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Estrogen Action on Hepatic Synthesis of Angiotensinogen and IGF-I: Direct and Indirect Estrogen Effects

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In the present study effects of estrogens (natural estradiol and synthetic ethinyl estradiol) on liver derived proteins (angiotensinogen, IGF-I) were investigated *in vivo* in ovariectomized rats and *in vitro* in a rat hepatoma cell line (Fe33). The aim of this study was to establish both an animal and an *in vitro* model for quantification of the hepatic activity of given estrogenic compounds, and to study underlying mechanisms as regards the question of direct or indirect mode of estrogen action. In ovariectomized rats subcutaneous (s.c.)-treatment for 11 days with either estradiol (E2) or ethinyl estradiol (EE) (dose range 0.1–3 μg/animal/day) induced a comparable dose-dependent increase in uterine weight indicating a similar estrogenic potency of the two estrogens. Equipotency was also found as regards the effects on IGF-I plasma levels which dose-dependently decreased by about 50% at the highest dose tested (3 μg/animal/day). The decrease in IGF-I serum levels was accompanied by a significant 40% decrease in liver IGF-I mRNA. In contrast angiotensinogen plasma levels were affected only by EE (60% increase for the 3 μg/animal/day dose) but not by E2. When rats, in addition to ovariectomy, were also hypophysectomized (substituted with human growth hormone and dexamethasone) angiotensinogen again increased by 80% upon administration of 3 μg/animal/day EE, whereas IGF-I remained unaffected by EE. In a rat hepatoma cell line (Fe33) which is stably transfected with an estrogen receptor expression plasmid, 10 nmol/l EE for 24 h caused a 2.4-fold increase in angiotensinogen mRNA level. We conclude from our studies that estrogen effects on angiotensinogen serum levels in the rat are direct effects via the hepatic estrogen receptor, whereas estrogen effects on IGF-I serum levels are indirect effects, the primary target of estrogen action being probably the pituitary. The changes in angiotensinogen serum levels in the rat model are comparable to the situation in humans indicating the rat model and the Fe33 model to be useful tools to study the hepatic activity of estrogenic compounds.

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INTRODUCTION

Oral estrogen therapy in women causes changes in several liver derived proteins like coagulation factors [1], steroid hormone binding proteins [2], angiotensinogen [3], somatomedin C (IGF-I) [4] and apolipoprotein A1 [5]. Interestingly the natural estradiol (E2) exerts reduced hepatic activity after parenteral compared to oral administration in humans [6], whereas the widely used synthetic ethinyl estradiol (EE) acts on liver derived proteins irrespective of the route of administration [7]. This different pharmacological action of EE compared to E2 is probably caused by the 17-ethinyl substitution, that protects EE against a rapid metabolism and leads to enterohepatic recirculation [8].

In order to establish a test model for studying direct estrogen effects on liver derived proteins we focussed in this study on IGF-I and angiotensinogen. Estrogen effects on IGF-I were demonstrated in humans [4] and rats [9, 10]. However, it is so far unclear whether the mechanism of estrogen action on this parameter is direct or indirect, e.g. by modulation of growth hormone secretion. As regards angiotensinogen it is suggested that estrogens stimulate its synthesis in the liver directly via the hepatic estrogen receptor [12].

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The aim of this study was to establish an animal and a suitable *in vitro* model for quantification of the hepatic activity of given estrogenic compounds and to study underlying mechanisms as regards the question of direct or indirect mode of action. Animal studies were performed in both ovariectomized (ovex) and ovari- and hypophysectomized (Hx) rats. In a separate approach Hep G2 (human hepatoma), primary rat hepatocytes, FTO-2B and Fe33 (both rat hepatoma) cells were studied in order to identify a useful *in vitro* system.

EXPERIMENTAL

Hormones

EE, E2 and dexamethasone (Dex) were provided by the medical chemistry of Schering AG (Berlin, Germany). Human growth hormone (hGH, Genotropin) was obtained from Kabi-Pharma GmbH (Erlangen, Germany). Glucagon was obtained from Sigma (München, Germany).

In Vivo Studies

Animals

Female rats (Wistar, Schering AG) weighing about 200 g were kept in macrolon cages with free access to water and were fed by a standard rat diet (Altromin) *ad libitum*. Light/dark-cycle was 14/10 h.

Animal treatment

The animals were ovex under ether narcosis 10 days prior to the beginning of application of compounds. Hx was performed under ether narcosis 4 days prior to the start of estrogen treatment. In order to maintain the hepatic estrogen receptor (ER) level, Hx animals were substituted with 12 U/day hGH by osmotic minipumps (Alzet 2ML2) and Dex (50 μg/animal/day s.c.) until the end of the experiment.

