain. Such results suggest that the high level of progesterone receptor at mid-cycle may act as a target for pharmacological inactivation in order to intercept processes leading to blastocyst implantation. None of the related preliminary observations in man* discourage to date the possibility of mid-cycle contraception [25].

Other recent work shows that the uterine estrogen receptor undergoes changes in early pregnancy in the rat [26]. The levels of estradiol and progesterone in the plasma, and the concentrations of the cytosol estradiol receptor measured separately in the myometrium and the endometrium are elevated. There are always more binding sites per DNA unit in the latter, and they increase markedly in the endometrium after the 3rd day, with a maximum at 5–6 days while the change is modest in myometrium. Implantation in the rat takes place on the 5th day and is not possible either before or after a narrow critical period [27]. On the basis of increased estradiol and progesterone concentrations in the blood in early pregnancy, hormones were given to castrated (at day 2 of pregnancy) rats after 3 weeks without treatment. The estrogen induced increase of estradiol receptor in both endometrium and myometrium is not unexpected. However, it is remarkable that progesterone does increase the estrogen receptor in the endometrium (not significantly in the myometrium), while when given with estradiol, it abolishes the receptor increase provoked by the latter in the myometrium. The relative concentrations of receptor in endometrium and myometrium are remarkably similar in both the physiological circumstances and the progesterone plus estrogen model. The antagonistic effect of progesterone on estradiol receptor induction in the myometrium may favour implantation by decreasing the estrogen dependent sensitivity to catecholamines and prostaglandin $F_{2\alpha}$ [28].

In conclusion, physiological and hormone induced changes of receptor concentrations have been demonstrated, and will probably be observed further in a variety of tissues and circumstances (including development [29, 30]. Indeed recent work with the insulin and growth hormone receptors [31] enlarges the concept to non-steroid hormones. Detailed studies of the relationship between receptor concentration and hormone action are now necessary, as well as analyses of qualitative changes, such as modification of hormone specificity and difference in receptor genome interactions, which may also occur and be of great importance.

DIFFERENT RECEPTORS FOR THE SAME HORMONE IN DIFFERENT TARGET CELLS

It is not known whether the receptor for the same hormone is the same in different target tissues. For instance is the estradiol receptor identical in the endometrium and in the cervical mucosa or in normal

* For instance, estrogen and progesterone receptors are found in the human endometrium, and whereas blood progesterone shows only a small increase at mid-cycle, there is a relatively high concentration of the hormone in the uterus (J. Ferin, personal communication).

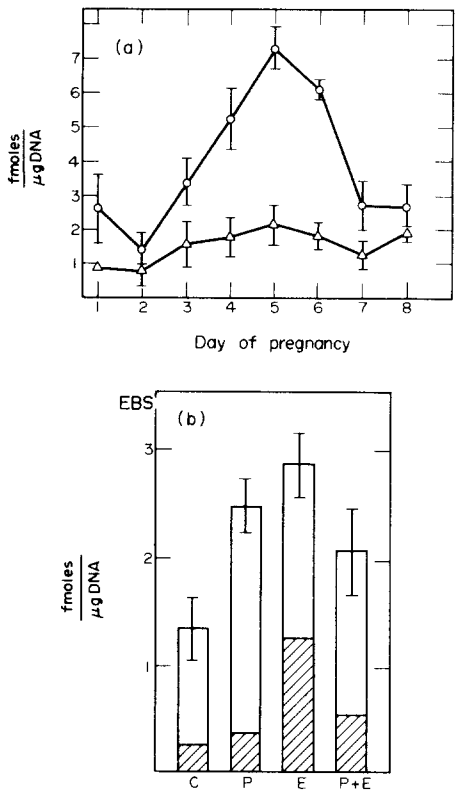

Fig. 4. Changes and hormonal control of estradiol receptor in rat uterus during early pregnancy. (a) Concentration of endometrium (top line) and myometrium (bottom line) estradiol receptor during early pregnancy. (b) Estradiol receptor in castrated rat endometrium (open bars) and myometrium (shadowed) and in control animals and after various hormonal regimes. It can be observed that (1) estradiol (E) increases receptor in both endometrium and myometrium, (2) progesterone (P) increases estradiol in endometrium but not in myometrium, (3) progesterone abolishes the estradiol induced increase of myometrium receptor. Therefore simultaneous administration of P + E gives a picture similar to that observed during early pregnancy (3–6 days) when both hormones are increased in the plasma.

and pathological mammary glands or in the hypothalamus and the anterior pituitary?

