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Androgen Regulation of the Insulin-like Growth Factor-I and the Estrogen Receptor in Rat Uterus and Liver

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For the first time testosterone is shown to be an important regulator of the insulin-like growth factor-I (IGF-I) in the rat uterus under *in vivo* conditions. In this study the regulation of IGF-I and the estrogen receptor (ER) by gonadal steroids in the uterus and liver of female rats was monitored. The ER level was assayed by hormone binding after treatment with testosterone, 5α-dihydrotestosterone or estradiol and specific mRNA species were analyzed by a solution hybridization/RNase protection assay using ^{35}S-labeled RNA probes. Ovariectomized rats restored uterine weight after treatment with testosterone. Uterine IGF-I mRNA was more than 20-fold higher in testosterone treated rats compared to untreated ovariectomized controls after 48 h treatment. The effects of testosterone on ovariectomized animals was followed in a timecourse study. Testosterone administration increased uterine IGF-I mRNA expression during the first 48 h and the maximally induced level was maintained throughout the duration of the experiment (168 h). Since induction of IGF-I mRNA by estrogen is transient, these data indicate that androgen and estrogen increase IGF-I mRNA by different mechanisms. Regulation of IGF-I mRNA by gonadal steroids was also studied in hypophysectomized animals. The rats were given either testosterone, 5α-dihydrotestosterone or estradiol, and uterine IGF-I mRNA was measured after 1 week of treatment. At this timepoint estrogen treated rats showed levels of IGF-I mRNA not significantly different from those of hypophysectomized controls. In contrast testosterone and 5α-dihydrotestosterone increased the IGF-I mRNA level 30 and 40 times, respectively, relative to hypophysectomized control animals. Since 5α-dihydrotestosterone is not convertable to estrogen, the induction by testosterone was considered to be a true androgenic phenomenon.

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INTRODUCTION

Female sex steroids are important regulators of uterine growth and differentiation [1]. The precise mechanism of all steps in their action is unknown (for a review, see Malayer and Gorski [2]). One possibility which has recently been considered is that estrogens control growth indirectly by regulating the synthesis of polypeptide growth factors and their receptors. As previously shown [3, 4] estrogen induced growth of the uterus might be controlled by growth factors acting in a paracrine and/or autocrine fashion. One such factor is insulin-like growth factor-I (IGF-I).

It has previously been reported that testosterone induces uterine growth [5]. Thus, Schmidt and Katzenellenbogen [6] showed that testosterone in high doses could act via the estrogen receptor, since concurrent administration of antiestrogens inhibited the effects of this androgen. In contrast, testosterone in low doses seemed to act via the androgen receptor since antiandrogens, but not antiestrogens, inhibited uterine growth [6].

In the present study, experiments were performed to investigate whether the uterine growth promoted by androgens might involve induction of IGF-I mRNA. Since testosterone can be metabolized to estradiol by aromatases in the liver, the androgen 5α-dihydrotestosterone, which is not metabolized, was used as a marker of direct androgenic action.

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MATERIALS AND METHODS

Animals and tissue preparation

Adult, 55- to 60-day-old, Sprague–Dawley rats were used in all experiments. The animals weighed 180 ± 20 g at the beginning of the experiments, and were purchased from Eklunds (Vallentuna, Sweden). The female rats were ovariectomized and then allowed to rest for 1 week before hormone treatment. Female hypophysectomized rats, body weight 200 g at the time of operation, and normal controls, were obtained from Möllegaards Breeding Centre Ltd (Denmark). The animals were housed in a controlled environment at 20°C on an illumination schedule of 12 h of light and 12 h of darkness each day. Standard pellet food and water (containing 0.9% NaCl and 5% glucose when offered to the hypophysectomized animals) were provided *ad libitum*. Each group of rats consisted of 4–6 animals.

