Advances in the Biosciences 7

Schering Workshop on Steroid Hormone 'Receptors'

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Opening Address

G. Raspé

Schering AG, 1 Berlin 65, Germany

Ladies and Gentlemen:

It is a pleasure to welcome you all to Berlin. This seventh meeting in our series "Advances in Biosciences" differs very much from the previous workshops in that no lectures are being held. Consequently, this workshop had to be planned in a sequence different from previous ones in order to avoid any idle time. The idea of this experiment stems from Etienne Baulieu.

As to the topic "Steroid Hormone Receptors," we are also indebted to our dear friends Etienne Baulieu and Peter Jungblut.

The quotation marks around the word "Receptors" may play a role within the next few days.

My sincerest gratitude goes to the authors who by writing their manuscripts and handing them in *on schedule* made this experimental workshop possible.

Another group of vital importance is that of the moderators. They started their activities immediately upon arrival and have timed the discussive comments for each section. The success of this workshop will depend a great deal on their preparatory work. We all can help to make this meeting successful by adhering to the workshop rules.

I think I do not need to mention who made you suffer by deadlines, galley proofs, reminders, etc. It is our colleague Silke Bernhard who has organized this conference.

All discussions will be tape-recorded. For this reason, we have stopped all building activities on our new laboratories for the duration of the workshop. In case any of you are bothered by those activities sustained, please let us know.

Every discussant will receive a rough copy and may decide what part of it should be printed. The findings of this workshop are to be published quickly in order to make them known to those who could not attend today. Authors will find appropriate galley proofs in their hotel rooms.

We have invited Etienne Baulieu and his group to act as the editorial board, and I want to thank him for his very kind cooperation.

I extend to all of you my best wishes for a successful workshop.

Now, Etienne Baulieu will address you.

Introduction

E.-E. Baulieu

Unité de Recherches sur le Métabolisme Moléculaire et la Physio-Pathologie des Stéroides de l'Institut National de la Santé et de la Recherche Médicale, Département de Chimie Biologique, Faculté de Médecine de Paris Sud, 78 Avenue du Général Leclerc, 94 Bicêtre.
Postal address: Lab Hormones – 94 Bicêtre – France.

After a memorable discussion with *Peter Jungblut* and *Silke Bernhard*, it was decided that the main contributions would be circulated among participants beforehand and not read at the meeting. Following a brief presentation, each submitted manuscript will be critically discussed and then edited by the authors and the Editorial Board.

The outcome of this workshop may, in future years, be considered as one of the major contributions of the last decade to our understanding of steroid hormone action. The reports in this volume will be unique in that nearly all major findings up to December 1970 will be reported and discussed by the investigators themselves.[1])

The title STEROID HORMONE "RECEPTORS" was the choice of the Editorial Board and the format of the symposium was designed to be experimental.

The word receptor• (marked with a point) is used in several presentations and discussions as a convenient short term to describe intracellular specific binding proteins, and is not meant to imply the definition of receptor (unmarked) used in pharmacology or endocrinology. The latter use would imply that hormone binding is directly coupled to an executive site or mechanism, that is, connected to the first response of the target cell machinery after which the hormone is no longer directly involved. Those authors who believe that this is the case with the steroid binding proteins were asked to use the unmarked word receptor.

I would like to thank especially the moderators of the sessions: *I. Edelman, A. Munck, S. Liao, B. O'Malley, G. Mueller* and *G. Tomkins*, and those scientists who, while personally not working on steroid receptors•, have brought us their knowledge and experience.

Without *Gerhard Raspé*, this conference would not have been possible. It was only through his attention to all things happening in Science that this meeting became reality. Also aiding greatly to the success of the workshop was *Silke Bernhard* who really was *receptive* to any problem we had. Our thanks go also to all other members of Schering AG, Berlin.

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[1]) Unfortunately, *E. Jensen, J. Gorski, J. Mester*, and *B. Flerkó* could not attend the meeting.

