PROPERTIES OF A UTERINE OESTRADIOL RECEPTOR

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The existence of specific receptor-proteins for oestradiol in the uterus is now well established (Talwar et al., 1964 ; Noteboom and Gorski, 1965 ; Alberga and Baulieu, 1965 ; Toft and Gorski, 1966 ; Jensen et al., 1966 ; Toft et al., 1967 ; Jungblut et al., 1967 ; Baulieu et al., 1967). Jensen et al. (1968) have described two oestradiol-receptors in the calf-endometrium : one in the nucleus, and the other in the cytoplasm. We summarize here some results of our studies on the latter.

I. The receptor-oestradiol-^{3}H complex has a sedimentation constant of 8 S. It has a tendancy to form heavier aggregates. Calf-endometrium was homogenised with 0,04 M Tris buffer, pH 7,4, containing 0,0015 M EDTA and 0,002 M 2-mercaptoethanol and centrifuged at 100,000 x g for 90 minutes. The supernatant, (20mg protein/ml) was incubated overnight with 5×10^{-9} M oestradiol-^{3}H (30,2 curies/mM) and analysed by ultracentrifugation on 5-20 % sucrose gradient the next day. All manipulations were carried out at 0-4°C. We find a single symmetrical radioactive peak, with a sedimentation constant of 8 S (Fig. 1A, curve 2). The same values were obtained from extracts prepared and labelled under identical conditions from pig and sheep endometrium and from the uterus of the immature rat. About 10 % of the radioactivity always sediments ahead of the 8 S peak, whilst the small pellet at the bottom of the tube also contains radioactivity. Aggregate formation is increased by freezing the tissue before the extraction, freezing the extract itself, concentration of the extract

(ultrafiltration, ammonium sulfate precipitation, dialysis against Carbowax), storing the extract at 0°C for more than a week, incubation at 30°C for 1 hour, or by filtration through Sephadex G 200 columns. In certain cases, all of the bound radioactivity sediments as aggregates heavier than 8 S (Fig. 1 B, curve 4).

II. The state of aggregation of the receptor-oestradiol complex depends upon ionic strength : The labelled extract was incubated overnight in presence of different concentrations of KCl, and analysed on sucrose gradients containing the corresponding amounts of KCl. Up to 0,05 M KCl, the position and pattern of the radioactive peak remains unchanged, i.e. the sedimentation constant is still 8 S. Between 0,3-1,0 M KCl the radioactive complex sediments as a peak with a sedimentation constant of 5 S (Fig. 1 C). Incubation with KCl does not change the amount of oestradiol-^{3}H bound by the receptor, and in fact the results are identical whether the extract is labelled before, simultaneously or after addition of KCl. NaCl has the same effect as KCl. To test the reversibility of the KCl effect, we incubated a labelled extract with 1 M KCl overnight and then dialysed for 24 hours against a buffer without KCl. Centrifugation of the dialysate on a sucrose gradient, in the absence of KCl, showed that not more than 10 % of the radioactivity now sediments in 5 S region, the rest sedimenting as heavier aggregates. Heavy aggregates formed under the conditions described under (I), were partly dissociated after 24 hours incubation with 1 M KCl, yielding the complex which sedimented as a 5 S peak (Fig. 1 B, curve 5).

III. A pancreatic lipase "dissociates" the receptor molecule. We incubated a labelled extract with 0,25 mg/ml lipase NBC 448, for 3-24 hours. Sucrose gradient ultracentrifugation of the treated extract revealed a symmetrical radioactive peak with a sedimentation constant of 4 S (Fig. 1 D, curve 11). As lipase NBC 448 is contamined with trypsin, we incubated the labelled extract with lipase in the presence of 100 µg/ml crystalline, soybean trypsin inhibitor. The result was the same as with lipase alone. Incubation for 60 minutes with 40 µg/ml crystalline trypsin

yielded a result quite dissimilar to that with the lipase treatment, as shown in Fig. 1 D, curve 9. It is not yet known whether the observed "dissociation" is due to the effect of the lipase alone or to the presence of proteolytic enzymes, other than trypsin, acting with lipase. Aggregates formed under conditions described under (I), were "dissociated" by lipase treatment. Wheat germ lipase Sigma has no effect on the receptor-oestradiol complex.

