Effects of temperature, testosterone and estradiol-17β on the testis composition in frog

Treatment	Gonosomatic index	Dry weight (%) (% water)	mg protein/g dry tissue	mg lipids/g dry tissue
Initial value	0.26 ± 0.02	7.5 ± 2.5 (92.5)	1485 ± 237	12.7 ± 3.1
28 °C	0.32 ± 0.02*	20.7 ± 2.6 (79.3)*	541 ± 46*	18.1 ± 4.9
15 °C	0.20 ± 0.06	12.4 ± 1.5 (87.6)*	1108 ± 154	23.7 ± 5.8*
4 °C	0.23 ± 0.02	10.9 ± 4.3 (89.1)	1089 ± 91*	27.3 ± 7.2*
15 °C + testosterone	0.21 ± 0.04	22.5 ± 1.9 (77.5)*, **	544 ± 69*, **	10.3 ± 2.6**
15 °C + estradiol-17β	0.22 ± 0.04	14.5 ± 0.4 (85.5)*	818 ± 67*	12.8 ± 3.3**

*Significantly different (student's t-test) from the initial value;
**significantly different from values obtained in 15 °C-treated frogs.

tion of the testis of these frogs are similar to the values obtained in frogs at 28 °C (table). Similar changes are observed for the testis lipids also. Thus testosterone inhibits the low temperature-induced increase in the testis lipids and proteins. Administration of estradiol-17β, on the other hand, does not alter the testis weight (wet or dry) but impedes the rise in testicular lipid concentration. It may not be out of line to mention that high temperature provokes degranulation of the pituitary gonadotrops whereas low temperature induces hypertrophy[3,4]. Thus in future, consideration must be given to the testis composition which, at least in part, reflects the influence of environmental and hormonal factors upon the testis.

Purification of the cytosol oestradiol-receptor complex from foetal guinea-pig uterus using electrofocusing on polyacrylamide plates[1]

J. R. Pasqualini and C. Cosquer-Clavreul

C.N.R.S. Steroid Hormone Research Unit, Foundation for Hormone Research, 26, boulevard Brune, F-75014 Paris (France), 8 June 1977

Summary. The ^{3}H-oestradiol receptor complex obtained from the cytosol fraction of foetal guinea-pig uterus was purified by the following steps: column chromatography in Sephadex G-15 and Ultrogel, and by electrofocusing on polyacrylamide plates. In the final step a concentration of 15–17% of the foetal uterine oestradiol receptor protein was obtained. The isoelectric point (pl) of this receptor was determined to be 6.1–6.2.

Recently it has been found that the foetal uterus of guinea-pig contains specific cytosol and nuclear receptors for oestradiol[2]. These specific oestradiol binding sites increase significantly during foetal developement, particularly at the end of gestation when values reach 500–800 fmoles/mg cytosol protein[3]. Purification of the oestradiol receptors from the uteri of immature calves or rats was obtained by ammonium sulfate precipitation, gel filtration and ion exchange chromatography[4] or by affinity chromatography[5]. In this paper the purification of ^{3}H-oestradiol receptor complex from the foetal uterus of guinea-pig by electrofocusing on plates is described.

Materials and methods. The uteri of Hartley Albino guinea-pig foetuses (55–62 days of gestation) were used. 500–600 mg (10–14 fetal uteri) were homogenized in 4 ml of 0.01 M Tris, HCl–0.001 M $CaCl_2$ (pH 7.4), centrifuged at 900 × g and the cytosol fraction was obtained after ultracentrifugation of the supernantant at 200,000 × g. The ^{3}H-oestradiol receptor complexes were prepared by incubation of this cytosol fraction (containing 4 mg protein/ml) with 9×10^{-8} M, 6,7-^{3}H-oestradiol (s.a. 60 Ci/mmole). Parallel incubations were carried out using the same concentration of ^{3}H-oestradiol plus a 100fold excess of non-radioactive oestradiol. The incubations were carried out at 25 °C for 20 min.