The Regulation of Uterine Concentration of Estrogen Binding Protein

Jack Gorski, Mary Sarff* and James Clark**

Depts. of Physiology and Biophysics and Biochemistry, University of Illinois, Urbana

Summary: The concentration of the estrogen binding protein in the rat uterus has been studied in three stages of uterine development. A 3- to 4-fold increase in concentration of estrogen binding protein occurs between days one and ten after birth. The concentration appears to remain relatively constant from this point on, with an estimated synthesis rate of about 80 binding sites/hr/cell. The half-life of the protein is approximately 5–6 days, which is compatible with a relatively stable protein.

After estrogen is injected, the receptor concentration in the cytoplasm goes into a three-stage cycle. (1) Initially, there is a loss of binding protein, followed by (2) a stage sensitive to inhibitors of protein and RNA synthesis, and (3) a replenishment period that is not affected by the inhibitors.

Introduction

Studies in this laboratory on estrogen-binding protein started as a result of the publication of the elegant work of *Jensen* and his colleagues in the early 60's [5]. *Noteboom* and *Gorski* [6, 7] showed that the binding of estrogen in the uterus was principally in the nucleus and cytosol, was stereospecific, and probably associated with a protein. *Toft* and *Gorski* [13, 14]showed that a cytosol protein that bound estrogen could be resolved on sucrose gradients. This was followed up by *Toft, Shyamala* and *Gorski* [15]who showed that the binding of estrogen could be carried out in cell-free systems. A possible role for the binding protein was demonstrated by the finding that estrogen appeared to cause the binding protein to migrate into the nucleus [4, 11, 12]. The thinking in this laboratory about the estrogen receptor is presented in reviews [4; Current Topics in Developmental Biology, 1969, ed. *Monroy & Moscona*].

Suggested effects of the receptor on gene expression have been previously reported. [3].

The regulation of estrogen binding protein concentration appears to involve four periods in which different conditions prevail. The first period occurs during development and would start with the development of uterine primordia. At the present time, only the postnatal period in the rat has been studied [2].

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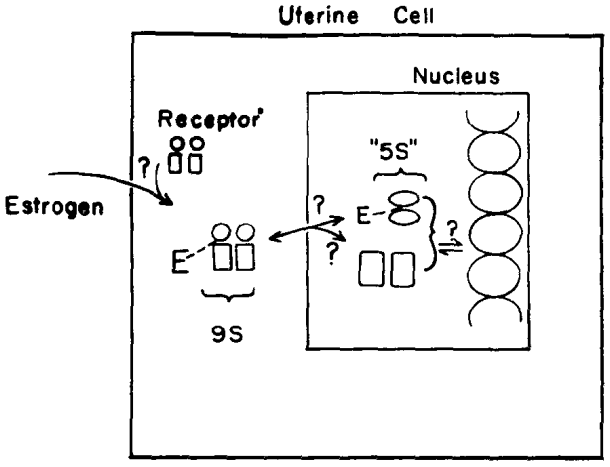

Fig. 1
Hypothetical model for estrogen interaction with uterine cell [11].

Following development, the next period in the rat runs from about 10 days to sexual maturity in the rat. This is a period of relatively little change in receptor numbers per cell, and synthesis and turnover are in equilibrium.

The third stage occurs when the uterus is exposed to estrogen (Fig. 1). The hormone is bound to the large binding protein (8S) in the cytoplasm and then appears to move into the nucleus [3]. As a result of this movement, the binding protein is depleted in the cytoplasm and is then gradually replenished. This replenishment process has turned out to be very complex and possibly reflects complexities in the structure of the binding protein. This is most dramatic when estrogen is injected, but also occurs during the natural rise and fall of estrogen levels during the estrous cycle.

The fourth and final period is that which occurs following continued estrogen exposure, such as in pregnancy. We have not looked at this stage to any great extent, and we will therefore exclude this period from the following discussion.