Experiment 1. Ovex rats were treated s.c. for 11 days with either the synthetic estrogen EE or the natural hormone E2 with the following doses: 0.1, 0.3, 1 and 3 μg/animal/day (the vehicle used was benzylbenzoate–castor oil, 1:4, v/v). Autopsy was on day 12 after beginning of treatment, uterine weight was determined and angiotensinogen and IGF-I serum levels were measured by radioimmunoassay (RIA). For IGF-I determination a commercially available RIA was used (Nichols Institute Diagnostics, San Juan, U.S.A.). Before quantification, IGF-I was separated from IGF-I binding proteins by acidification of serum samples and by acid gel filtration [13] according to the manufacturer's advice. The detection limit was 0.122 ng/ml and intraassay variance was 9.7%, the interassay variance was 7.5%.

Angiotensinogen was measured using the RIA for angiotensin I before and after the generation of angiotensin I in presence of an excess of hog renin, at 37°C for 1 h. Under these conditions angiotensin I will be completely cleaved from angiotensinogen within 10 min. Free serum angiotensin I was determined by RIA according to Menard and Catt [14] as described previously [15]. The cross-reactivity of the angiotensin I antiserum and angiotensin II was $<5 \times 10^{-4}$. The intraassay variation was $4.29 \pm 1.37\%$ ($n = 7$) and the interassay variation was $4.02 \pm 1.22\%$. The assay system was calibrated using a standard for angiotensin I and II (Nat. Biol. Standards Board, Holly Hill, London, England).

Statistical analysis of IGF-I and angiotensinogen measuring data was performed by the Dunnett-test.

Experiment 2. Beginning on day 4 after hypophysectomy ovex + Hx rats were treated for 6 days with EE (3 μg/animal/day s.c.). The experiment comprised the following treatment groups:

group 1 = ovex
group 2 = ovex + Hx
group 3 = ovex + Hx, hGH
group 4 = ovex + Hx, hGH + Dex
group 5 = ovex + Hx, hGH + EE
group 6 = ovex + Hx, hGH + Dex + EE

Animals of all groups were treated with all vehicles.

For Dex and estrogens the vehicle consisted of benzylbenzoate–castor oil, 1:4, v/v, for hGH administration we used 100 mmol/l phosphate buffer, pH 7.4, containing 250 mmol/l glycine, as a vehicle. Autopsy was on day 10 after hypophysectomy. Changes in the liver ER level were determined in cytosol fractions of liver tissue by ligand binding experiments using [^{3}H]E2 as tracer (50 nmol/l) and E2 (5 μmol/l) for determination of non-specific binding. ER was determined in pooled samples of all treatment groups. The detection limit was 2 fmol/mg protein, intra- and interassay variations were below 10%. IGF-I and angiotensinogen were determined as described.

Experiment 3. Female ovex rats were treated for 6 days with EE 0.3–3 μg/animal/day s.c. dissolved in benzyl benzoate castor oil. Animals were killed, desanguinated and total RNA was isolated from liver tissue according to the guanidinium thiocyanate–phenol–chloroform extraction method of Chomczynski and Sacchi [16]. The preparation of polyA + -RNA was carried out according to Medcalf *et al.* [17]. "Northern" blot analysis was performed as described by Sambrook *et al.* [18]. For detection of IGF-I mRNA a commercially available DNA probe was used (engineered gene for mature human IGF-I, British Bio-technology, England), for detection of GAPDH a rat cDNA [32] was used. Hybridization was carried out according to a protocol for rapid hybridization (Stratagene, Heidelberg, Germany). Statistical analysis of scanning data was performed by the Dunnet-test.

In Vitro Studies

The preparation of isolated rat hepatocytes was carried out according to the collagenase perfusion method of Seglen [19]. Approximately 10^7 intact hepatocytes were spread on fibronectin/collagen G-coated 15 cm Petri dishes.

