EFFECTS OF PROLONGED ETHANOL ADMINISTRATION ON THE HEPATIC ESTROGEN RECEPTOR IN THE RAT

Tomas CRONHOLM and Håkan ERIKSSON
Department of Chemistry, Karolinska Institutet, Box 60 400, 104 01 Stockholm, Sweden

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1. Introduction

Male alcoholics with liver disease are frequently feminized [1]. In addition to clinical signs such as gynecomastia, biochemical changes have been reported. Thus, the hepatic synthesis of several estrogen-dependent proteins is increased [2,3]. Due to difficulties to explain these changes merely on the basis of changes in plasma estrogen levels, it was hypothesized that the amount of estrogen receptors was increased [4]. Evidence for the presence of a receptor protein for estrogens in liver tissue has been presented for chickens [5], amphibians [6] and mammals [7,8]. The receptor is present in livers from rats of both sexes [8,9]. Male rats fed an ethanol-containing diet develop hypogonadism and have been suggested as experimental models for the feminization seen in male alcoholics [10]. Here, male and female rats were given the liquid ethanol-containing and control diets described in [11]. The cytosolic estrogen receptor in liver was measured after 7 or 24 days. In the ethanol-treated male rats the receptor content, expressed as fmol/mg cytosolic protein, was significantly higher (~50%) than in the controls. In female rats the amounts were higher than in male rats, but no effect of ethanol was seen.

2. Experimental

2.1. *Animals*

Sprague-Dawley rats were fed the liquid diet described in [11] for 7 and 24 days. The alcohol diet which provides 36% of the energy from ethanol, and the control diet with carbohydrate replacing ethanol, were obtained from BioServ Inc. (Frenchtown NJ). Alcohol was introduced step-wise, and the controls were pair-fed [11]. Each group consisted of 5 rats. Initial weights were chosen to give about equal weights at the end of the feeding period. At the start, the rats in the 7 day experiments weighed 160–175 g (male) and 200–220 g (female), and in the 24 day experiments 110–120 g (male) and 175–190 g (female). The weight changes were small in the 7 day experiments, and in the 24 day experiments the controls had gained 85 ± 2 (SD) g (male) and 28 ± 10 g (female), whereas the ethanol-treated rats had gained 69 ± 11 g (male) and 20 ± 4 g (female). The amount of lipid in the livers from male rats fed the liquid diets for 24 days was determined by Folch extraction [12]. Livers from ethanol-treated rats contained more lipid (580 ± 191 (SD) mg) than the controls (150 ± 16 mg) [11].

2.2. *Receptor determination*

A detailed description of the procedure will be given elsewhere (H. E., in preparation). All operations were performed under light ether anaesthesia. The liver was perfused in situ with saline–albumin, excised and homogenized in TE-buffer (0.01 M Tris–HCl–0.0015 M EDTA, pH 7.4 at 0°C) in a Potter-Elvehjelm homogenizer. The homogenate was centrifuged at 82 000 × *g* for 30 min, the lipid layer removed and the supernatant recentrifuged at 200 000 × *g* for 60 min. The cytosol fraction obtained was transferred to an E-flask and ammonium sulphate added to 35% saturation. The precipitate was harvested and dissolved in a small volume of TE-buffer and passed through a 1.5 ml column of Lipidex® 1000 (Packard Instrument Co., Downers Grove IL) equilibrated with buffer to remove endogenous steroids and lipids [13,14]. The eluted sample was diluted with TE-buffer to yield 5 mg protein/ml and used in binding experiments.

Saturation analysis of cytosol binding sites was

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performed with [^{3}H]Moxestrol as ligand. Binding data were analyzed according to Scatchard [15]. Regression analysis and Student's t-test of significance were performed on Hewlett Packard HP-85 computer. Protein was determined by the Lowry method [16]. Radioactivity was analyzed by scintillation counting using Instagel® (Packard Instrument Co.) as scintillator.

3. Results

The binding data obtained indicated the presence of estrogen high-affinity binding sites and non-saturatable binding in the liver cell cytosol. The former sites conformed to the characteristics of the classical estrogen receptor and displayed a K_d of 0.9 nM.