Ontogeny of the estrogen binding protein

Period 1: The development of uterine binding

The ability of the rat uterus to respond to estrogen increases during the first 10 days after birth [5]. If the estrogen binding protein has some relationship to tissue response, one might expect the binding protein concentration to increase in a manner corresponding to the change in uterine response. Fig 2 shows that the concentration of estrogen binding sites per unit of DNA or per cell increase about 4-fold during the first 10 days of postnatal development [2]. That this is a change in concentration and not physical

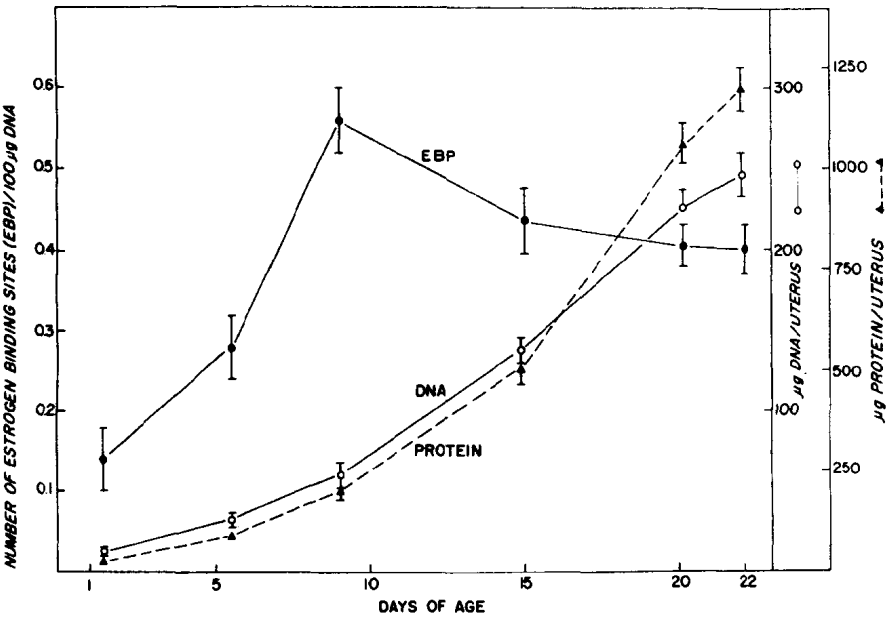

Fig. 2. The relationship between the quantity of estrogen binding protein (EBP) and uterine growth in the immature rat.
The number of EBP sites expressed as picomoles of EBP is based on using the 22–23 day old rat as a standard. Points on the graph represent the means ± S.E.M. of 3 to 4 experimental groups [2].

state of the binding protein is shown in Fig. 3. The binding protein has the same relative affinities at 4, 10, and 22 days, as estimated by Scatchard plots. The similarity in size is indicated by the sedimentation velocity of 8S in both 10 and 22 day old rats. The change in concentration of estrogen binding sites was shown not to be dependent on the rats' ovaries [2]. Other sites of control might be the pituitary or other endocrine glands, but no experimental evidence is yet available. We also have no information on binding site concentrations in prenatal uteri or in other species; either pre- or postnatal.

Period 2: Equilibrium

Once the rat reaches 10 days of age, estrogen binding site concentration per unit of DNA or per cell does not change, but rather it appears to reach an equilibrium between sythesis and degradation. The rate of synthesis is difficult to determine; however, an estimate of degradation rate can be obtained and, therefore, an indirect estimate of syn-

