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Effect of a mycooestrogen, zearalenone, and a phytooestrogen, coumestrol on the liver membrane insulin receptor of ovariectomized female rats

Leszek Nogowski, Paweł Maćkowiak and Krzysztof W. Nowak

Department of Animal Physiology and Biochemistry, Agriculture Academy, Wołyńska 35, 60-637 Poznań, Poland

Zearalenone and coumestrol, compounds of plant origin, when administered to animals, exert an oestrogen-like activity. Zearalenone, the most common mycooestrogen, is a secondary metabolite of many fungal species, mainly of the Fusarium genus, proliferating in poorly stored grain, oil seeds, and hay [1]. Coumestrol, in turn, is the most oestrogenic substance among the compounds produced by various legumes [2]. Both zearalenone and coumestrol cause many disorders in reproduction of livestock and laboratory animals [1], affect carbohydrate and lipid metabolism [3, 4] and alter the blood insulin level [3]. The aim of the present study was to compare the effect of coumestrol and zearalenone on the liver membrane insulin receptors in ovariectomized rats.

Female Wistar rats weighing 160 - 200 g were kept in standard room conditions with free access to LSM rat chow (Bacutil, Poland) and water. Ten days before the experiments the animals were anaesthetised with ether and ovariectomized through the dorsal access to eliminate the source of oestrogens. The examined compounds, dissolved in dimethylsulphoxide (Sigma) were injected subcutaneously (200 µg/day either of coumestrol - Eastman Kodak or zearalenone - Sigma). The animals of the control groups were treated with the solvent alone which, as checked previously, did not affect liver membrane insulin receptors.

After ten days, the rats were decapitated, the blood and liver were sampled, and the uterus was weighed immediately. Blood serum insulin was determined radioimmunologically with the RIA-Ins kit (Świerk, Poland). The liver membranes were prepared according to Havrankova *et al.* [5] and dissolved in the incubation buffer (20 mmol/l Tris/HCl, pH 7.4, 0.1% bovine serum albumin) to a final concentration of 0.5 mg of protein per 1 ml of the 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 the increasing amount of unlabeled porcine insulin up to 700 nmol/l. The non-specific binding was measured in the presence of 10 µmol/l of porcine insulin. After incubation the membranes were centrifuged off at 15000 × *g*, and the radioactivity was directly counted in a gamma counter. The binding capacity and dissociation constant were counted by using a microcomputer LIGAND program [6]. One way analysis of variance and the multiple range test were used to establish significant differences of the results.

The biological effect of plant oestrogens is most often demonstrated by measuring the enlargement of uterus in immature or ovariectomized animals [1]. Coumestrol and zearalenone caused a significant, similar increase in uterus weight of ovariectomized rats (Table 1); the effect of zearalenone seems to be a little smaller than that of coumestrol.

Despite almost the same oestrogenicity, coumestrol and zearalenone differ in their influence on insulin level (Table 1) and the liver membrane insulin receptors. Liver membranes of the coumestrol treated animals bound less insulin over the concentration range of 0.2 to 2.5 nmol/l compared with controls (Fig. 1A), whereas zearalenone had practically no effect

Table 1

Effect of coumesterol and zearalenone on uterus weight and blood insulin level in ovariectomized rats.
Each result represents mean ± S.E., n = 6 in each group

	Control	Coumestrol treated	Control	Zearalenone treated
Uterus weight (mg)	60 ± 8	167 ± 11*	66 ± 8	141 ± 7*
Insulin (μU/ml)	29 ± 3	28 ± 4	24 ± 3	34 ± 4*