However, there is already one example showing that a given hormone (testosterone) circulating in the blood may play its part in different target tissues through different receptors. The case is relatively easy to demonstrate since testosterone is active in target cells either as testosterone itself (in muscles, levator ani or skeletal [32, 33], and in kidney [34], or after transformation into metabolites such as androstanolone (dihydrotestosterone) in ventral prostate and seminal vesicles [35–37], or as estrogen(s) in hypothalamus [38]). These results suggest a difference between tissues engaged in responding to the same blood testosterone hormone, and such a diversity may be of pharmacological interest, since it may be possible to obtain dissociated activities for various androgens, and indeed empirical attempts to separate anabolic properties from virilizing effects of steroids are well known.

DIFFERENT RECEPTORS FOR DIFFERENT HORMONES IN THE SAME CELLS

The very fact that estradiol can induce the progesterone receptor (see above) suggests that uterine cells which synthetize it contain also an estradiol receptor.

Formal evidence for two types of receptors per cell comes from the analysis of cloned cells (MI_1) from a mammary tumour SHI-115 in mice, the growth of which is androgen dependent an effect that estrogens antagonize. There are two different receptors in these (single type hopefully) cells, E (estrogen) and A (androgen) receptors, binding estradiol and testosterone respectively, with very high affinity [39]. The E receptor does not bind testosterone, while the A receptor binds estradiol with relatively high affinity, however lower than that of the E receptor. Therefore, referring to the anti-androgenic effect of estradiol, it is not known whether or not it passes through a competitive binding for the A receptor which would not be activated as with testosterone, or if it implicates the E receptor which would either carry an antagonistic order to a particular site of the genome or would compete with the A receptor for its acceptor site.

DIFFERENT RECEPTORS FOR THE SAME HORMONE IN THE SAME CELL

The fact that estradiol binds to both the E and A receptors is evidence that two different receptors can bind the same steroid depending on the concentration: when the concentration of estradiol is low, the higher affinity E receptor is first occupied, while at a higher concentration the A receptor begins also to be saturated. If the effects promoted by the E receptor–estradiol and A receptor estradiol complexes are opposite, we may observe a dose response curve going up initially with increasing concentrations, until values at which the A receptor estradiol complexes become operative and invert the slope, reproducing a well-known pattern of pharmacological response not well explained until now.

Diethylstilbestrol (DES), a non-steroidal estrogen, binds to the E receptor similarly to the estradiol itself, while its interaction with the A receptor is very weak. DES has been known as a perfect estrogen when its activity has been tested on the uterus; it is also a widely used compound in cancer therapy [40]. Indeed the reported observation suggests that the effects of estradiol and DES can be dissociated while the presence of the E and A receptors binding estradiol can

Table 1. Presence of two steroid hormone receptors in S-115 MI_1 cells

	S-115 Tumor MI_1 cells	
	Receptor A	Receptor E
T	+ + + +	0
E_{II}	+ +	+ + + +
DES	0±	+ + + +

Crosses (+) indicate relative affinity of the two receptors for different ligands. A, androgen receptor; E, estrogen receptor. T, testosterone; E_{II}, estradiol; DES, diethylstilbestrol.

explain a change of slope when estradiol concentration increases, the binding of DES by the E receptor alone leads to the prediction that this change of slope will not be seen with DES.

NEW THERAPEUTICAL APPROACHES: RECEPTORS AND RECEPTIVITY, DISSOCIATION OF EFFECTS, AGONISM AND ANTAGONISM

From the above reported data, it follows that one can reexamine now, on biophysical grounds a series of pharmacological concepts of primary therapeutical importance.