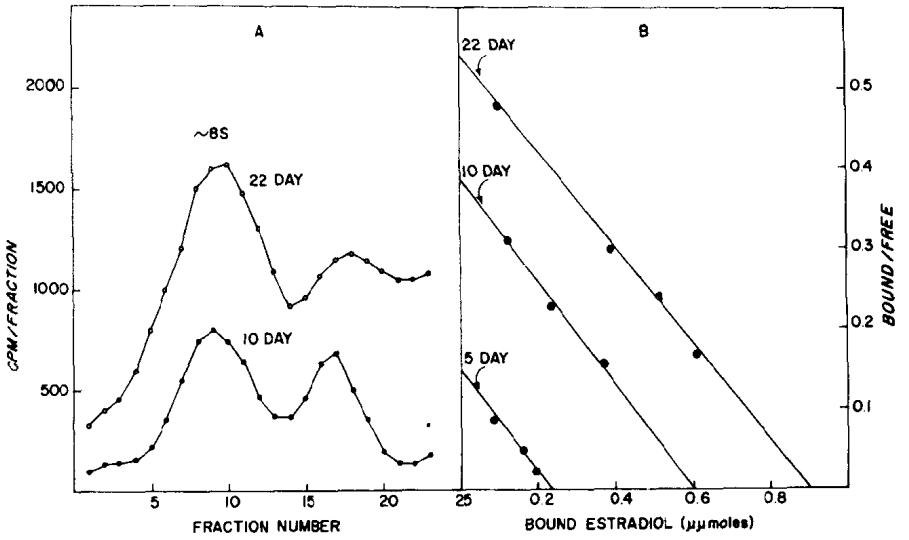

Fig. 3. Determination of the dissociation constants, number of binding sites and sedimentation characteristics of the uterine EBP at different ages.
A. Sucrose density gradient profiles of uterine cytoplasmic fractions from 10 and 22 day old rats. ^{3}H-estradiol (5×10^{-4} μg) was added to 0.2 ml of cytosol prepared from one rat uterus and layered on 5–20% sucrose gradients. Gradients were centrifuged at 35,000 rpm for 17.5 hr on a Model L. Spinco ultracentrifuge using a SW-39 rotor. B. Scatchard plots of EBP binding determined by the glass binding method. K_d for all three groups was approximately 3.0×10^{-9}M and the number of binding sites 0.9 μμ moles/one 22 day uterus, 0.6 μμ moles/two 10 day uteri, and 0.23 μμ moles/three 5 day uteri [2].

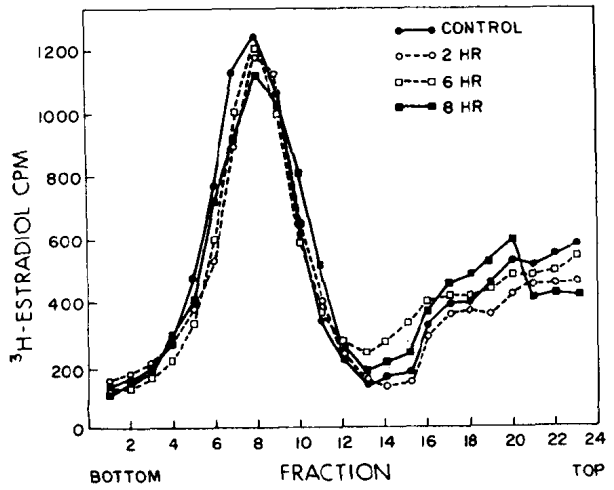

Fig. 4

Turnover of estrogen binding protein.
Protein synthesis was blocked by injecting I.P. 200 μg cycloheximide 2, 6, or 8 hr prior to killing. Estrogen binding protein in cytosol assayed on sucrose density gradients centrifuged at 220,000 x g for 15 hr [9, 10].

thesis at equilibrium [6]. The protein synthesis inhibitor, cycloheximide, can be used to block synthesis of the binding protein while degradation continues. Fig. 4 shows the sucrose gradient patterns of estrogen added to uterine cytosol from rats treated for various time periods with cycloheximide. Treatment for 8 hr resulted in only a 5% drop in binding capacity. A summary of several turnover studies using three different assays for specific estrogen binding is shown in Fig. 5. These data have been used in Fig. 6 to calculate the rate of synthesis and the 1/2 life of estrogen binding sites on a per cell basis. We have also shown an analysis of rates of synthesis and turnover calculated from the data shown in Fig. 2 and based on the formulations of *Berlin* and *Schimke* [1]. It can be seen that estimates of synthesis and turnover by the two methods are very similar and add to our confidence in using them. These calculations suggest that the developmental period involves the establishment of a new rate of synthesis at about the time of birth, which then reaches a new equilibrium at 10 days after birth.

The rates of synthesis calculated above are quite low and could be handled by one polysome unit (one messenger RNA with proper number of ribosomes), making peptide bonds at approximately 1/7 the rate of polysome that synthesize hemoglobin.