Primary cultures, FTO-2B (rat hepatoma cells, provided by Professor Ryffel, Essen, Germany) and HepG2 cells (American Type Culture Collection) were maintained in Dulbecco's minimal essential medium (DMEM, Serva, Heidelberg, Germany) without phenol red containing 5% charcoal-treated fetal calf serum (FCS, Serva, Heidelberg, Germany) and further supplements as described in the text. Fe33 cells (FTO-2B cells stably transfected with an ER expression plasmid by Kaling *et al.* [20]) were maintained in DMEM–Ham's F12 (1:1, v/v) without phenol red (Gibco/BRL, Berlin, Germany) containing 10% charcoal treated fetal calf serum (FCS) on gelatine-coated 15 cm Petri dishes. When serum free medium was used several supplements were added according to Edmunds *et al.* [21]. Total RNA was isolated from cell cultures according to the guanidinium thiocyanate–phenol–chloroform extraction method of Chomczynski and Sacchi [16]. The preparation of polyA + -RNA, "Northern" blot analysis and hybridization was carried out as described above. The angiotensinogen probe was taken from the clone pRag16 [22]. RT-PCR was

performed according to standard procedures [23]. Oligonucleotide primers for detection of ER transcripts were as follows: 5′-TGACCAACCTGGCAGACAGG-3′; (upstream) 5′-GCCTTTGTTACTCATGTGCC-3′ (downstream), yielding a 527 bp PCR product. As an internal control cyclophilin was used. Respective primers for RT-PCR were as follows: 5′-GGATTCATGTGCCAGGGTGG-3′ (upstream); 5′-CACATGCTTGCCATCCAGCC-3′, yielding a 213 bp PCR product. Determination of ER levels in cell culture cytosol preparations was performed by ligand binding assay as described or by using a commercially available enzyme immunoassay (Abbott, Wiesbaden, Germany).

RESULTS

In vivo studies

Experiment 1 (Fig. 1). In ovex rats s.c. administration of both, E2 and the synthetic estrogen EE, caused a comparable dose-dependent stimulation of uterine growth indicating that the two compounds exhibited similar estrogenic potency if applied s.c. Concomitantly IGF-I serum levels decreased dose-dependently to about 50% for the 3 μg dose of both, E2 and EE. In contrast a dose-dependent increase in angiotensinogen serum levels (up to 60% increase for the 3 μg dose) was produced by EE only, but not by E2. Treatment with 0.3 and 1 μg E2 and with 0.3 μg EE resulted in a marginal but significant decrease of angiotensinogen.

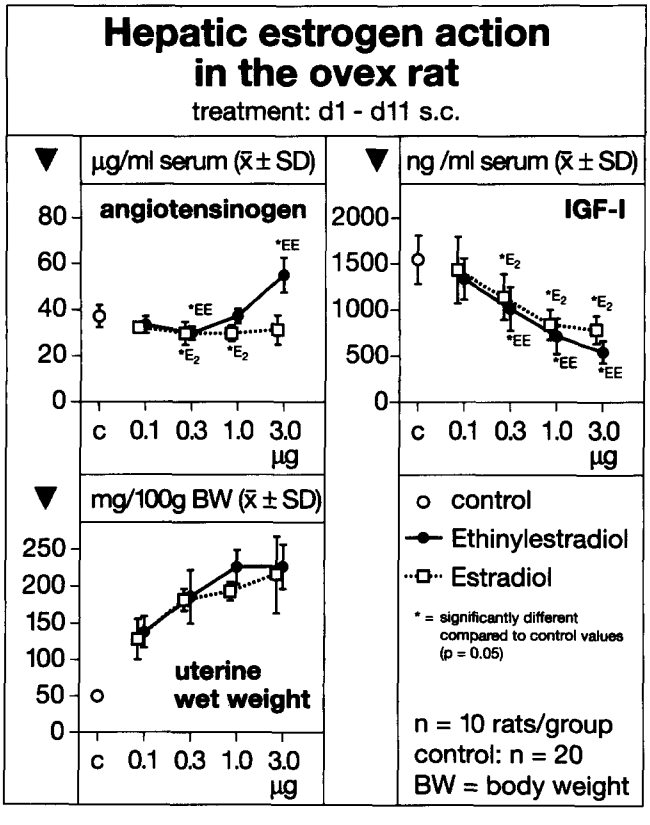

Fig. 1. Effects of E2 and EE on uterine growth, angiotensinogen and IGF-I serum levels in ovex rats treated s.c. with increasing doses of the respective estrogen for 11 days.

Comparable changes in IGF-I and angiotensinogen serum levels as observed after an 11 days treatment period with EE were also detected after 6 days of s.c. treatment (data not shown).

The different action of E2 and EE on angiotensinogen, but not on IGF-I, suggest, that these liver derived proteins are regulated by estrogens via different mechanisms. Angiotensinogen is supposed to be regulated directly via the hepatic ER [11, 12]. As IGF-I synthesis in the liver is controlled by growth hormone which is synthesized and secreted by the pituitary, the pituitary was suggested to be one possible candidate as primary target of estrogen action.

