EFFECT OF CALF ENDOMETRIUM NUCLEI ON HUMAN ESTRADIOL RECEPTOR COMPLEX; AN IN VITRO STUDY.

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Summary:

A heterogenous system was created containing purified calf endometrium nuclei and cytosol from adult human uterine tissue to test whether calf endometrium nuclei are able to convert the 4S-form of the estradiol receptor complex into the well known 5S form extracted under high salt conditions from uterine nuclei.
Quite in contrast to the receptor hormone complex from immature tissue the complex from mature uterine tissue is translocated in a temperature independent step into calf endomertium nuclei.

Introduction:

It has been well established that when immature and ovariectomized animals are given radioactive estradiol there is accumulation almost exclusively in target organ cell nuclei (1). The labelled hormone can be extracted from isolated nuclei bound to a protein fraction sedimenting at 5S in a sucrose gradient. In comparison uterine cytosol layered on a linear sucrose gradient is fractionated in different peaks ranging from 8S - 10S, 6S, 5S, 4S depending on the origin of the cytosol as well as on the salt concentration of the cytosol and the gradient. In the nuclei of target organs the estradiol-receptor-complex binds to the chromatin (4). However time course and location of the change in molecular size of the estradiol-binding-protein, and therefore an important part of the steroid hormone action in the cell are not accurately determined. It is still a matter of controversy whether the change takes place outside or inside the cell nucleus.

Experiments on this topic involving young animals give a complicated picture, since different forms of the receptor protein are found in the cytosol of young uterine tissue under different experimental conditions (2,3). We have found little uptake of radioactively labelled hormone by isolated nuclei in adult species and human uteri. We therefore combined the advantages of the two types of tissue. The cytosol of human uteri containing a clearly defined "4S" receptor (5,7) was mixed with calf endometrium nuclei, known to have a high uptake capacity of hormone-receptor-complex. Thereby we observed an uptake of the human (adult) receptor-hormone-complex in young nuclei.

Methods:

Calf uteri were obtained at the local slaughter house and carried back to the laboratory on ice. All manipulations were carried out at 4°C, except where indicated differently.

Calf endometrium nuclei were prepared by a procedure modified from that of Glasser et al (6). The endometrium tissue was scratched out from the slit open calf uteri and homogenized in TKMS-buffer (0.01 M TRIS·HCl (pH 7.7)-0.2M KCl-0.01 M $MgCl_2$-2.2 M Sucrose). The homogenate was filtered through one layer of Nylon gauze and the filtrate then centrifuged at 75'000xG in a Spinco SW 50.1 rotor for 30 minutes. The supernatant was discarded and the pellet containing the nuclei was gently stirred with a glass rod in the original buffer and recentrifuged under the same conditions. This procedure yielded clean nuclei as checked by phase contrast microscopy. No detergent was necessary to attain this result. The pellet was then suspended in GTM buffer (25% Glycerol-TRIS·HCl (pH 7.7)-0.01 M $MgCl_2$) and was used immediately.

Cytosol containing the receptor protein was prepared from calf endometrium tissue or from human uteri removed by hysterectomy. The latter were used immediately after operation or kept deep frozen (-20°C) for about 2 weeks. The homogenate from human uterine tissue was prepared from samples containing both myometrium and endometrium tissue. The tissues were homogenized in different buffer systems (see below) (3 parts buffer: 1 part tissue) and the

resulting homogenates centrifuged at 105'000xG for 90 minutes to obtain the cytosol used in our experiments. Homogenates were prepared in either TS-buffer (0.01 M TRIS·HCl (pH 7.7)-0.3 M Sucrose); TK_{15} buffer (TRIS·HCl (pH 7.7)-0.15 M KCl) or TK_{40}-buffer (TRIS·HCl (pH 7.7)-0.4 M KCl).

The hormone-receptor-complex was prepared by incubating the cytosol with $\{^3H\}$-E_2 (NEN) ($5x10^{-9}$ M) at 4°C from 1 to 16 hours. Excess $\{^3H\}$-E_2 was removed by adding 15 volumes of a charcoal-Dextran mixture (0.5% charcoal Norit A (Serva), 0.05% Dextran 60 (Serva) in 0.01 M TRIS·HCl (pH 7.7) to the samples and shaking in the cold for 15 minutes. The charcoal was removed by centrifuging the mixture for 10 min. at 4°C at 20'000 rpm in a Beckmann J-21 centrifuge. The supernatant fraction was either used in incubation experiments or layered as controls on a sucrose gradient. The incubation of nuclei with $\{^3H\}$-E_2 treated cytosol lasted 1/2 hour at either 4°C or 25°C. Following this incubation the suspension was centrifuged and the nuclear pellet washed twice in TK_{15} buffer. The pellet was resuspended in TK_{40} buffer and homogenized by means of a sonicator (Branson) until all nuclei were disrupted (checked microscopically). The suspension was centrifuged at 10'000 rpm (Beckmann, J-21 centrifuge). The supernatant, containing the nuclear extract, was layered on a 5-20% sucrose gradient containing 0.4 M KCl and centrifuged at 50'000 rpm in a Spinco SW 50.1 rotor for 18h.

