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ESTRODİOL RESEPTÖRÜN, DOMUZ UTERUSUNDAN ELDE EDİLEN LİZOZOMCA ZENGİN FRAKSİYON TARAFINDAN İNAKTİVE EDİLMESİ

IN VITRO INACTIVATION OF THE ESTRODIOL RECEPTOR BY A LYSOSOME–ENRICHED FRACTION FROM PIG UTERUS

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SUMMARY

Cytosol and lysosomes were prepared from pig uterus. Inactivation of estrodiol receptor in cytosol was investigated with and without lysosomes from pigendometrium. Lysosomes fraction of pigutrus inactivated the binding site of estrodiol receptor significantly.

KEYWORDS : lysosomes/estrodiol receptor/pig uterus

ÖZET

Sitosol ve lizozom domuz uterusundan hazırlandı. Sitosol içindeki estrodiol reseptörun inaktivasyonu kendi halinde veya domuz endometriumundan hazırlanan lizozom ile araştırıldı. Domuz uterusundan elde edilen lizozom estrodiol reseptorun bağlanmasını önemli ölçüde inaktive etti.

INTRODUCTION

Research about estrodiol receptor began in 1957 (1). The first specific steroidbinding protein in the cell was found by Jensen et al (2, 3). This protein was extracted with buffer and characterized by sucrosgradient zentrifugation and named as receptor. In 1966 Jungblut extracted an estrodiol receptor from the nucleus fraction of uteri. After injection estrodiolsolution, takes uteruslumen very quickly. In a few minutes an acumulation of receptor and steroid flow in the cell nuclei (4). In this time cytosol receptor is reduced. After 5–7 hours cytosolreceptor concentration

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increases again. However in the cell nuclei estrodiol receptor sinks during this time. Nearly 40 % of estrodiol receptors come again in the cell nuclei, but the rest 60 % is synthesized new in the cytoplasma. It has been shown that estrodiol modifies the level of its receptor in both the rat (Jensen et al 1969 (5), Gorski et al 1970 (6), and the pig uterus (Jungblut et al 1976 (7) by enhancing receptor biosynthesis. Invitro studies by Coulson and Pavlik 1977 have indicated that cytosol is not involved in receptor degradation, while Peck et al (1973) (8), suggested that the instability of the estrogenreceptor might result from its susceptibility to lysosomal attack. How does estrodiol receptor metabolize, and is them for it or is it the work of the proteasen enzym? Do lysosomal enzyms play a role in the inactivation of estrodiol receptor? We isolated lysosomes from pig uterus and incubated estrodiol receptor in cytosol. We inwestigeted the inanctivation of estrodiolreceptor with and without lysosomal enzyms.

MATERIAL AND METHODS

The preparation of cytosol, electrophoresis were carried out to Hekim, N and Jungblut P.W (9). The preparation of mitochondria–lysosomas and lysosomes fraction and determination of enzyms were done according to Sierralta, W et all (10).

Radioactive estrodiol came from Amersham, agar purum and the protein determination kit from Behringwerke, the rest of chemicals were purcased from merck.

Measurement of radioactiviy : Samples of extracts and fraction of density gradient analysis were counted in polyethylene vials after addition of 15 ml of scintillation fluid (80 g naphthalene, 5 g PPO, 50 mg POPOP/1000 ml xylol–dioxane 1:2) in Packard Tri–Carbs model 3320 with trityum efficiencies of 40–46%.

RESULTS AND DISCUSSION

Isolation of lysosomes from pig uterus : The pig uterus homegenat in sucrosgradient was centrifuged and fractionalized. We used N–acetyl β–glucosaminidase for lysosome and ICDH for mitochondria as markerenzym which were measured in each fraction. The peaks collected as lysosome, and mitochondria–lysosomes fraction respectivly.

Lysosomes was separeted from pig uterus and tested as flowing.