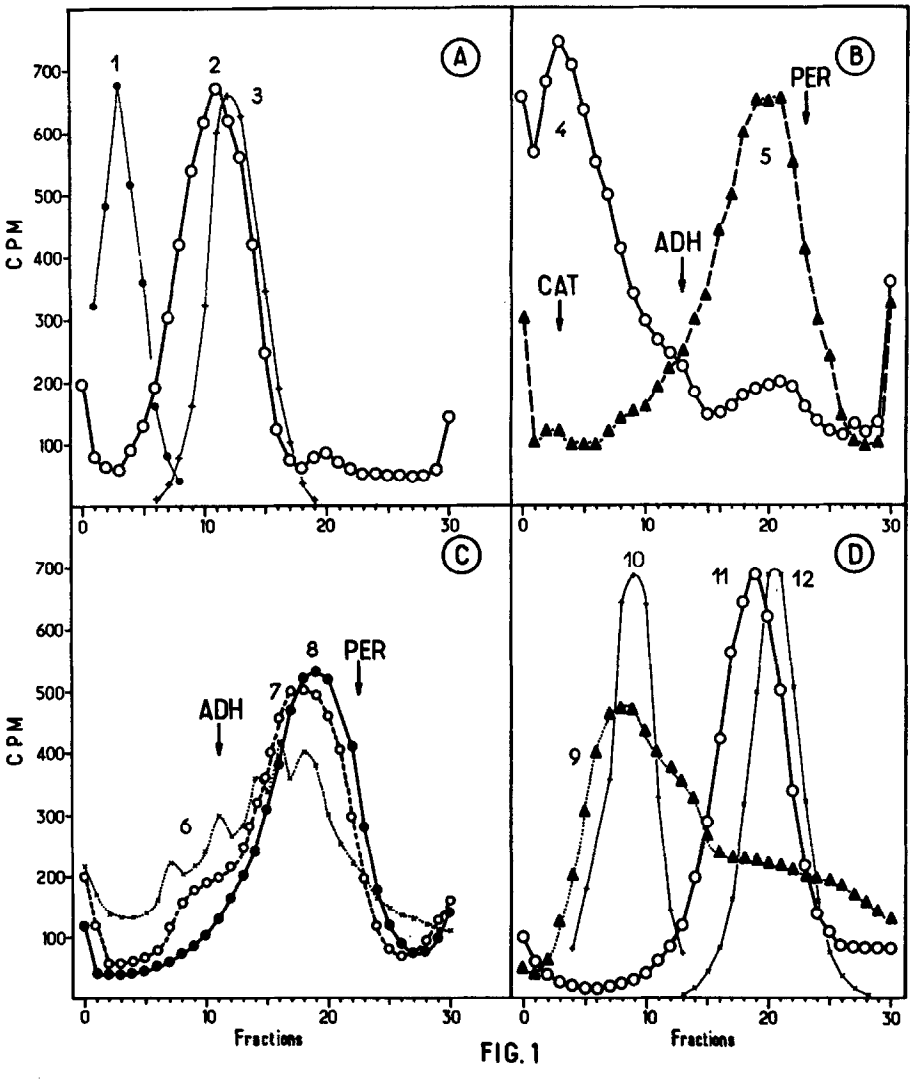

FIG. 1

Fig.1. 0,2 ml labelled extract was layered on a 4,3 ml 5-20 % linear sucrose gradient and centrifuged in Spinco L 40 ultracentrifuge, rotor SW 39, at 3°C, in exp. A, B, C : for 18 hours, in exp. D : for 20 hours. 30 fractions were

collected, and 0,1 ml aliquots were counted in Bray's solution with 23 % eff. The bottom ($\frac{1}{2}$ cm) of the tube was cut off, and put directly in Bray's solution ; fraction "0" in figures. Beef heart catalase (CAT), yeast alcohol dehydrogenase (ADH) and horse radish peroxidase (PER) was added either directly to the labelled extracts or run separatelly with identical results. Enzyme-activities are expressed in arbitrary units (A and D) or only the peak of activity is indicated by arrows (B and C). All figures are normalised for 30 fractions.

A) Curve 1) : catalase ; curve 3) : ADH ; curve 2) : labelled extract in 0,04 M TRIS buffer.

B) A labelled extract was kept in 0,04 M TRIS buffer for 24 hours at -15°C, thawed and kept for another 24 hours at 0°C either in 0,04M TRIS buffer only : curve 4) or in presence of 1 M KCl : curve 5).

C) A labelled extract was kept 24 hours in 0,2, 0,3 or in 1M KCl, and centrifuged on sucrose gradient in presence of the appropriate concentrations of KCl. Curves 6, 7 and 8 resp.

D) Curve 9) : The labelled extract was incubated with 40 µg/ml cryst. trypsin for 60 mins at 0°C before ultracentrifugation ; curve 10) : ADH ; curve 11) : The labelled extract was incubated with 250 µg/ml lipase for 6 hours at 0°C before ultracentrifugation ; curve 12) : peroxidase.

IV. Upon filtration on a Sephadex G 200 column the receptor-oestradiol-^{3}H complex is eluted close to the exclusion volume, and is retarded in presence of 1 M KCl or if the extract was treated with lipase. Fig.2. It was not possible to calculate exact K_d values : In the case of the untreated extract almost only the assymetry of the peak (Fig. 2 A, curve 3) indicated that not all radioactivity is eluted with the exclusion volume ; in presence of 1 M KCl or if the extract was treated with lipase the peaks were extremely broad (Fig. 2 B curves 4 and 6 resp.). Tentatively K_d values of 0,02, 0,14 and 0,43 resp. were calculated.