Results:

The human uterine cytosol was subjected to ultracentrifugation on sucrose gradients with different salt concentrations to determine whether the salt concentration itself used in the extraction step of the nuclei would affect the sedimentation behavior of the receptor i.e. convert the 4S into 5S form. There is no difference between the sedimentation patterns carried out in high (0.4 M KCl) and low (0.15 M KCl) salt. The sedimentation pattern of human uterine cytosol incubated with $\{^3H\}$-E_2 is characterized by two peaks (4.5 S and 3.3 - 3.7 S) relative to bovine serum albumine (BSA) as internal marker (Fig.I & III). The 4.5S peak can totally be

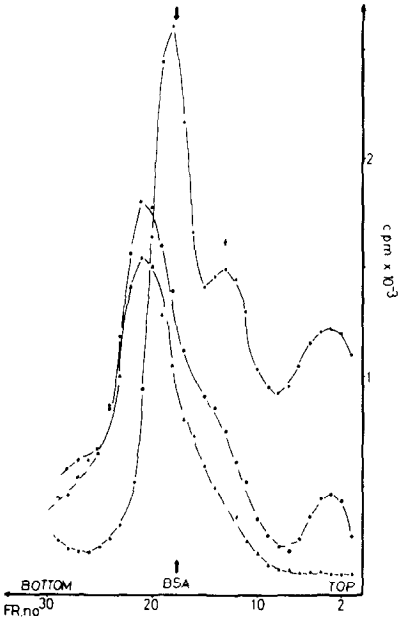

Fig.I: Human uterine cytosol {^{3}H}-Estradiol-receptor-complex layered on a 5-20% sucrose gradient containing (0.4M KCl) (-●-). Extracts from calf endometrium nuclei at 4°C (-▲-) and at 25°C (-△-) after incubation with human uterine cytosol.

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abolished by charcoal treatment of the cytosol incubated with {^{3}H}-E_2, whereas the peak at 3.3 - 3.7S, known from the literature as "4S", is abolished by treatment with antiestrogen (results not shown). Therefore the 4.5S peak must be attributed to unspecific, the "4S" peak however to specific binding.

Calf endometrium cytosol incubated with {^{3}H}-Estradiol and run on sucrose gradients containing low and high salt show the well known distribution of large amounts of 8S - 10S and little 5S in low salt and in high salt no 8S - 10S and practically all receptor in the 5S form (result not shown and 8). Calf endometrium nuclei incubated with calf endometrium cytosol in vitro yield upon extraction in high salt a 5S form which is not distinguishable from the "salt converted" form of the receptor.

Human "4S" receptor brought together in vitro with calf

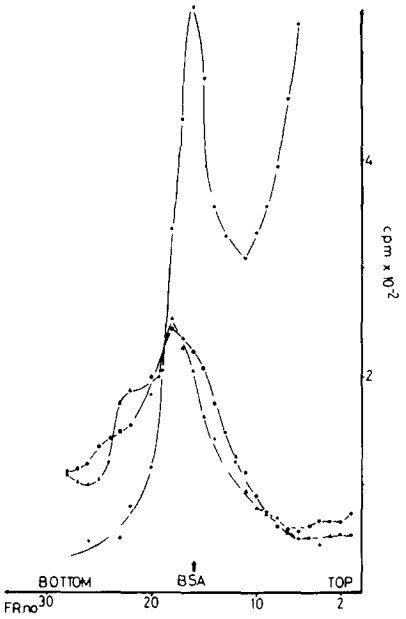

Fig.II: (-O-) Human uterine {^{3}H}-Estradiol-receptor-complex extracted from calf endometrium nuclei not treated and treated with (-▲-) charcoal and layered on a linear 5-20% sucrose gradient (0.4M KCl) and human uterine cytosol (-●-) as control.

endometrium nuclei is extracted from the latter sedimenting in the 5S region of a sucrose gradient (Fig.I).

From experiments with calf endometrium receptor transferred into calf endometrium nuclei we know the uptake of the complex in the nucleus to be temperature dependent. However such temperature dependence does not exist when using estradiol receptor in human cytosol brought together with calf endometrium nuclei (Fig.I). The conversion of 4S to the 5S form nevertheless takes place.

To determine the specificity of estradiol binding to the nuclear extract a charcoal treatment was included. As one can see (Fig.II) no difference in the sedimentation pattern between untreated and with charcoal treated extracts exists.