Preparation of subcellular fractions

Detailed outlines of the procedures for preparation of subcellular tissue fractions are given in Refs [7, 8]. In short, the vena cava was cannulated and the portal vein severed. The liver was perfused *in situ* with 10 ml of body warm saline solution and then rapidly excised, weighed and trimmed. It was drained in cold saline for 5 min, dried briefly on Kleenex, a portion was removed for mRNA analysis and all were put in liquid nitrogen. It was stored at −70°C until used. After thawing it was cut into smaller pieces and homogenized in 5 volumes of Tris–EDTA (TE) buffer pH 7.4 in a Potter–Elvehjelm homogenizer with a Teflon pestle. The homogenate was centrifuged at 5000 *g* for 20 min in a Beckman JA-20 rotor. The lipid layer was removed and the supernatant recentrifuged at 184,000 *g* for 80 min in a Beckman 70Ti rotor. A small portion of the supernatant was removed for protein determination and the remainder transferred to a JS-13 tube. Solid ammonium sulfate was added under constant stirring to 35% saturation. The solution was stirred for 60 min. The precipitate was collected following centrifugation at 12,000 *g* for 15 min in a Beckman JS-13 rotor. The precipitate was dissolved in 7 ml of TE buffer and used in binding experiments. Unless otherwise stated all procedures were performed at 4°C.

The uterus was removed, stripped of adhering fat and connective tissue, weighed, cut in two equal halves and immediately frozen in liquid nitrogen and stored at −70°C until analyzed. After thawing one half was analyzed with respect to receptor content. It was homogenized in TE-buffer in a glass–glass homogenizer, and the homogenate centrifuged at 1200 *g* for 20 min in a Beckman JA-20 rotor. The supernatant was recentrifuged at 192,000 *g* for 65 min in a Beckman SW55 rotor and the highspeed supernatant used for determination of "cytosolic" receptors. Cytosolic receptors, i.e. referring to presumably loosely bound nuclear ER that is found in the cytosol as a result of cell fractionation procedures. The nuclear pellet obtained after the first centrifugation was used for determination of nuclear receptors.

Hormone treatment

In ovariectomized rats, testosteronenanthate was administered as a depot. In the time study the animals were given an i.m. injection of 20 mg in 150 μl ol. ricini. Hypophysectomized rats were treated with estradiol by constant infusion at 4 μg/h for 7 days, testosterone and 5α-dihydrotestosterone at 20 μg/h for 7 days. Unfortunately the way of administration had to be changed compared to the experiment with the ovariectomized rats as no depot preparations of estradiol and 5α-dihydrotestosterone were available, therefore all substances were administered via osmotic minipumps. The estradiol dose given to the hypophysectomized rats was 40-fold higher than the dose given to the ovariectomized rats. This was done to exclude the possibility that the testosterone effect was due to a superphysiological stimulation of estradiol due to the aromatization of testosterone to estradiol in the liver. The hormones were dissolved in 99.5% ethanol at a high concentration and then diluted with propyleneglycol to the proper concentration. The final ethanol concentration in the infusion solution was less than 10%. The hormones were administered in Alzet osmotic pumps (Alza, Palo Alto, CA). The pumps were placed subcutanously in the neck region under light ether anesthesia.

Radioimmunoassays of hormonal blood levels

Blood samples were taken from all animals at the time of sacrifice. They were left in room temperature for 1 h, centrifuged and kept at −20°C until analyzed. These serum samples were later assayed for testosterone using an antisera from the WHO. The radioimmuno assay (RIA) was performed according to the WHO manual [9]. Antisera (S-741 No. 7) for 5α-dihydrotestosterone was purchased from Dr Guy E. Abraham (Division from Reproductive Biology, Harbor General Hospital, Torrance, CA). This antisera cross-reacts with testosterone to 100%. The technique used for this RIA is described by Aso *et al.* [10].

Chemicals

Diethylstilbestrol (DES), 17β-estradiol, testosterone and 5α-dihydrotestosterone were purchased from Sigma Co. (St Louis, MO). Testoviron-Depot® (135 mg/ml) for the timestudy was from Schering AG (Berlin, Germany). [2,4,6,7-^{3}H]Estradiol-17β [3β,17β-dihydroxy-1,3,5(10)-estratriene], sp. act. 92.0 Ci/mmol, was obtained from New England Nuclear (Boston, MA). Radioactivity was determined in an LKB-Wallac 1215 Rackbeta liquid scintillation counter using Readysafe (Beckman Instruments Inc., Fullerton, CA) as scintillation fluid. Dextran T70 was

bought from Pharmacia (Uppsala, Sweden). Activated charcoal was purchased from Sigma.

Analytical methods

Binding studies were performed in the presence of a wide range of [^{3}H]estradiol concentrations (0.4–20.0 nM) ± 100-fold excess of cold DES. Tubes were vortexed and incubated at 4°C for 16 h and the measurement of specific estrogen binding was performed by the dextran-coated charcoal method [11].

