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Comparison of Phytooestrogen-Coumestrol and Oestrone Effects on the Liver Membranes Insulin Receptors in Ovariectomized Female Rats

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The presence in plants of oestrogens, compounds which interfere with normal reproductive processes has been shown for over sixty decades (Farnsworth et al. 1975). Phytooestrogens have been detected in a variety of plants (Price and Fenwick 1985); coumestrol first isolated from ladino clover and alfalfa (Bickoff et al. 1957), is the most oestrogenic of such compounds. Its amount in the plant is changeable and depends in the large extenton the stage of growth (Bickoff et al. 1960), infection with foliar pathogens (Kain and Biggs 1980) or environment pollution (Baruch et al. 1983). Reproductive disorders attributed to ingestion of food containing coumestrol have been reported in many animals (Adler and Trainin 1960; Newsome and Kitts 1980; Le Bars and Hurard 1982; Burroughs et al. 1985). However, coumestrol like oestrogens can affect not only animal reproduction but also may influence on carbohydrate and lipid metabolism (Beguin and Kincaid 1984; Nogowski 1987; Nogowski 1990). It was also shown to decrease the blood insulin level in the young immature female rats while introduced in food (Nogowski 1987).

The effect of insulin on the animal metabolism is a result of insulin secretion, its binding to the specific membrane receptor and post receptor events (Gammeltoft 1984; Grunberger 1991). The intent of present study was to determine if coumestrol given to the ovariectomized female rats would change the characteristics of liver membrane insulin receptors and if the observed changes are comparable to those of oestrone.

MATERIALS AND METHODS

Female Wistar rats weighing 140-160 g were kept in standard room conditions with free access to LSM rat chow (Bacutil - Poland) and water. The animals were ovariectomized to eliminate endogenous source of oestrogens. Ten days after ovariectomy the rats were divided into three groups

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with six in each group. The rats of groups E and C were injected subcutaneously with either 20 mg of oestrone (Polfa - Poland) or 200 mg of coumestrol (Eastman - Kodak), per day, for 14 days period. Both compounds were dissolved in DMSO (dimethylsulphoxide - Sigma). The control group (K) was treated with just the solvent. On the 15th day of experiment the rats were eliminated by decapitation and the blood and liver were taken. Blood serum insulin was determinated radioimmunologically with RIA-Ins (Swierk - Poland) kit. The liver membranes were prepared according to Havrankowa et al. (1978) and dissolved in the incubation buffer (20 mmol/l TRIS - HCl, pH 7.4, 0.1 % BSA) to the final concentration of 0.5 mg of protein per 1 ml of incubation mixture. Liver membranes were incubated in triplicate at 4°C for 16 h in the presence of 0.03 nmol/l of ^{125}I-insulin (OPIDI - Świerk, Poland) with increasing amounts of unlabeled porcine insulin up to 700 nmol/l. The non-specific binding was measured with 10 µmol/l of native insulin. After the incubation the tubes were centrifuged at 15000g, the supernatant was discarded and the membranes were directly counted in a gamma counter. The counting results were used to construct displacement curves and Scatchard plots. The binding capacity and dissociation constant were counted by using a microcomputer program LIGAND PC v.3.1 (Munson and Rodbard 1980). One way analysis of variance and multiple range test were used to establish the differences between groups.

RESULTS AND DISCUSSION

The Fig.1 presents ^{125}I-insulin binding to the liver membranes from control and experimental rats. The liver membranes of coumestrol treated rats bound less insulin over its concentration range of 0.2 to 25 nmol/l. Oestrone did not show this effect. However the upward tendency in the insulin binding was observed at higher insulin concentration both after coumestrol and oestrone administration. Fig. 2 shows the Scatchard plots for the different groups of rats. In the control and oestrone treated groups the plots are almost superimposed. The plot for the coumestrol treated rats was somewhat steeper in the region of low insulin concentration. This suggest that these animals had fewer binding sites. The results in Tab. 1 support this suggestion. The binding capacity of high affinity insulin receptors of coumestrol treated rats was statistically significant less than those of control group. Simultaneously increase in receptor affinity was noticed.

Scatchard plots (Fig. 2) for control, coumestrol and oestrone treated animals are similar in their shape to those previously observed for both isolated and membrane insulin receptor in rat liver (Gammeltoft 1984). The curvilinearity of the plots can be due to the presence of more than one class of receptors or site-site interactions, or both. Assuming there are two classes of receptors

Table 1. Effect of coumestrol and oestrone on serum insulin level and binding parameters of liver plasma membranes insulin receptors.