Experiment 2 (Fig. 2). In order to test whether estrogen treatment causes changes in angiotensinogen and IGF-I serum levels in the absence of the pituitary a study with Hx + ovex female rats was performed. To maintain the hepatic ER level, Hx animals were substituted with hGH or hGH + Dex [24]. As expected the ER level dropped upon hypophysectomy, it increased upon substitution with hGH and the level of pituitary intact animals could be almost restored by treatment with hGH + Dex. IGF-I serum levels

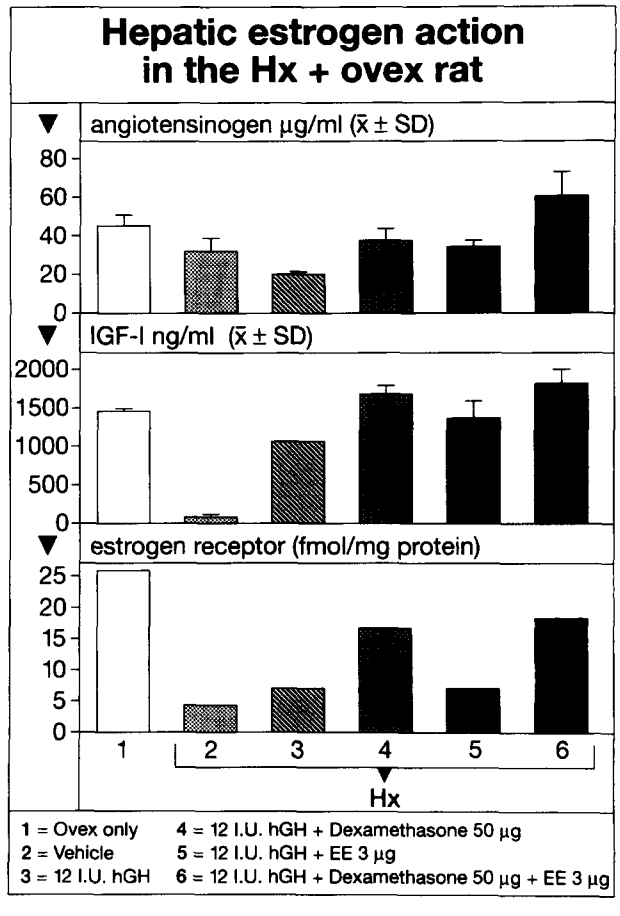

Fig. 2. Impact of hypophysectomy, and hormonal substitution with hGH, Dex and EE on angiotensinogen and IGF-I serum levels and on the hepatic ER level in ovex female rats. EE treatment started on day 4 after hypophysectomy for 6 days with 3 μg/animal/day s.c. Dex and hGH treatment started immediately after hypophysectomy for the rest of the experiment.

decreased upon hypophysectomy of ovex rats and reincreased to almost the level of pituitary intact animals in ovex rats treated with 12 IU hGH. In this experiment, EE-treatment (3 μg/animal/day for 6 days), caused an about 80% increase in angiotensinogen serum levels in the presence of hGH but it did not cause a decrease in the serum level of IGF-I (Fig. 2, bars 3 compared to 5 and 4 compared to 6). The increase in angiotensinogen was found in both, hGH substituted animals (ER level about 7 fmol/mg protein) and in rats substituted with hGH + Dex (ER level about 17 fmol/mg protein which was close to the level in pituitary intact rats).

Experiment 3 (Fig. 3). Aiming to answer the question whether the estrogen induced decrease in IGF-I plasma levels in pituitary intact animals was accompanied by a decrease in the IGF-I mRNA level in the liver, IGF-I mRNA levels were determined in ovex control animals and ovex rats treated with EE (0.3 or 3 μg/animal/day, respectively) for 6 days. The result of a "Northern" experiment is documented by Fig. 3. A statistically significant decrease in the liver IGF-I mRNA level (by 40%) was observed in animals treated with 3 μg/animal/day EE for 6 days. Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) mRNA (detected by a rat cDNA probe [32]) was used as a reference.