Livers from ethanol-treated rats showed significantly higher weights than those from control animals (table 1). This difference is most likely due to accumulation of triglycerides in livers of treated rats [11]. Thus, no difference was observed between ethanol-treated and control rats with respect to liver cytosol protein content (table 1). For these reasons the quantity of estrogen receptors is not related to liver weight but to the content of cytosolic protein and expressed as fmol/mg protein.

The receptor concentration was higher in female than male rats ($P < 0.002$ and $P < 0.10$ in the 7 day and 24 day experiments, respectively). The variation was higher in the female rats than in the males.

The ethanol-treated male rats had ~50% higher receptor concentration than the corresponding controls. This effect was seen both after 7 days and 24 days of treatment and was highly significant ($P < 0.01$ in both studies). No difference was seen between ethanol-treated and control female rats, with respect to receptor content.

The level of non-specific, low-affinity, estrogen binding in the liver cytosol was lower in ethanol-fed rats than in controls. This finding is similar to the effects of castration [17] and indicates that ethanol-feeding either lowers the capacity or affinity of non-specific sites for the ligand.

4. Discussion

The pronounced accumulation of lipids during prolonged feeding of an ethanol-containing diet [11] causes difficulties in the quantitation of hepatic receptors for lipid-soluble hormones. Thus, it was considered necessary to remove lipids which were not removed by the first, low-speed, centrifugation, by chromatography of the cytosol preparation on a column of Lipidex® 1000 [13,14]. Furthermore, by a combination of ammonium sulphate precipitation and use of the synthetic estrogen Moxestrol as receptor ligand, the non-specific binding could be reduced considerably, permitting accurate determinations.

Both in the 7 day and the 24 day feeding experiments the male rats given ethanol had significantly higher levels of estradiol receptors in the liver than the male control rats. This ethanol effect tended to

Table 1
Effect of ethanol feeding on the hepatic estrogen receptor

Sex	Days	Group	Liver wt (g)	Cytosolic protein (mg)	Receptor (fmol/mg protein)
Male	7	Control	6.5 ± 0.6	258 ± 32	15 ± 4
Male	7	Ethanol	7.6 ± 0.7[a]	254 ± 29	23 ± 3[b]
Male	24	Control	7.1 ± 0.4	340 ± 61	16 ± 1
Male	24	Ethanol	8.5 ± 0.9[b]	301 ± 23	23 ± 4[b]
Female	7	Control	6.8 ± 0.4	329 ± 32	35 ± 9
Female	7	Ethanol	7.6 ± 0.4[b]	355 ± 36	33 ± 9
Female	24	Control	7.5 ± 1.1	280 ± 34	22 ± 10
Female	24	Ethanol	8.8 ± 0.9[a]	278 ± 54	29 ± 13

All values are mean ± SD; [a,b] significant ($P < 0.05$ and $P < 0.01$, respectively) differences between ethanol and control groups

eliminate the sex difference with respect to receptor content that was observed in the control rats.

The mechanisms regulating the receptor content in liver tissue have not yet been fully elucidated. Both prolactin and growth hormone have been suggested to be responsible for the maintenance of a normal receptor concentration [18]; glucocorticoids were found necessary for this response [19]. Alcoholics with gynecomastia and cirrhosis have elevated basal prolactin levels in blood [20], and ethanol administration to human volunteers has been reported to cause an elevation of growth hormone levels [21]. Acute ethanol administration causes an increase in blood glucocorticoids, and chronic administration might attenuate this response and change the diurnal rhythm of the glucocorticoids [22]. Thus, there are several possible mechanisms by which ethanol could cause the increase of the hepatic estradiol receptor. Prolonged ethanol administration to rats and humans causes an increase in the activity of the hepatic 3-oxo-5α-steroid:NADP Δ^4-oxidoreductase (EC 1.3.1.22) [23]. This enzyme which catalyzes inactivation of testosterone is induced by a pituitary factor that may be identical with prolactin or growth hormone [18]. An increased level of estradiol receptors might be responsible for the increased synthesis of sex-steroid binding globulin, cortisol-binding globulin and thyroxine-binding globulin in alcoholic men [2,3]. If the increase is not limited to the receptor in liver it might also explain other signs of feminization in alcoholic men. Thus, in breast tissue it could cause gynecomastia by increasing the responsiveness to prolactin [24].

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