Data were plotted according to Scatchard [12]. Protein was quantitated spectrophotometrically by the method of Lowry *et al.* [13].

The solution hybridization analysis of specific mRNA was performed as described previously [14, 15]. For measurement of mRNA, RNA probes were synthesized *in vitro* and radiolabeled with [^{35}S]UTP (Amersham, Bucks., England). The *in vitro* synthesis of radioactive cRNA was performed essentially as described by Melton *et al.* [16] using reagents supplied by Promega Biotech (Madison, WI). [^{35}S]UTP cRNA was hybridized (20,000 cpm/incubation) at 70°C to TNA samples as described previously [17]. Incubations were performed in micro-centrifuge vials (Treff AG, Switzerland) in a volume of 40 µl containing 0.6 mol/l NaCl, 20 mmol/l Tris–HCl, pH 7.5, 4 mmol/l EDTA, 0.1% SDS, 10 mmol/l dithiothreitol and 25% formamide. After overnight incubation, samples were treated with RNase for 45 min at 37°C by adding 1 ml of a solution containing 40 µg RNase A and 2 µg RNase T1 (Boeringer-Mannheim, Mannheim, Germany) and 100 µg calf thymus DNA to each sample. Radioactivity protected from RNase digestion was precipitated by the addition of 100 µl 6 mol/l trichloroacetic acid (TCA) and collected on a filter (Whatman GF/C). The hybridization signal of a sample was compared to the signal of the ovariectomized or hypophysectomized control, which was set to 100%.

The probe used for IGF-I mRNA determinations derived from a genomic clone of mouse IGF-I [17]; a 160 bp *Ban* I–*Pvu* I fragment from exon 3 (assuming homology to human IGF-I) was subcloned into the RNA expression vector pSP64.

The probe used for estrogen receptor (ER) mRNA determinations derived from pMOR101, an *Eco* RI fragment of 1.9 kb containing the whole open reading frame of the mouse ER [18] inserted into a pSP64 vector. Restriction of this plasmid with *Bgl* II allows the synthesis of a probe corresponding to nucleotides 1470–2062 which encode the C-terminal half of the steroid binding domain (E) and all of domain F.

Thus, ER mRNA and IGF-I mRNA were detected in the rat tissues using heterologous mouse probes. Rat and mouse IGF-I showed a high degree of homology. It is feasible to use the mouse IGF-I probe to specifically detect rat IGF-I mRNA as demonstrated by Möller *et al.* [19]. The rat ER mRNA and mouse ER mRNA show 97% homology [20] and specific binding was obtained when the mouse ER probe was hybridized to rat liver RNA on a Northern blot [14].

β-Actin mRNA hybridizations were performed with a 51 basepair probe, corresponding to aminoacid 331–347 in exon 6 of the rat cytoplasmatic β-actin gene.

A 1300 basepair cDNA of rat glyceraldehyde-3-phosphate dehydrogenase (GAP DH), subcloned into the RNA expression vector pIBI-30, was used to synthesize RNA probes [21].

The results are shown as means ± standard errors of mean (SEM) and the statistical calculations were done by ANOVA and significances evaluated according to Scheffé [22], in Statgraphics computer program (Statistical Graphics Corporation, Rockville, MD). Significances of $*P < 0.05$ and $**P < 0.01$ are presented. The RIA determinations of the hormonal serumlevels are presented as geometrical means and 95% confidence limits.

Internal standards

It is very difficult to find a reliable internal mRNA control marker that is not affected by variations in hormone levels or other stimuli. Hsu and Frankel [23] have shown that β-actin is stimulated by estradiol in immature rat uteri, and others [24, 25] comment that β-actin and GAP DH themselves are subject to considerable variation in mammalian tissues. For these reasons both β-actin and GAP DH mRNA were measured as internal markers in the present study. Several different mRNA species were measured in the same samples and the increase or decrease in the level of a certain mRNA species was compared to the levels of other mRNAs studied. This made it possible to discriminate between specific hormone induction and a general variation in the whole mRNA population.