*Significant at $P < 0.05$ in comparison to the control group

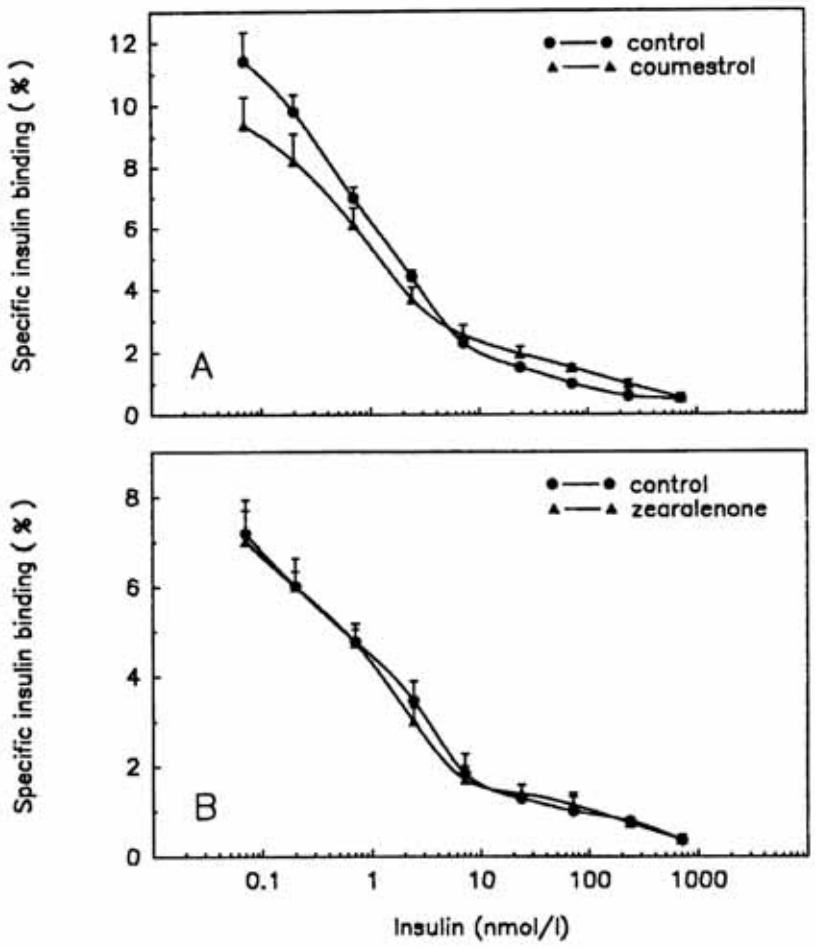

Fig. 1. Displacement of ^{125}I-insulin in rat liver plasma membranes by unlabeled pork insulin after subcutaneous injections of coumestrol (A) and zearalenone (B).
The membranes protein (0.25 mg) were incubated at 4°C for 16 h with 0.03 nmol/l of ^{125}I-insulin in the presence of increasing concentration of unlabeled insulin. Values expressed as mean ± S.E. (n = 6)

(Fig. 1B). Scatchard plots for the control and zearalenone treated rats are superimposed (Fig. 2B). The plot for the coumestrol treated group is somewhat steeper in the region of low insulin concentration (Fig. 2A). This suggests that the coumestrol treated animals had fewer sites available for insulin binding. The binding capacity of high affinity insulin receptors (HAIR) of the latter group was significantly lower than that of the control group (Table 2). At the same time the receptor affinity (measured as the dissociation constant, K_D) was increased.

The mechanism whereby coumestrol or zearalenone influence the insulin receptors is still unknown. The response of insulin receptors is