Receptivity to a given hormone may indeed depend on the amount of the corresponding receptor in target cells. The lack of response of secondary sex organs to testosterone in the testicular feminizing syndrome [41], or in lymphoma cell mutants resistant to corticosteroid [42] are interesting examples, since no receptor is found in these tissues. There are good correlations also between the hormonal response of breast cancer and the estradiol receptor content [43] and of lymphoblastic leukaemia and corticosteroid receptors [44], results which are of obvious theoretical and practical interest. Whether subtle pharmacological manipulations will be of therapeutical and practical interest, as proposed above in the case of contraception, remains to be demonstrated.

The induction of steroid receptor by steroid hormones is of great value for explaining 'priming', a type of synergism acquired over a well defined time sequence. The negative influence of steroids on receptor induction [26] is conversely a way of explaining certain antagonisms between hormones, while other possibilities are the simultaneous presence of different receptors [39] or their competition for the same receptor [45].

Finally, the case is made that one can dissociate responses ordinarily (physiologically) linked by the very nature of a natural circulating hormone. We have seen that testosterone effects are mediated by different steroidal products in target cells, and that all estradiol effects may not be shared by the synthetic DES: these results may lead to new therapeutical advances.

Regulatory proteins, the steroid hormone receptors, provide some of the most advanced models for rational physico-chemical and physiological approaches of pharmacological and therapeutical problems. Their purification and complete characterization will undoubtedly lead to even more progress in clinical medicine.

Acknowledgements From many references, it is clear that I am indebted to my colleagues for their competent collaboration. The work has been supported partially by the Ford Foundation and the Délégation Générale à la Recherche Scientifique et Technique. I thank Dr. R. Sutherland for help in writing this manuscript.