RESULTS

Hormonal concentrations in serum after treatment

The diurnal serum levels of testosterone and 5α-dihydrotestosterone + testosterone in normal male rats

Table 1. *Diurnal serum levels of androgens in normal male rats*

Time of day	Testosterone (nmol/l)	5α-Dihydrotestosterone + testosterone (nmol/l)
8–9 a.m.	6.37	8.91
(n = 6)	(1.76–23.1)	(2.98–26.6)
12–1 p.m.	11.4	13.1
(n = 6)	(4.63–27.8)	(5.78–29.8)
4–5 p.m.	12.3	13.0
(n = 6)	(6.14–24.4)	(5.93–28.7)
8–9 p.m.	2.40	2.28
(n = 6)	(1.62–3.56)	(1.86–2.78)
12–1 a.m.	4.85	3.94
(n = 6)	(2.46–9.57)	(1.75–8.87)
4–5 a.m.	5.84	5.94
(n = 6)	(3.18–10.7)	(3.10–11.4)

The results are presented as geometrical means and 95% confidence limits.

Table 2. Hormonal serum levels in female hypophysectomized rats after treatment with estradiol, testosterone or 5α-dihydrotestosterone for 1 week

Treatment	Testosterone (nmol/l)	5α-Dihydrotestosterone + testosterone (nmol/l)
Normal control	0.403 (0.263–0.618) ($n = 6$)	1.07 (0.772–1.48) ($n = 6$)
Hypophysectomized (Hx) control	0.040 (0.009–0.190) ($n = 6$)	0.419 (0.311–0.565) ($n = 6$)
Hx + estradiol (100 μg/day)	0.286 (0.223–0.366) ($n = 4$)	0.245 (0.208–0.289) ($n = 3$)
Hx + testosterone (500 μg/day)	59.4 (41.8–84.4) ($n = 4$)	59.7 (41.6–85.6) ($n = 4$)
Hx + 5α-dihydrotestosterone (500 μg/day)	1.41 (1.13–1.76) ($n = 3$)	41.3 (24.7–69.0) ($n = 4$)

The results are presented as geometrical means and 95% confidence limits.

were determined to obtain physiological reference levels for comparison to the hormone treated animals (Table 1). The testosterone level in males varied between 2.40 and 12.3 nmol/l during the day. The 5α-dihydrotestosterone + testosterone concentration did not differ from the testosterone level. In the ovariectomy experiment blood samples from two normal female rats showed a mean value of 0.361 nmol/l (not shown), which is very close to the concentration of 0.406 nmol/l seen in the normal female in the experiment with hypophysectomized animals (Table 2). The 5α-dihydrotestosterone + testosterone concentration in the normal controls was 1.07 nmol/l, i.e. 2.5-fold the testosterone concentration. (Blood samples from female controls were taken between 8 and 9 a.m.) (Table 2).

The testosterone levels in ovariectomized rats treated with a testosteronenanthate depot were about 15 times higher after 48 h than those of the male rats at 4–5 p.m. (Table 3). The hypophysectomized rats treated with testosterone or 5α-dihydrotestosterone had serum levels 5 times that of the normal males at 4–5 p.m. (Table 2).

Effect of testosterone administration in ovariectomized rats

Continuous administration via a depot of 20 mg testosteronenanthate to ovariectomized rats for a week, stimulated uterine growth identical to a continuous infusion of 2.5 μg/day of estradiol (Fig. 1).

The IGF-I mRNA level in the uterus increased more than 15-fold during the first 24 h. The increase continued for the next 24 h, and was maintained thereafter on a more than 20-fold induction level for the rest of the experimental period of 168 h (Fig. 2). Uterine ER mRNA was not significantly altered during testosterone treatment (Fig. 3). However, ER mRNA was decreased compared to the variation in GAP DH mRNA and β-actin mRNA (Fig. 3). When the alterations of GAP DH mRNA and β-actin mRNA were taken into account the effect of testosterone on IGF-I mRNA remained significant. Testosterone treatment did not give significant changes of the uterine ER levels (data not shown).

In the livers of ovariectomized animals no significant effects on IGF-I mRNA, ER and ER mRNA could be observed after testosterone administration (data not shown).

Effects of testosterone treatment in hypophysectomized rats

To verify whether the results presented above could be reproduced in hypophysectomized (hx) rats and to compare the effects of 5α-dihydrotestosterone to those of testosterone, a 1 week study of hormone treatment

Table 3. Serum levels of testosterone in female ovariectomized (OVX) rats after treatment with an intramuscular depot of 20 mg of testosteronenanthate

Time (hours)	Testosterone (nmol/l)
0 h	0.226
OVX control ($n = 4$)	(0.154–0.332)
6 h	76.4
($n = 6$)	(40.6–144)
24 h	129
($n = 6$)	(83.7–200)
48 h	189
($n = 6$)	(151–237)
72 h	137
($n = 5$)	(78.0–242)
96 h	121
($n = 6$)	(63.6–230)
168 h	32.3
($n = 6$)	(18.2–57.1)

The results are presented as geometrical means and 95% confidence limits.