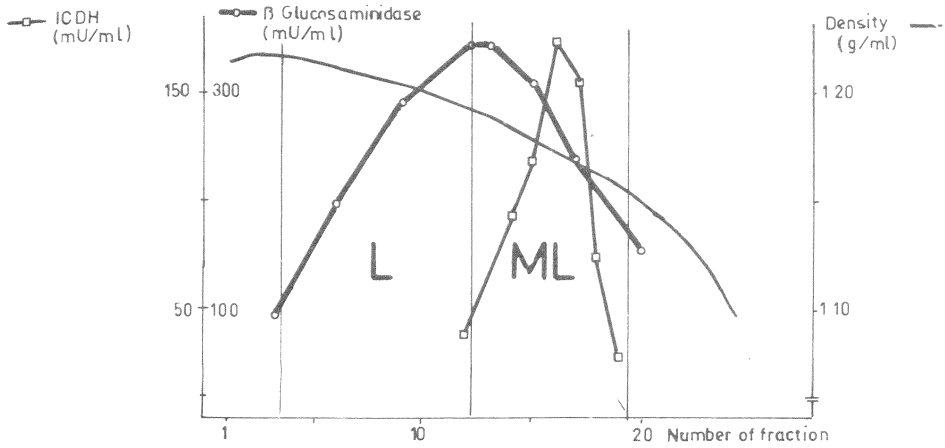

Fig – 1 : Isopycnic subfraction of a mitochondrial/lysosomal fraction from pig endometirium was homegenized with buffered sucrose and separated on a 25–52 %, w/w sucrosgradienten. 2 ml of a 1:6 suspension of a 17500 g pellet from endometriumhomegenat, in 50 mM tris, 3mM EDTA, 0.25 M sucrose pH 7.5 was carried out on a 27 ml of a 25–52 % in sucrosgradienten. After 15 hours centrifugation at 10 000 rpm 1 °C the activity of enzyms were measured in the fraction and then pooled. The density was calculated with refraktion index.

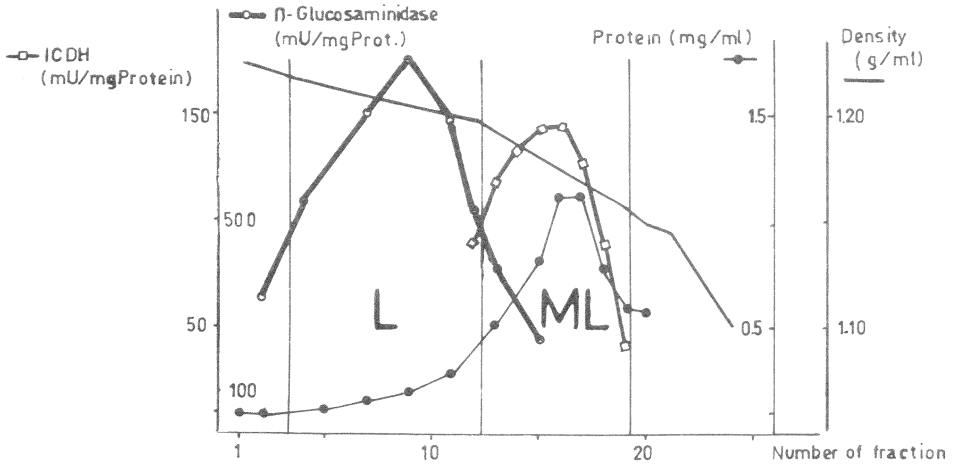

Fig – 2 : The specific activity of β– glucosaminidase and ICDH of a ML and L. The Specific activity of fraction was calculated enzymactivity and protein concentration.

Table – 1 : HM : Homegenat, ML : Mitochondria–Lysosomes, L : Lysosomes

	Protein concentration (mg/dl)	N–acetyl–β–glukose-aminidase (mu/ml)	(mu/mg prot)	ICDH (mu/ml)	(mu/mg prot)	PURIFICATIO fold
HM	45	1890	42	1350	30	1
ML	14	2590	185	1446	103	4.4
L	0.72	610	847	–	–	20

2 pellets of L containing 0.72 mg protein were dissolved in 250 µl of the buffer (10 mM Tris + 4mM EDTA + 5 mM DTT + 0.5 % surfynol) and incubated for 24 hr, at 4 °C, then frozen and thawed three times. After then cytosol was incubated with this lysosomes suspension or mitochondria–lysosomes fraction from pig uterus endometrium.

Table – 2 : These mixtures (I, II, III) were incubated at room temperature for 1 hr, then in a cold room for 24 hr, then were done electro–phoresis. Agargel was cut in pieces. The pieces were measured in sicintilation liquid in the counter.

	I	II	III
Cytosol	100 µl	100 µl	—
Lysosomes sus	100 µl	—	100 µl
E2 (2.10^{-7})	12.5 µl	12.5 µl	12.5 µl
Buffer	37.5 µl	137.5 µl	137.5 µl
total	250 µl	250 µl	250 µl

Table – 3 : Inactivation of estrodiol receptor in cytosol with lysosomes fraction.

	(+) site of gel	(–) site of gel	total
Cytosol	2736 cpm	2358 cpm	5904 cpm
cyt+lys	564 cpm	2076 cpm	2640 cpm
Lysosomes	202 cpm	882 cpm	1084 cpm
Inact.	79.4 %	12. 9 %	48.2 %

CONCLUSION

The effect of lysosomes enzyms on estrodiol receptor is more at (+) site than (–) site of the agar gelectrophoresis. CPM in lysosomes may occur during the contamination of estrodiol receptor with lysosome–enriched fraction but may however come from the background of counter.

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