Data of Sephadex-filtration experiment together with the values of the sedimentation constants calculated from the results of sucrose gradient ultracentrifugation yield enough information to estimate the molecular weights and frictional ratios of proteins even in impure systems (Siegel and Monty, 1966).

Taking our present data as they stand, we calculated the following MW and f/f_o values for the receptor-oestradiol

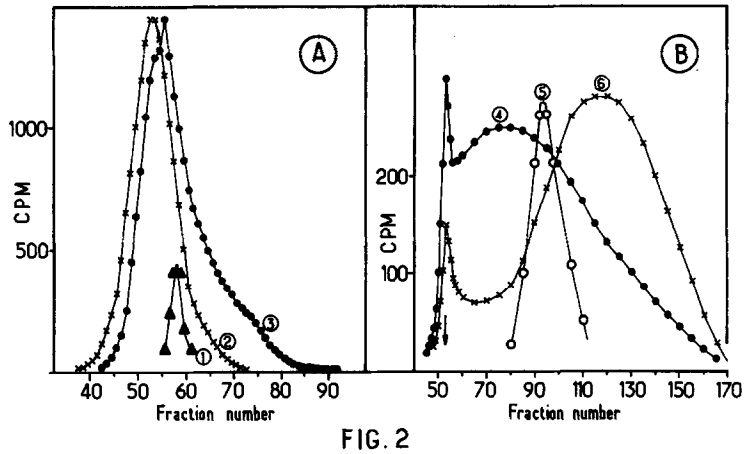

FIG. 2

Fig.2 : A 1,5 ml sample of a labelled extract was applied to a 30 mm x 480 mm Sephadex G-200 column. (Siegel and Monty, 1966). Fractions of 1,5ml were collected, and 0,5ml aliquots were counted in Bray's solution with 20 % efficiency Radioactivity is expressed in CPM ; the activity of catalase and optical density of Blue Dextran and fibrinogen are given in arbitrary values.

A) The column was equilibrated with 0,04 M Tris buffer (pH 7,4) containing 0,0015 M EDTA and 0,05 M KCl. The labelled extract was in the same buffer. Curve 3) : radioactivity. Blue Dextran 2000, (curve 2) determined by A 260 measurement, and fibrinogen (curve 1) determined by A 280 measurement were obtained in separate experiments.

B) Curve 4 : radioactivity of a labelled extract, kept 24 hours in the presence of 1M KCl. The column was equilibrated with 1M KCl as well. Catalase (curve 5) was added with the samples. The arrow marks the positions of the Blue Dextran peak. Curve 6 : radioactivity of a lipase-treated labelled extract, buffer as in A.

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complex : In dilute Tris-buffer : MW 360,000, f/f_o 2,6 ; in presence of 1 M KCl : MW 140,000, f/f_o 2 ; after lipase-treatment : MW 65,000, f/f_o 1,4. As the values in the first case are certainly overestimated (ultracentrifugation shows that Sephadex-filtration increases aggregate formation) it is tempting to suggest that the original molecule is dissociated into 2 fragments by 1 M KCl and into 4 fragments by lipase treatment. The initial step of "dissociation" might be identical whether KCl or lipase is used, because the ultracentrifugal patterns were identical whether the extract was treated with lipase alone, or with lipase in presence of 1 M KCl.

All these date concern the behaviour of the receptor-oestradiol complex, and not the free receptor itself, and all our experiments were carried out on crude extracts. As aggregation and dissociation phenomena might be different with purified material, confirmation of the above results must await the purification of the receptor-substance, which is now in progress.

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References

Alberga,A., and Baulieu,E.E., C.R. Acad.Sci.Paris, 261, 5226 (1965)
Baulieu,E.E., Alberga,A. and Jung,I., C.R.Acad.Sci.Paris, 265, 354 (1967)
Jensen,E.V. et al., in "Colloque sur la Physiologie de la Reproduction chez les Mammifères" ed by Jost,A. (1966 b.,) C.N.R.S. Paris.
Jensen,E.V. et al., Proc.N.A.S. 59, 632 (1968)
Jungblut,P.W. et al., in Wirkungsmechanismen der Hormone", 18 Mosbacher Colloquium, (Springer Verlag, Heidelberg), p.58 (1967)
Noteboom,W.D. and Gorski,J., Arch.Biochem.Biophys., 111, 559 (1965)
Siegel,L.M. and Monty,K.J., Biochim.Biophys.Acta, 112, 346 (1966)
Talwar,G.P. et al., Proc.N.A.S. 52, 1059 (1964)
Toft,D. and Gorski, J.Proc.N.A.S. 55, 1574 (1966)
Toft,D., Shyamala,G. and Gorski,J. Proc.N.A.S. 57, 1740 (1967)