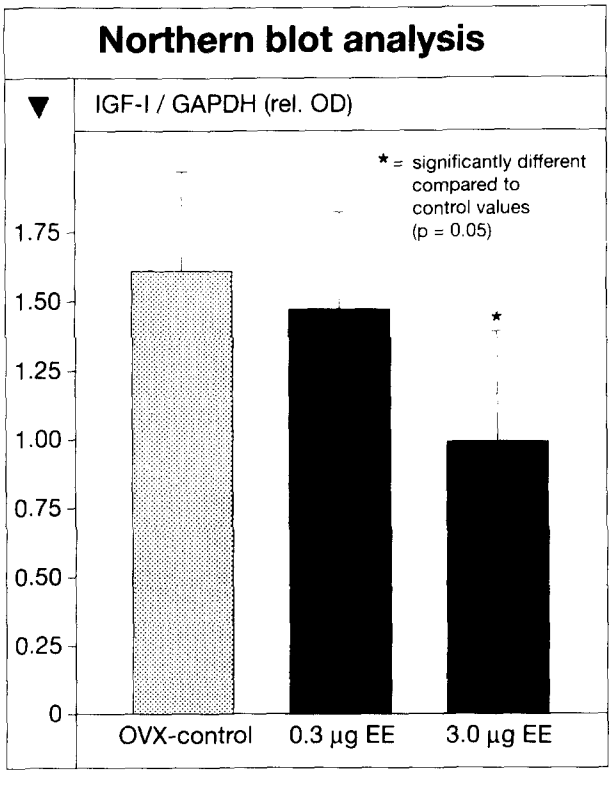

Fig. 3. Effect of chronic treatment of ovex rats with different doses of EE for 6 days on the liver IGF-I mRNA level. The mRNA levels of IGF-I and the standard glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) were determined by "Northern" blot analysis. The data were obtained by laser densitometric analysis of autoradiographies and are presented as relative optical density (rel. OD).

In vitro studies

The human hepatoma celline HepG2 is described in the literature as an *in vitro* system to study estrogen effects on liver protein synthesis [21]. However we found that HepG2 cells do not express detectable amounts of ER. The determination was performed by ligand binding assay and by enzyme immunoassay. Also, ER mRNA was not detectable by "Northern" analysis (data not shown). We therefore turned to primary hepatocytes and to Fe33 cells for our studies on estrogen action on angiotensinogen synthesis.

Primary rat heptocytes

An increase in the angiotensinogen mRNA level could be observed in primary rat hepatocytes upon administration of dexamethasone/glucagon to the culture medium (time of incubation 24 h; Fig. 4) indicating that the promoter for the angiotensinogen gene was intact in these cells. In contrast EE had no effect on the angiotensinogen mRNA level (incubation with 10 nmol/l EE for 24 h; Fig. 4) nor on angiotensinogen protein in cell culture supernatants (data not shown). The same result was obtained even if using different times of incubation with EE (1 up to 24 h) and/or administering increasing concentrations of EE (up to 1 μmol/l for 1 up to 24 h). In order to clarify the lack of EE-effects on angiotensinogen synthesis we determined the ER-mRNA level within the first 5 h of cultivation of these hepatocytes. As demonstrated in Fig. 5 we found the ER-mRNA to be rapidly downregulated in the presence and absence of 10 nmol/l EE in the culture medium. In accordance with this, ER protein was below detection limits in hepatocytes cultured for 5 h whereas the receptor concentration in freshly isolated rat liver tissue was 20–30 fmol/mg protein (determination by ligand binding assay; data not shown).

Fe33/FTO-2B cells

Fe33 cells are derived from the hepatoma cell line FTO-2B which is stably transfected with an ER expression plasmid [20]. The incubation with 10 nmol/l EE for 24 h caused an increase in the angiotensinogen mRNA level (Fig. 6) in Fe33 cells, whereas FTO-2B cells did not respond (Fig. 6). In contrast, glucocorticoid treatment (Dex, 100 nmol/l; incubation for 24 h) caused an increase in angiotensinogen mRNA levels in both cell lines (Fig. 6).

The time course of the EE-induced change in the angiotensinogen mRNA in Fe33 cells reveals an increase in angiotensinogen mRNA starting 3 h after addition of EE to the culture medium (Fig. 7).

DISCUSSION

In vivo studies

The study with ovex rats (experiment 1) revealed that E2 and EE affect angiotensinogen serum levels differently if applied s.c., whereas the two estrogens