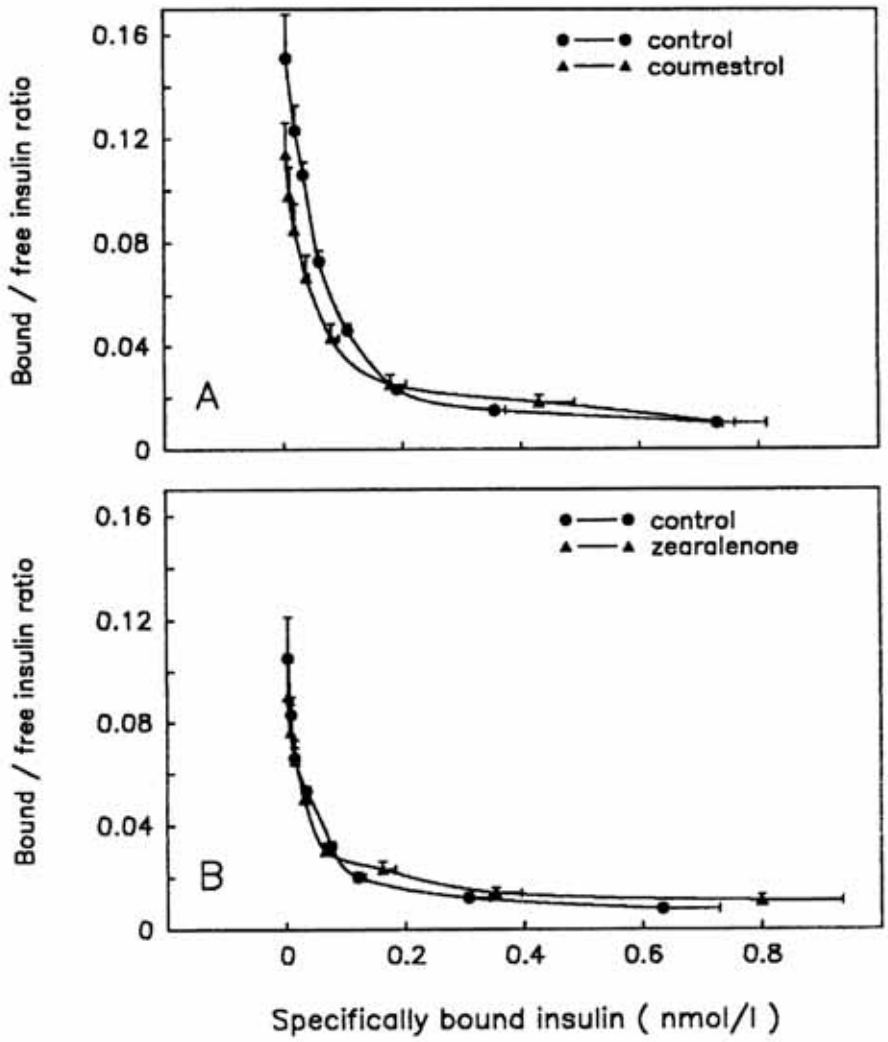

Fig. 2. Scatchard plots of ^{125}I-insulin binding to rat liver plasma membranes after subcutaneous injections of coumestrol (A) and zearalenone (B).
Values expressed as mean ± S.E. (n = 6)

Table 2

Effect of coumestrol and zearalenone on binding parameters of high and low affinity insulin receptors (HAIR and LAIR) of the liver plasma membranes.
Results expressed as mean values ± S.E.; n = 6 in each group

	Control	Coumestrol treated	Control	Zearalenone treated
HAIR				
B_{max} (fmol/mg protein)	189 ± 40	74 ± 8*	113 ± 19	100 ± 16
K_D (nmol/l)	0.73 ± 0.19	0.34 ± 0.03	0.77 ± 0.20	0.79 ± 0.32
LAIR				
B_{max} (pmol/mg protein)	3.9 ± 1.8	6.5 ± 1.5	9.2 ± 3.8	8.3 ± 2.4
K_D (nmol/l)	256 ± 90	131 ± 56	399 ± 194	225 ± 80

*Significant at $P < 0.05$ in comparison to the control group. B_{max}, binding capacity; K_D, dissociation constant

regulated by insulin itself and/or by heterologous hormones [7]. In the present study, zearalenone increased the blood insulin level (Table 1) and, possibly in this way, diminished slightly the binding capacity of liver membrane insulin receptors. The influence of insulin on its own receptor is regarded as a negative cooperativity or the "down regulation" effect [8]. However, a much stronger decrease in the binding capacity of the liver membranes observed in the coumestrol treated rats can not be explained by the "down regulation" effect because no changes in the blood insulin level were found (Table 1). Some studies indicated that many hormones could exert a regulatory effect on insulin receptors in various cells: e.g. glucocorticoids raise the number of insulin receptors on the surface of cultured human lymphocytes [9] and decrease binding of insulin to the rat liver membranes [10]. Insulin binding in adipocytes is increased by oestradiol [11] whereas progesterone could either increase [12] or decrease [11] insulin binding in these cells. We have observed in our previous experiments that coumestrol altered binding capacity of insulin receptors in rabbit erythrocytes [13].

The results of the present study suggest that coumestrol, a phytooestrogen, influences the liver membrane insulin receptors in ovariectomized rats, whereas the effect of the mycooestrogen, zearalenone, appeared to be negligible.

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