The ^{3}H-oestradiol macromolecule complexes were first obtained after column chromatography on Sephadex G-15 (Pharmacia, Uppsala, Sweden) (30 cm high, 0.9 cm diameter). These ^{3}H-oestradiol protein complexes were re-chromatographed in a column of Ultrogel AcA-34 (L. K. B., Broma, Sweden, 0.30 cm high, 0.9 cm diameter). Both chromatographies were carried out at 2 °C. The peak containing the oestradiol receptor was submitted to thin layer gel electrofocusing in a polyacrylamide gel plate (L. K. B. 2117 Multiphor, Broma, Sweden) containing ampholytes with a pH range of 3.5–9.5 for 90 min at 2 °C. The isoelectric point (pl) was calculated by measuring the pH in different gel sections, using a pH meter (Radiometer PHM 62). Furthermore, in order to estimate the sedimentation coefficient of the ^{3}H-oestradiol receptor complex, aliquots (0.2 ml) of the cytosol fraction from the Sephadex and Ultrogel columns were layered on a sucrose density gradient (5–20% w/v) in a 0.01 M Tris, HCl (pH 7.4) solution, containing 0.0005 M dithiothreitol and 0.01 M EDTA and centrifuged at 200,000 × g for 18 h at 2 °C.

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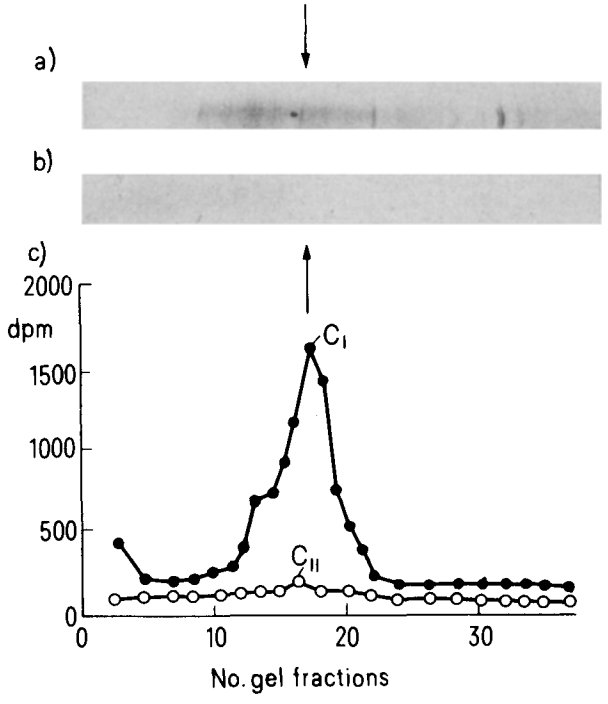
Proteins were evaluated by the method of Lowry et al.[6] and the different bands of protein on the plate after electrofocusing were detected using Coomassie Brilliant R (No. B 0630) (St Louis, Mo. USA) as follows: the proteins were fixed with a solution of 0.35% (w/v) sulfosalicylic acid and 1.15% (w/v) trichloroacetic acid in 70% methanol for 30 min at room temperature. They were then stained with a solution of 0.115% (w/v) Coomassie Brilliant R dissolved in a mixture of water: acetic acid: methanol (13.4:1.5:5 per vol.) for 10 min at 60 °C. The gels were destained using the same mixture overnight at room temperature. The stained protein bands in the gel plate were evaluated using an absorptiometer (ISCO, Nebraska, USA, model U-A-5) with a 660 nm filter. The amount of protein in the bands in the gel plates was calculated using a standard protein, bovine fibrinogen (pI: 5.8). The area under the recorded peak of absorption at 660 nm which represented the band containing the cytosol ^{3}H-oestradiol receptor was compared with the area from a quantity of standard protein which had been electrofocused in parallel. Another parallel strip containing the cytosol ^{3}H-oestradiol receptor complexes was cut into 2-mm sections which were dissolved in Instagel (Packard, SA) and the radioactivity counted in Packard Scintillation Spectrometer Model 3330. Correction for the dissociation of the ^{3}H-oestradiol receptor complex was carried out as follows: The ^{3}H-oestradiol receptor fraction was isolated by Sephadex G-15 column chromatography. Subsequently, at different periods of time, binding was again determined and the initial binding was calculated by extrapolation to time zero.