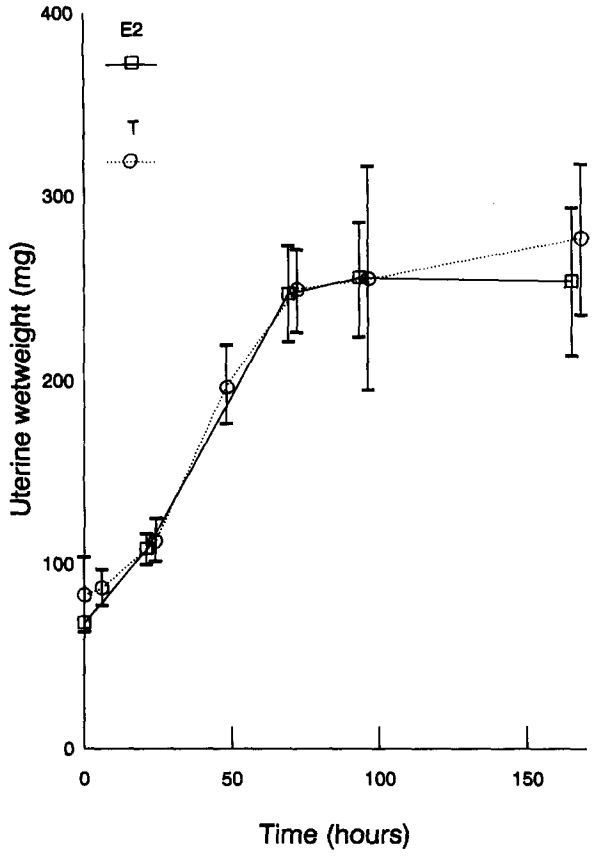

Fig. 1. Effects of testosterone and estradiol on the uterine wet weight. Ovariectomized rats were in two different experiments given either a testosterone depot of 20 mg testosterone or 2.5 μg estradiol/day in an osmotic mini pump. Six animals in each experiment were OVX controls at time 0. Then the testosterone treated rats were sacrificed after 6, 24, 48, 72, 96 and 168 h, 6 at each timepoint. The estradiol treated animals were sacrificed after 21, 69, 93 and 165 h, 6 at each timepoint. The means ± 1 SEM in uterine wet weight for each group is plotted in this figure.

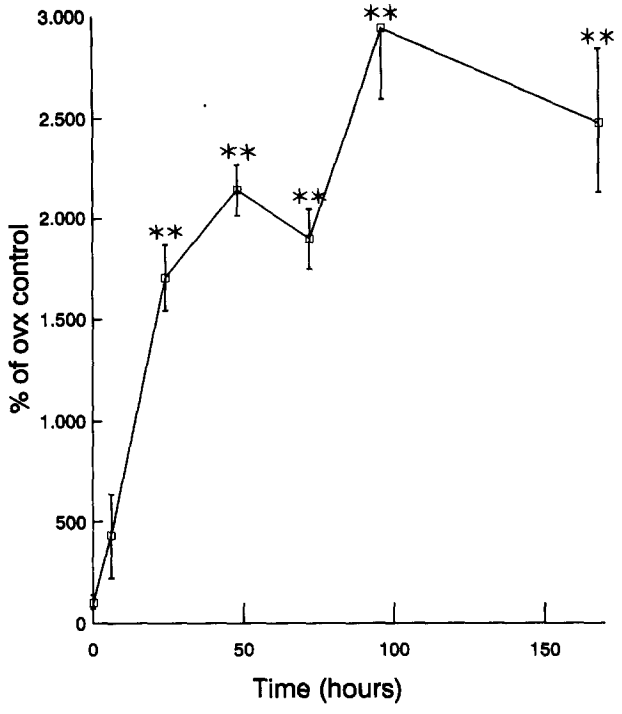

Fig. 2. Time-course of the effect of testosterone on IGF-I mRNA in the uteri of OVX rats. Testosterone (T) was administered i.m. as a depot of 20 mg at time 0. Total nucleic acids (TNA) were analyzed for the presence of specific IGF-I mRNA using a solution hybridization technique. OVX rats were used as controls and their mRNA contents set to 100%. Values are expressed as means ± 1 SEM. $^{**}P < 0.01$ compared to OVX control. $n = 6$ rats for every timepoint.

of hypophysectomized rats were performed. The rats were given either testosterone, 5α-dihydrotestosterone or estradiol. After treatment the uterine weights of the animals in all three groups were not different from those of normal intact controls (Table 4).