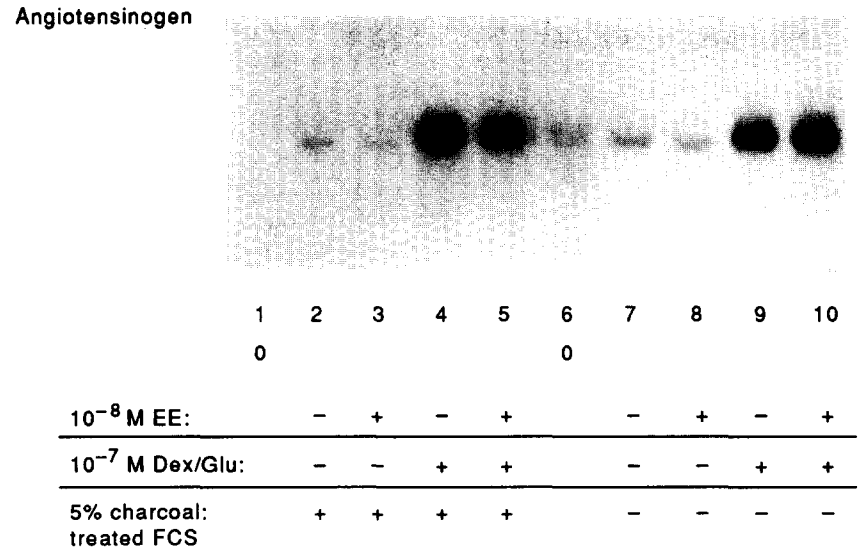

Fig. 4. "Northern" blot analysis to demonstrate hormonal effects on expression of angiotensinogen mRNA in primary rat hepatocytes grown in the presence and absence of charcoal treated FCS. Hepatocytes were cultured for 24 h in the absence of hormone (lanes 2 and 7) in the presence of 10 nmol/l EE (lanes 3 and 8), in the presence of 100 nmol/l Dex/glucagon (Dex/Glu; lanes 4 and 9) or in the presence of EE (10 nmol/l) and Dex/Glu (100 nmol/l; lanes 5 and 10). Lanes 1 and 6 represent the angiotensinogen mRNA level in freshly isolated hepatocytes before cultivation.

caused similar changes in IGF-I serum levels. The two compounds were equipotent with regard to stimulation of uterine growth. Rat liver angiotensinogen synthesis was shown recently to be regulated directly via the hepatic ER [11, 12]. The difference with regard to the effect on angiotensinogen upon application of the two estrogens could be explained by the difference in metabolic stability of the two compounds. Subcutaneous application of up to 3 μg/animal/day E2 may

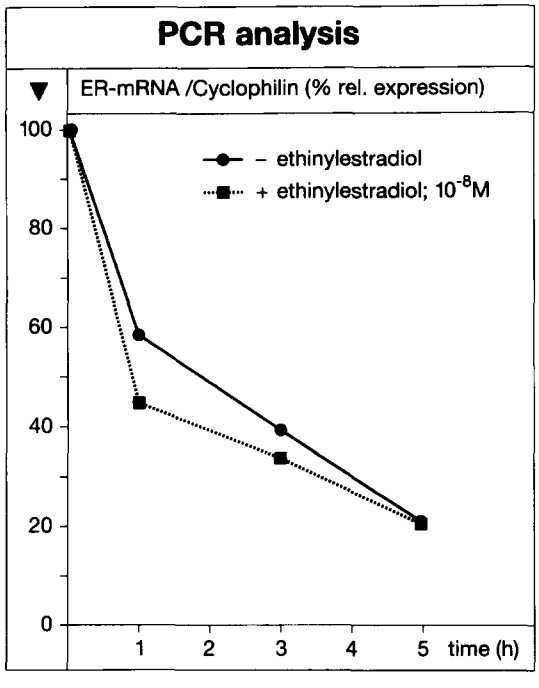

Fig. 5. RT-PCR analysis of the time course of ER mRNA decline in rat hepatocyte primary cultures during the first 5 h of cultivation. ER and cyclophilin mRNAs were amplified by RT-PCR at different time points after treatment with or without 10 nmol/l EE and in the presence of 100 nmol/l Dex/glucagon (Dex/Glu). Hepatocyte ER mRNA derived RT-PCR products in relation to the respective cyclophilin mRNA derived RT-PCR products after quantification of autoradiographic bands by laser densitometry are presented graphically. The value for zero hours of cultivation was designated arbitrarily to be 100%.