Specific activity (dpm/mg protein) in the successive steps of purification of the ^{3}H-oestradiol receptor complexes from the cytosol fraction of the foetal uterus of guinea-pig

	dpm/mg protein	Percentage of purity of the ^{3}H-oestradiol receptor complex*
I) Chromatography on Sephadex G-15	403, 000	0.07
II) Chromatography on Ultrogel AcA-34	2, 321, 000	0.41
III) Electrofocusing (pH range: 3.5–9.5) Band pI: 6.1–6.2	92, 840, 000	16.37

* Assuming a mol. wt of 240,000 daltons for the receptor protein molecule[7] and 1 specific binding site. The dissociation of the ^{3}H-oestradiol-receptor complex was corrected for in each step.



a 20 µl of the ^{3}H-oestradiol receptor complex obtained after chromatography in Sephadex G-15 and Ultrogel AcA-34 were electrofocused in pH range of 3.5–9.5, and *b* the zone with a pI of 6.1–6.2 which contained the ^{3}H-oestradiol receptor was electrofocused a 2nd time. Part *c* indicates the radioactivity in the different gel sections and represents the data obtained after incubation of the cytosol of fetal guinea-pig uterus 9×10^{-8} M ^{3}H-oestradiol in the absence (C_I: -●-●-) or in the presence of a 100 fold excess of non-labelled oestradiol (C_{II}: -▲-▲-). The proteins in the gel were stained and the radioactivity was measured as indicated in the text.

Results and discussion. The sedimentation profile after ultracentrifugation in sucrose density gradient shows the presence of a ^{3}H-oestradiol receptor complex component with a sedimentation coefficient of 8.5 S, confirming previous studies in the same foetal tissue[2]. The radioactivity of this peak is abolished in the presence of a 100fold excess of unlabelled oestradiol.

2 aliquots (20µl, 20–30µg protein) of the cytosol fraction from the Ultrogel column which contained the ^{3}H-oestradiol receptor complex were electrofocussed in parallel. 1 strip was stained with Coomassie Brilliant R (figure, a) and the area of the other strip, which contained the ^{3}H-oestradiol receptor, was electrofocussed again and stained (figure, b). In a parallel series of experiments, uterine cytosol fraction was incubated with ^{3}H-oestradiol in the presence and absence of a 100fold excess of non-radioactive oestradiol. After incubation, the cytosols were chromatographed on Sephadex G-15 and Ultrogel columns and submitted to electrofocusing. The distribution of radioactivity in the electrofocussed strips is shown in the figure, c. C_I (without the addition of non-radioactive oestradiol) and C_{II} (with the addition). The area containing the ^{3}H-oestradiol receptor corresponds to a pI of 6.1–6.2.

The specific activities (dpm/mg protein) in the successive purification steps of the ^{3}H-oestradiol-receptor complexes are indicated in the table. In the Sephadex G-15 column 80–85% of the proteins were eluted in the excluded volume. In the ultrogel column, the ^{3}H-oestradiol receptor complex was eluted in the 8–10.4-ml fraction. To calculate the recovery in this column, a protein of mol. wt close to the 8.5 S component[7], human α-globulin (Calbiochem, Los Angeles, California, USA) (mol. wt: 200,000) was used. This protein was eluted in the same fraction as the ^{3}H-oestradiol receptor complex and its recovery was 80–90%.

As is indicated in the table, the specific activity of the purified ^{3}H-oestradiol receptor complex after electrofocusing is 92,840,000 dpm/mg of cytosol protein. As the specific activity of ^{3}H-oestradiol used is 60 Ci/mmole (2.04 pg of ^{3}H-oestradiol/1000 dpm) it is calculated that 0.19 µg of ^{3}H-oestradiol is associated with 1 mg of protein. Assuming that the mol. wt of the 8.5 S component is 240,000 daltons[7], and that 1 binding site is present per receptor protein molecule, it is calculated that 15–17% of the proteins contained in this band consist of the oestradiol receptor. It is concluded that electrofocusing on polyacrylamide plates is a useful method for a rapid and partial purification of the oestradiol receptor complex.

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