The uterine IGF-I mRNA decreased by 85% after hypophysectomy (Fig. 4). Testosterone administration resulted in a 30-fold increase in IGF-I mRNA compared to the hx control and 5α-dihydrotestosterone in a corresponding 40-fold increase, whereas estradiol treatment for 1 week did not cause a significant increase relative to the hypophysectomized control level (Fig. 4).

The uterine ER mRNA was not significantly affected by hypophysectomy or subsequent steroid treatment. The cytosolic estrogen receptor level in the uterus increased 2-fold in hx animals (Fig. 5). The cytosolic ER was unaffected by 5α-dihydrotestosterone whereas it was reduced below the normal level by estradiol and testosterone. For the nuclear fraction no significant changes were observed.

Hypophysectomy lowered liver IGF-I mRNA (Fig. 6), to less than 10% of the control level. Steroid treatment did not influence this mRNA. The hepatic ER mRNA level was decreased to 25% of the normal control. Estrogen or testosterone treatment did not alter this situation. In contrast to this, administration of 5α-dihydrotestosterone to hx rats elevated ER mRNA (Fig. 6).

Hypophysectomy caused a decrease of the hepatic ER to 25% of the normal level (Fig. 7). Similar to the effect on ER mRNA, treatment with 5α-dihydrotestosterone increased estrogen receptors to about half of the normal level, whereas estradiol and testosterone did not significantly affect the ER level.

DISCUSSION

In the present study the mechanisms for the regulation of uterine growth by gonadal steroids was studied. Both estradiol and testosterone administration resulted in similar growth stimulatory effects on the uterus. As previously shown, continuous estradiol treatment stimulates an increase in uterine IGF-I mRNA of ovariectomized rats. Estradiol induced IGF-I expression is transient with a maximum after 24 h. Despite continued estradiol administration the IGF-I mRNA then decrease and is close to its initial level after

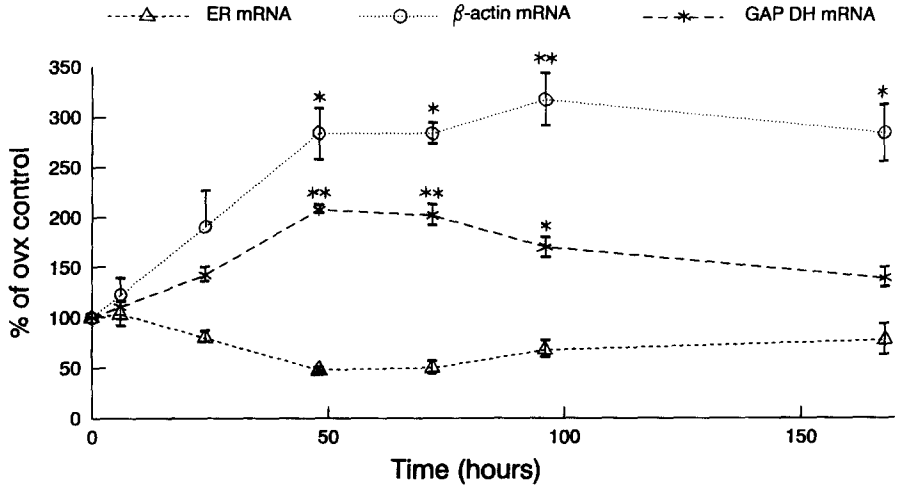

Fig. 3. Time-course of the effect of testosterone on uterine ER mRNA, β-actin mRNA and GAP DH mRNA in OVX rats. Total nucleic acids (TNA) were analyzed for the presence of specific ER mRNA (- - - △ - - -), β-actin mRNA (· · · ○ · · ·) and GAP DH mRNA (– – * – –) using a solution hybridization technique. For comparison the levels of OVX rats were used as controls and their mRNA contents set to 100%. Values are expressed as means ± 1 SEM. *$P < 0.05$ and **$P < 0.01$ compared to OVX control. $n = 6$ rats for every timepoint.