	Control	Coumestrol	Oestrone
Insulin (μU/ml)	40 ± 3	39 ± 4	41 ± 3
HAIR			
R_O (fmol/mg protein)	189 ± 40	74 ± 8	161 ± 31
K_D (nmol/l)	0.73 ± 0.19	0.34 ± 0.03	0.58 ± 0.16
LAIR			
R_O (pmol/mg protein)	3.9 ± 1.8	6.5 ± 1.5	8.7 ± 3.4
K_D (nmol/l)	256 ± 90	131 ± 56	404 ± 180

HAIR - high affinity insulin receptor, LAIR - low affinity insulin receptor
R_O - binding capacity, K_D - dissociation constant
$p < 0.05$; Values expressed as mean ± SE, n = 6 in each group

(high affinity, low capacity - HAIR and low affinity, high capacity receptor - LAIR), the affinities of both types of receptor did not changed significantly after oestrone treatment (Tab. 1). However, the binding capacity of the HAIRs was significantly reduced by phytooestrogen, with simultaneous increase in their affinity. The mechanism whereby coumestrol influences on the insulin receptor is yet unknown. The insulin receptors have been shown to be regulated by insulin itself or/and by heterologoues hormones (Andersen 1990). Considering that coumestrol administration did not change the blood insulin level, the first possibility of regulation can be rather excluded. However, some studies have indicated that many hormones may have a regulatory effect on insulin receptor. For example insulin receptor binding in adipocytes is increased by oestradiol (Ryan and Enns 1988), whereas progesteron may increase (Mendes et al. 1985) or decrease insulin receptor binding (Ryan and Enns 1988). Glucocorticoides have been shown to enhance the number of insulin receptors on the cultured human lymphocytes surface (Fantus et al. 1982) as well as to decrease the insulin binding to the rat liver membranes (Kahn et al. 1978). Finally, we observed in our previous experiments (Nogowski et al. 1992) that coumestrol changed the rabbit red blood cells insulin receptors. The results presented in this paper suggest that phytooestrogen - coumestrol may, like steroid hormones, have a direct effect on the insulin receptor. On the other hand, while the HAIR capacity was