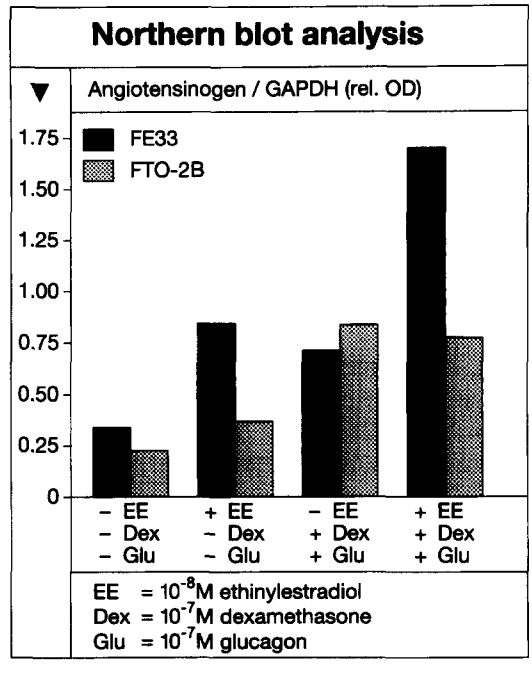

Fig. 6. "Northern" blot analysis of the angiotensinogen mRNA level in Fe33 cells and in FTO-2B cells after cultivation in serum free medium for 24 h in the absence of steroid hormone, in the presence of EE (10 nmol/l), of Dex/glucagon (Dex/Glu; 100 nmol/l) or of the combination of EE (10 nmol/l) and Dex/Glu (100 nmol/l). The data were obtained by laser densitometric analysis of autoradiographies and are presented as relative optical density (rel. OD).

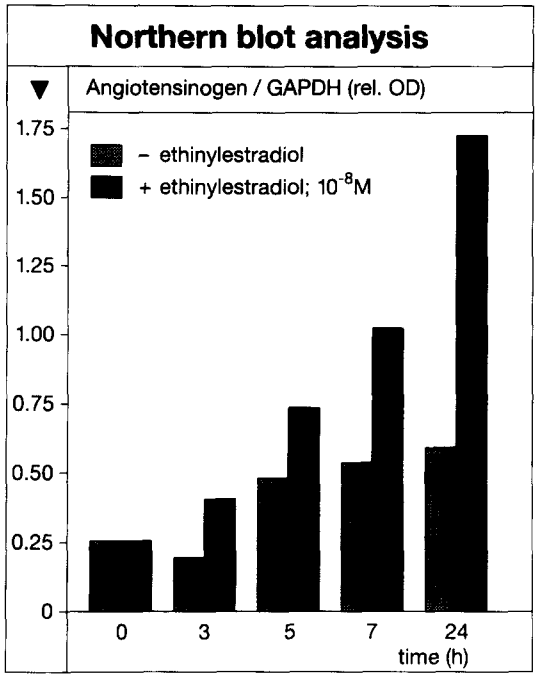

Fig. 7. Time course of the angiotensinogen mRNA level in Fe33 cells after different times of incubation with EE (10 nmol/l) and Dex/glucagon (Dex/Glu; 100 nmol/l) as determined by "Northern" blot analysis. The data were obtained by laser densitometric analysis of autoradiographies and are presented as relative optical density (rel. OD).

not lead to a sufficiently high intrahepatic concentration of estrogen to affect liver protein synthesis. Higher E2 doses can lead to an increase in angiotensinogen serum levels as was reported by others [25]. In contrast to E2, EE undergoes the enterohepatic recirculation [8]. The EE concentration is therefore probably sufficiently high to cause changes in liver protein synthesis.

The changes in angiotensinogen serum levels in the rat model are comparable to the situation in humans. Clinical studies showed that after oral application of high doses of E2, increases in angiotensinogen serum levels occurred, whereas after parenteral (transdermal) application of E2, angiotensinogen serum levels remained unaffected. This difference was observed although after both ways of administration similar E2 serum levels were obtained [6]. On the contrary, EE caused changes in hepatic parameters irrespective of the way of application, oral or parenteral [7]. Therefore we suggest the rat model to be useful for studies, with relevance for the human situation, aimed to investigate the impact of the way of estrogen administration on their hepatic effects.

In contrast to angiotensinogen, IGF-I seems to be regulated by estrogens via an indirect mechanism, because IGF-I serum levels decreased to the same extent upon application of both, EE and E2. This interpretation was supported by the studies in ovex + Hx rats (experiment 2). Hypophysectomy induced a dramatic reduction in the IGF-I plasma level, whereas angiotensinogen decreased only moderately upon removal of the pituitary. It is known that hGH is a major regulator of IGF-I synthesis in the liver [9]. Accordingly the substitution of the Hx animals with hGH stimulated the IGF-I levels to a similar level as measured prior to hypophysectomy. In contrast, hGH substitution did not cause an increase in the angiotensinogen serum level to the level in pituitary intact controls. However, the moderate effect of hypophysectomy on angiotensinogen can be explained by the lack of ACTH, the pituitary derived hormone regulating adrenal glucocorticoid synthesis. Glucocorticoids on the other hand stimulate angiotensinogen synthesis in the liver [26].