168 h [26]. A major finding in the present study is that testosterone stimulated IGF-I mRNA formation, to the same degree as estradiol at 24 h, but the induction was maintained at this level and even increased during the remaining experimental period. It thus appears that the two structurally related growth promoting steroids regulate the IGF-I expression in the uterus in a temporally different manner.

β-Actin mRNA and GAP DH mRNA were also stimulated by testosterone but to a much lesser extent than IGF-I mRNA. ER mRNA showed no significant variation but compared to the two internal standards it was downregulated. Autologous downregulation of the estrogen receptor is known [27] and as testosterone is convertable to estrogen, this relative downregulation in ER mRNA could be due to estrogen action.

In another experiment hypophysectomized rats were given estradiol, testosterone or 5α-dihydrotestosterone. Also in this situation did androgens increase IGF-I mRNA, 30- and 40-fold relative to the initial value. The effect of estradiol on IGF-I mRNA after 168 h was marginal, although the given dose was 40-fold that given to ovariectomized rats. This indicates that androgens have an effect on the synthesis of IGF-I mRNA separate from estrogens, and that this effect of androgens is not mediated via the pituitary. In fact, even though the serum levels of androgens are higher in the ovariectomized rats than in the hypophysectomized, the effect of androgens is more pronounced in hypophysectomized as compared to ovariectomized animals. A similar observation has been made by Murphy and Ghahary [28] for the IGF-I mRNA induction by estrogen. These findings exclude pituitary factors, e.g. GH, as mediators of the uterine IGF-I mRNA induction and also the possibility that the testosterone effect was caused by an aromatization of this hormone to estradiol, as 5α-dihydrotestosterone was equally effective. The induction of IGF-I mRNA after testosterone and 5α-dihydrotestosterone administration was clearly significant and our data suggests that androgens play an important role in the regulation of the uterine IGF-I expression.

Table 4. Uterine weight after treatment of hypophysectomized rats with estradiol, testosterone or 5α-dihydrotestosterone for 1 week

Treatment	Uterine weight (mg)
Normal control $n = 6$	395 ± 20.8**
Hypophysectomized (hx) control $n = 6$	96.7 ± 8.03
Hx + 100 μg estradiol/day $n = 4$	418 ± 32.8**
Hx + 500 μg testosterone/day $n = 4$	368 ± 35.0**
Hx + 500 μg 5α-dihydrotestosterone/day $n = 4$	333 ± 18.0**

**$P < 0.01$ compared to hx controls.

The different stimulatory effects of estradiol and testosterone on IGF-I expression in the rat uterus demonstrated in this study might be explained by several possible mechanisms involving both the ER and androgen receptor (AR) as well as some pathways which are only partly known. The presence of AR in the uterus is known [29, 30]. The simple explanation would be that the two hormones act exclusively via their respective receptors, ER and AR, which induce IGF-I mRNA through interaction with regulatory elements that exercise their control by different mechanisms. Testosterone in doses as those used in the time study do not interact with the ER to any significant degree, see Clark and Peck, p. 7 [1], but the possibility of aromatization to active estrogens must be considered.