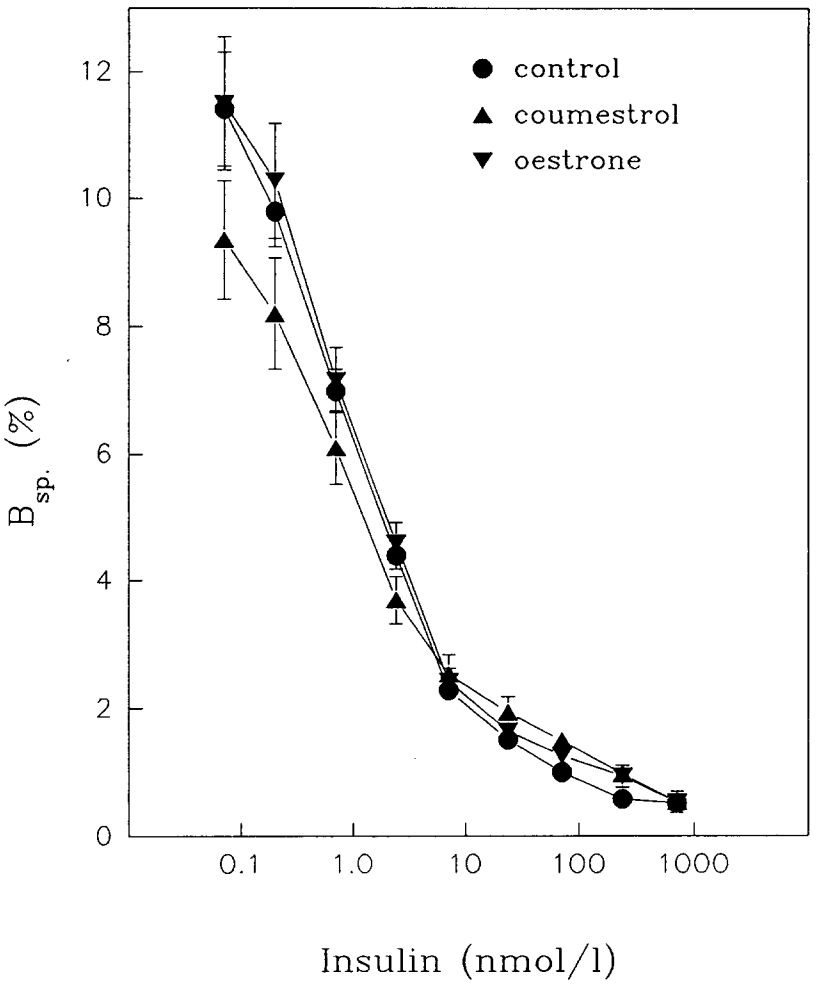

Figure 1. Displacement of ^{125}I-insulin binding to rat liver plasma membranes by unlabeled pork insulin after oestrone or coumestrol subcutaneous injections (0.5 mg of membranes protein was incubated at 4°C for 24 hrs with 0.03 nmol/l of I-insulin in the absence or presence of increasing concentration of unlabeled insulin). Values expressed as mean ± SE (n = 6).

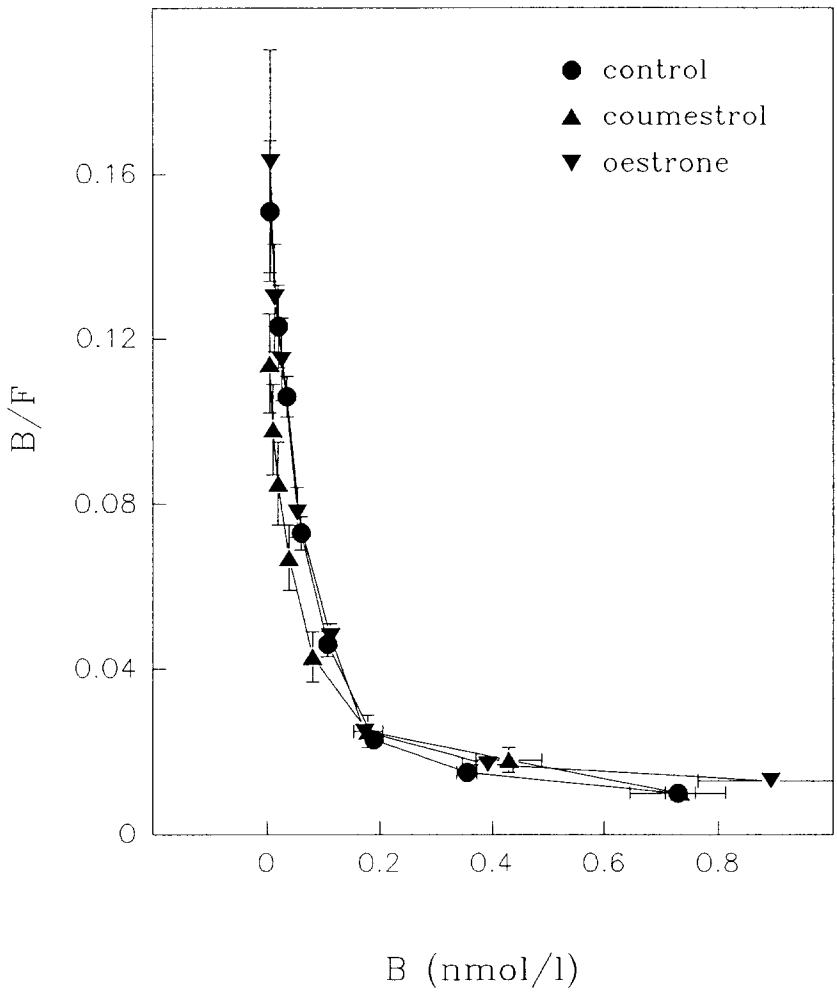

Figure 2. Scatchard plots of the ^{125}I-insulin binding to the rat liver plasma membranes after oestrone or coumestrol subcutaneous injections. B - insulin bound to the liver plasma membranes, F - free insulin (unbound to the liver plasma membranes). Values expressed as mean ± SE (n = 6).

decreased after coumestrol administration to ovariectomized rats, the upward tendency in LAIR was observed (Tab. 1). Some authors suggest (Corin and Donner 1982) that the presence of two pools of insulin binding sites with different affinities (measured as K_A) to ligand, is a result of transformation of HAIRs into LAIRs, possibly through the intermediate insulin receptors. Perhaps coumestrol is somehow involved in this transition.

The influence of coumestrol on human metabolism has not yet been ascertained, although this phytooestrogen can enter the human body directly via common food plants or indirectly by the residues in animal products and milk from livestock grazing on oestrogenic pasture (Price and Fenwick 1985). The results obtained in different species should be considered separately and extrapolation to the human should be done only with great caution. However this work points to the disirability of monitoring the contents of coumestrol in these products for human consumption witch are suspected to contain phytooestrogens.

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