REFERENCES

1. E.-E. Baulieu, *Proc. 4th Int. Congress of Endocrinology*, Washington 18–24 June 1972, (Ed. R. O. Scow) p. 30. ICS 273, Excerpta Medica, Amsterdam (1973).
2. E. V. Jensen and H. I. Jacobson, *Rec. Progr. Hormone Res.* **18**, 387 (1962).
3. R. F. Glasscock and W. G. Hoekstra, *Biochem. J.* **72**, 673 (1959).
4. E. V. Jensen, T. Suzuki, T. Kawashima, W. E. Stumpf, P. W. Jungblut and E. E. De Sombre, *Proc. natn. Acad. Sci. U.S.A.* **59**, 632 (1968).
5. D. Toft and J. Gorski, *Proc. natn. Acad. Sci. U.S.A.* **55**, 1574 (1966); D. Toft, G. Shyamala and J. Gorski, *Proc. natn. Acad. Sci. U.S.A.* **57**, 1740 (1967).
6. J. Gorski, D. Toft, G. Shyamala, D. Smith and A. Notides *Rec. Progr. Hormone Res.* **24**, 45 (1968).
7. G. P. Talwar, S. J. Segal, A. Evans and O. W. Davidson, *Proc. natn. Acad. Sci. U.S.A.* **52**, 1059 (1964).
8. A. Alberga and E. E. Baulieu, *C.R. Acad. Sci. Paris* **261**, 5226 (1965).
9. E. E. Baulieu, A. Alberga and I. Jung, *C.R. Acad. Sci. Paris* **265**, 354 (1967).
10. E. E. Baulieu, A. Alberga, I. Jung, M. C. Lebeau, C. Mercier-Bodard, E. Milgrom, J. P. Raynaud, C. Raynaud-Jammet, H. Rochefort, H. Truong and P. Robel, *Rec. Progr. Hormone Res.* **27**, 351 (1971).
11. G. Raspé (Ed.) *Advances in the Biosciences*, Vol. 7. Pergamon Press Vieweg, Oxford (1971).
12. E. Milgrom, M. Atger and E. E. Baulieu, *Biochim. biophys. Acta* **320**, 267 (1973).
13. E. Milgrom, M. Atger and E. E. Baulieu, *Biochemistry* **12**, 5198 (1973).
14. D. D. Fanestil and I. S. Edelman, *Proc. natn. Acad. Sci. U.S.A.* **56**, 872 (1966).
15. H. Rochefort and E. E. Baulieu, *C.R. Acad. Sci. Paris* **267**, 662 (1968).
16. C. Raynaud-Jammet and E. E. Baulieu, *C.R. Acad. Sci. Paris* **268**, 3211 (1969).
17. S. Mohla, E. R. De Sombre and E. V. Jensen, *Biochem. biophys. Res. Commun.* **46**, 661 (1972).
18. E. E. Baulieu, A. Alberga, C. Raynaud-Jammet and C. R. Wira, *Nature New Biol.* **236**, 236 (1972).
19. E. Milgrom and E. E. Baulieu, *Biochem. biophys. Res. Commun.* **40**, 723 (1970).
20. J. Anderson, J. H. Clark and E. J. Peck, *Biochem. J.* **126**, 561 (1972).
21. E. Milgrom, M. Atger, M. Perrot and E. E. Baulieu, *Endocrinology* **90**, 1071 (1972).
22. E. Milgrom, M. Luu Thi, M. Atger and E. E. Baulieu, *J. biol. Chem.* **248**, 6366 (1973).
23. R. Deanesly, *J. Reprod. Fertil.* **1**, 242 (1960).
24. R. Courrier, in *Vitamins and Hormones* (Eds. R. S. Harris and K. V. Thimann) Vol. 8, p. 197. Academic Press, New York (1950).
25. E. E. Baulieu, *Eur. J. Obst. Gynecol. Reprod. Biol.* **4**, 161 (1975).
26. J. Mester, D. Martel, A. Psychoyos and E. E. Baulieu, *Nature* **250**, 776 (1974).
27. A. Psychoyos, in *Vitamins and Hormones* (Eds. R. S. Harris, E. Diczfalusy, P. L. Munson and J. Glover) Vol. 31 p. 201. Academic Press, New York (1973).
28. M. Baudouin-Legros, P. Meyer and M. Worcel, *Br. J. Pharmac.* **50** (1974), in press.
29. J. H. Clark and J. Gorski, *Science* **169**, 76 (1970).
30. G. Michel, I. Jung, E. E. Baulieu, C. Aussel and J. Uriel, *Steriods* **24**, 437 (1974).
31. J. R. Gavin, J. Roth, D. M. Neville, P. De Meyts and D. N. Buell, *Proc. natn. Acad. Sci. U.S.A.* **71**, 84 (1974).
32. I. Jung and E. E. Baulieu, *Nature New Biology* **237**, 24 (1972).
33. G. Michel and E. E. Baulieu, *C.R. Acad. Sci. Paris* **279**, 421 (1974).
34. L. P. Bullock and C. W. Bardin, *Endocrinology* **94**, 746 (1974).
35. N. Bruchovsky and J. D. Wilson, *J. biol. Chem.* **243**, 2012 (1968).
36. K. M. Anderson and S. Liao, *Nature* **219**, 277 (1968).
37. E. E. Baulieu, I. Lasnitzki and P. Robel, *Nature* **219**, 1155 (1968).

38. K. J. Ryan, F. Naftolin, V. Reddy, F. Flores and Z. Petro, *Rec. Progr. Hormone Res* **31,** (1975), in press.
39. I. Jung-Testas and E. E. Baulieu, *C.R. Acad. Sci. Paris* **279,** 671 (1974).
40. C. Huggins, *Science* **156,** 1050 (1967).
41. L. P. Bullock and C. W. Bardin, *J. Clin. Endocr.* **35,** 935 (1972).
42. C. H. Sibley and G. M. Tomkins, *Cell* **2,** 221 (1974).
43. E. V. Jensen, G. E. Block, S. Smith, K. Kyser and E. R. De Sombre, *Nat. Cancer Inst. Monogr.* **34,** 55 (1971).
44. M. E. Lippman, R. H. Halterman, B. G. Leventhal, S. Perry and E. B. Thompson, *J. Clin. Invest.* **52,** 1715 (1973).
45. C. Geynet, C. Millet, H. Truong and E. E. Baulieu, in *Hormones and Antagonists: Gynec. Invest.* (Eds. P. O. Hubinont, S. M. Hendeles and P. Preumont) Vol. 3, p. 2, (1972).