In the absence of the pituitary EE caused an increase in the angiotensinogen serum level, whereas the IGF-I serum level was unaffected by EE (compare Fig. 2 columns 3 vs 5 and 4 vs 6). In this context it is important to have information on the effect of hypophysectomy on the ER level in the liver. In accordance with the study of Norstedt *et al.* [24] we found that ER levels comparable to those in ovex controls were only achieved by the substitution of Hx rats with the combination of hGH and the glucocorticoid Dex (Fig. 2). As regards IGF-I, EE did not affect the IGF-I plasma level of hypophysectomized animals substituted with hGH or hGH + Dex. Therefore we conclude that the estrogen effects on IGF-I in pituitary intact animals (experiment 1) are indirect effects via the pituitary. However it remains unclear whether other hormones which are regulated by the pituitary, as thyroid or adrenal derived hormones, are involved in hepatic IGF-I regulation.

In order to answer the question whether the estrogen induced decrease in IGF-I plasma levels can be attributed to a reduced production of IGF-I by the liver, we studied the effect of estrogen treatment on liver IGF-I mRNA levels. The decrease in IGF-I serum levels observed upon estrogen treatment was accompanied by a decrease in liver IGF-I mRNA (experiment 3, Fig. 3), indicating that the change in serum IGF-I is probably caused by reduced IGF-I synthesis by the liver. Similar results were obtained by others [10].

Estrogen induced changes in IGF-I binding proteins could also affect indirectly the plasma level of free IGF-I. However, before quantification, serum IGF-I was separated from IGF-I binding proteins by acidic extraction and acid gel filtration (compare Experimental, according to [13, 27]). Thus, even though plasma IGF binding proteins are probably subject to hormonal regulation in our experiments—e.g. growth hormone [28]—the method of IGF-I determination applied is supposed to reduce effects of changes in IGF binding proteins on IGF-I measuring data to a minimum.

In vitro studies

Our *in vitro* studies with three hepatoma cell lines and primary rat hepatocytes were directed to identify a useful *in vitro* system to study hepatic effects of estrogens. Such a cell system is suggested to be useful

to monitor direct estrogenic effects on synthesis of liver derived proteins. The *in vitro* studies were restricted on angiotensinogen gene expression because IGF-I mRNA levels were below detection limits in all four *in vitro* systems studied. The human hepatoma cell line Hep G2 had been considered as being an attractive model to monitor direct estrogen effects on liver protein synthesis for the following reasons. The cells were described to express the ER [5, 21]. Furthermore several liver proteins like coagulation factors and inhibitors [29, 30] as well as angiotensinogen [31], sex hormone-binding globulin [21] and apolipoprotein A1 [5] were reported to be synthesized and secreted by this cell line. Surprisingly we could not detect the ER on protein or transcript levels using different analytical techniques (data not shown). Consequently we did not detect estrogen effects on angiotensinogen gene expression in this cell line.

The angiotensinogen gene of isolated primary rat hepatocytes was upregulated by administration of Dex, demonstrating the functionality of the endogenous promoter. In contrast the addition of EE, even if using different culture conditions and incubation times (1–24 h), did not influence the expression of the gene which is incompatible to the *in vivo* situation.

Even if using unphysiologically high concentrations of EE (1 μmol/l) in analogy to a study published by Klett *et al.* [11] we could not detect a regulatory effect of EE on the angiotensinogen gene in isolated rat hepatocytes. This is probably due to a rapid loss of the ER mRNA which is documented by Fig. 5. We conclude that primary rat hepatocytes are no useful system to study effects of estrogens on rat liver *in vitro*, because the ER expression is unstable in this system.

Using identical conditions of cultivation as used for primary hepatocytes, FTO-2B cells, which do not express the ER, are responsive to Dex but not to EE (Fig. 6). In contrast, in Fe33 cells, which do express the ER, the angiotensinogen mRNA level increased upon application of both, Dex and EE (Fig. 6). This finding fits to the suggested *in vivo* regulation of the gene in rat liver by glucocorticoids and estrogens and indicates a direct regulation of the promoter via the ER as shown earlier by Feldmer *et al.* [12]. A drawback of this cell line is that the integrated ER contains a pointmutation which affects the binding affinity of E2 and EE.

We conclude from our studies that estrogen effects on angiotensinogen serum levels in the ovex rat are direct effects via the hepatic ER. Estrogen effects on IGF-I are indirect effects, the primary target of estrogen action being probably the pituitary. If applied parenterally, E2 is different from EE in that it does not influence the indicator of direct hepatic estrogen action in the rat, angiotensinogen, in the dose range tested.

The human hepatoma cell line HepG2 and primary rat hepatocytes are not suitable to study estrogen effects on liver protein synthesis, because the ER is not detectable (HepG2) or is rapidly downregulated (hepatocytes). Fe33 cells are a useful model to study the regulation of the angiotensinogen gene by estrogens. The applicability of Fe33 cells to study estrogen effects on liver protein synthesis in general has to be tested.

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