Period 3: Depletion-replenishment cycle after estrogen

The model of estrogen interaction with the uterus shown in Fig. 1 indicates that the estrogen binding protein after combining with estrogen in one compartment (the cytosol) moves to another compartment (thought to be the nucleus). Therefore, the con-

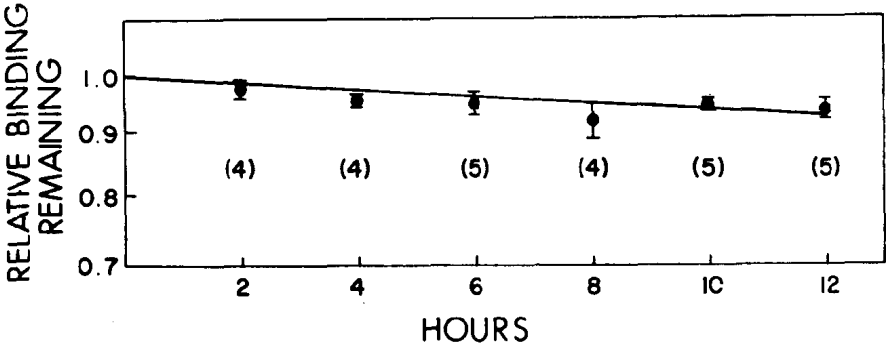

Fig. 5. Turnover of estrogen binding protein.
Relative ^{3}H-estradiol-17β binding capacity in uterine cytosol of immature rats after exposure to cycloheximide. Data from ten experiments are expressed as % of control (mean ± S.E.). *In vivo* controls were saline-injected, and *in vitro* controls were incubated without cycloheximide for the same period as the experimental groups. Assays for ^{3}H-estradiol-17β binding were done either by sucrose density gradients, the glass pellet binding assay, or Sephadex G-100 columns. Eight, 10 and 12 hr means are significantly less than control at 0.01 level. () indicate number of experiments used to determine mean [9].

SYNTHESIS AND DEGRADATION OF ESTROGEN BINDING SITES

At Equilibrium:

Synthesis (S) = Degradation (kP)

$\sim$80 sites/hour/cell = .005 (k) x 16,000 moles/cell

1/2 life of protein ($T_{1/2}$) = $\frac{\ln 2}{k}$ = 5–6 days

Postnatal Development:

P increases from 5,000 at age 1 day to 21,000 sites/cell at 10 days

$\frac{dP}{dt} = S - kP$ S = 151 sites/hour/cell

$T_{1/2}$ = 1/2 the time it takes to reach equilibrium $\therefore T_{1/2} = \sim 5$ days

Fig. 6. Equilibrium calculations based on data from Fig. 5. Postnatal development calculation based on data from Fig. 2. Formulation for calculations based on methods outlined by [1].

centration of binding protein in the cytosol is depleted after an estrogen injection as illustrated in Fig. 7 [7]. About half of the cytosol binding sites are lost after administration of 0.1 μg of estradiol into immature female rats. The depletion period reaches a low about 4 hr after estrogen and is significantly increased above the low by 8 hr after estrogen. The replenishment of binding sites continues to about 24 hr after estrogen injection and shows a marked overshoot of binding sites. This overshoot is correlated with the increased protein content per uterus, but probably is not dependent on this increase. The replenishment period results in an increase of binding sites at a rate 5 to 10 times the rate of binding protein synthesis calculated for either development or equilibrium, and raises several questions.

One question concerns the relationship of replenishment of cytoplasmic binding to nuclear binding. Studies summarized in Fig. 8 indicate that when whole uteri are incubated in a tissue culture medium, estrogen remains bound in the nucleus for 24 hr in contrast to the slow but exponential loss from the nucleus *in vivo*. Further cytoplasmic binding capacity does not increase *in vitro*, whereas *in vivo* the replenishment discussed above occurs. The loss of bound estrogen from the nucleus occurs prior to the replenishment of cytoplasmic binding sites, but as yet no direct relationship between these two events has been shown. The replenishment process could be due to the synthesis of binding protein or it could be due to re-activation of nuclear receptors which have discharged their bound estrogen [7].

Studies to determine the role of protein synthesis in replenishment gave the surprising data shown in Fig. 9. Cycloheximide, a protein synthesis inhibitor, blocked the replenishment of binding protein when administered before estrogen injection or up to 2 hr after. However, when administered 6 hr after estrogen, at the start of the replenishment