Control experiments with non-target organ nuclei (calf dia-

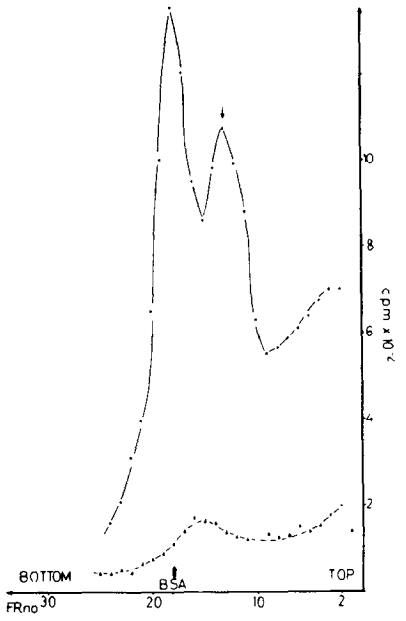

Fig.III: Human uterine cytosol {^{3}H}-Estradiol-receptor-complex (-●-) mixed with calf Diaphragma nuclei. The diaphragma nuclei extract layered on a 5-20% salt containing sucrose gradient (-▲-).

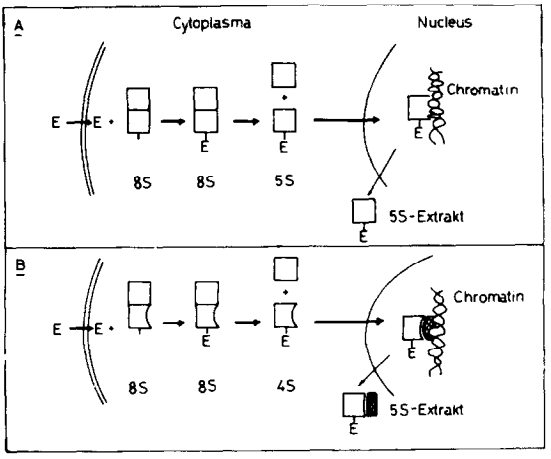

Fig.IV: Possible pathways of Estradiol-translocation in a juvenile target organ cell.

Table 1: Forms of Estradiol receptor complex as found under different experimental conditions.

Experimental conditions	Calf endometrium cytosol receptor	Human uterine cytosol receptor
low salt (0.15M KCl)	8-10S	4S
high salt (0.4M KCl)	5S	4S
after incubation with calf endometrium nuclei	5S	5S

phragma) at 4°C and 25°C are negative with respect to uptake and conversion of human uterine receptor. Only some background radioactivity runs throughout the whole gradient (Fig.III).

Discussion:

Concerning the mechanism of estradiol action in target organs several different proposals have been brought forward about hormone receptor-complex entering the nucleus. King and Mainwaring (8) have summarized the different possible pathways and have singled out the two following mechanisms as most likely:

1) The 8S receptor-estrogen-complex is converted in the cytoplasm into the 5S form which then enters the nucleus, or,

2) The 8S form is converted in a first step to "4S", binds estrogen and enters the nucleus and is only then converted to 5S after a probable attachment to the chromatin.

Working with juvenile uterine tissue the question of conversion of the 8S form in the cytosol into the 5S form extracted from the

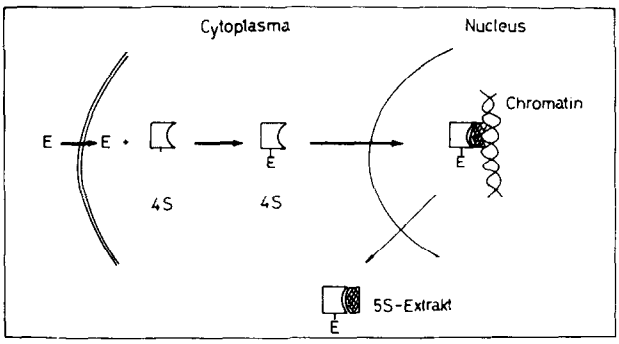

Fig.V: Possible pathway of Estradiol-translocation in a adult target organ cell.

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nuclei is complicated by the fact that the high salt conditions.

By using directly the 4S form of the complex as found in humans we circumvent the difficulties mentioned above. The 4S form is unaffected by the salt concentration of the medium and therefore no interference by artificially converted hormone-receptor-complex has to be taken in account.

The results presented in this paper support strongly the second point as indicated by King and Mainwaring (above):

The 4S form of the receptor complex in the adult animal and human is assumed to be derived from the 8S form under the influence of estrogen and body temperature. It enters the nucleus independent of temperature to the same extend at $2^{o}C$ and $25^{o}C$ (Fig.I) which indicates that temperature and hormone is necessary in the juvenile system only to convert the receptor (8S) to its 4S form and not to enter the nucleus (Fig.V). Obviously an intra-nucleic component is then necessary, as the experiments involving diaphragma nuclei show, to yield the 5S complex extracted from the nuclei.

A second not unimportant result of these experiments is just the finding of the "juvenile" nucleus to be able to convert the receptor complex within itself. It proves the quite general properties of the receptor protein and of the nuclear acceptor sites or both. Bearing in mind that the diaphragma nuclei are not able to convert the receptor complex indicates however that the specificity for the binding lies in the nucleus of the target organ more than with the receptor protein.

A third point which is to be mentioned is the conversion of the 4S to the 5S receptor complex in a heterologous system, i.e. human uterine cytosol and calf endometrium nuclei. This conversion seems therefore to be a general step in the action of estradiol and is conserved throughout development.

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