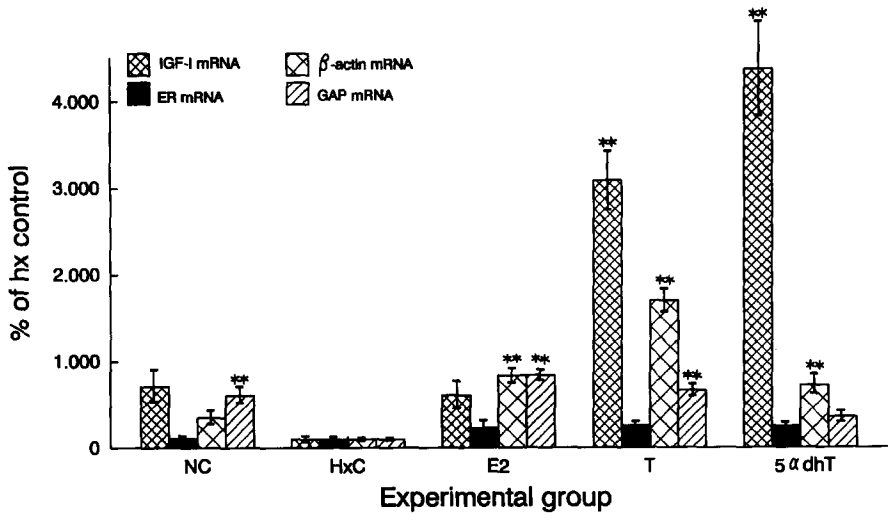

Fig. 4. The effect of estradiol (E2), testosterone (T) and 5α-dihydrotestosterone (5αdhT) on the uterine mRNA levels of IGF-I, ER, β-actin and GAP DH in hypophysectomized (hx) rats. Total nucleic acids (TNA) were analyzed for the presence of the specific mRNAs using a solution hybridization technique. Hypophysectomized rats were used as controls and their mRNA content set to 100%. Values are expressed as means ± 1 SEM. **$P < 0.01$ compared to hx control. $n = 4$ rats in the treatment groups, $n = 6$ in normal and hx controls.

Phillip *et al.* [31] have shown that IGF-I serum levels do not increase in male hypophysectomized rats after testosterone treatment, nor are their hepatic IGF-I gene expression stimulated. This indicates that the androgenic (as the estrogenic) stimulation of uterine IGF-I expression is tissue specific. In the human prostate the IGF-I receptor seems to be under negative androgenic regulation, indicating a potential role for the growth factor in the mechanism of response to the castration induced regression of androgen dependent tissue. After treatment with a GnRH analog there was a significant increase in IGF-I receptors, which could be explained by a decrease in the prostatic concentration of IGF-I [32]. This would indicate androgenic regulation of IGF-I in prostatic tissue. 5α-Dihydrotestosterone has been shown to stimulate 5α-reductase activity via IGF-I in human and rat scrotal fibroblasts *in vitro* [33].

Another mechanism to explain the persistent high level of IGF-I mRNA seen after androgen stimulation could involve non-ligand dependent receptor activation similar to those described by O'Malley's group [34, 35] and Ignar-Trowbridge *et al.* [36]. The latter group showed that EGF, in the absence of steroids, can

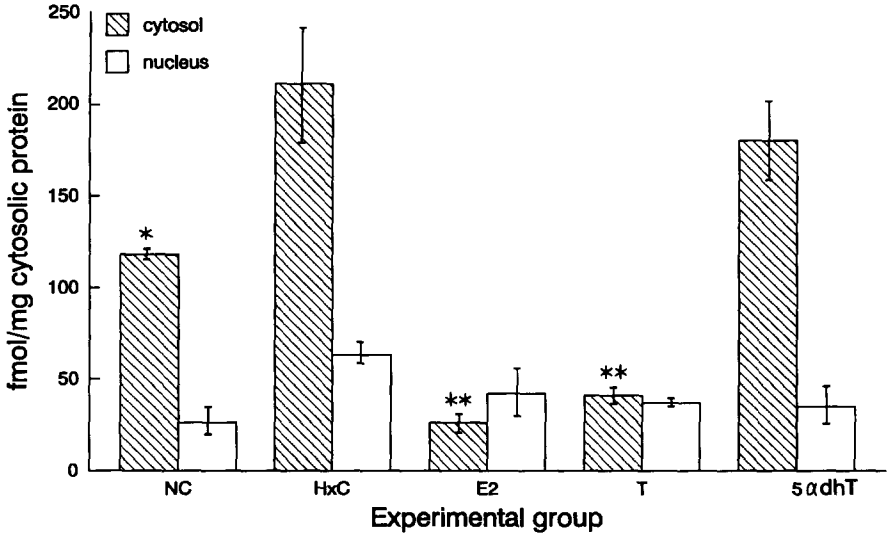

Fig. 5. The effect of estradiol (E2), testosterone (T) and 5α-dihydrotestosterone (5αdhT) on the uterine ER levels in hypophysectomized (hx) rats is shown. The receptor content in the cytosol (i.e. referring to presumably loosely bound nuclear ER that is found in the cytosol as a result of cell fractionation procedures) was analyzed by the dextran coated charcoal method and the nuclear receptor population by the nuclear exchange assay. Values are expressed as means ± 1 SEM. *$P < 0.05$ and **$P < 0.01$ compared to hx control. $n = 4$ rats in the treatment groups, $n = 6$ in normal and hx controls.

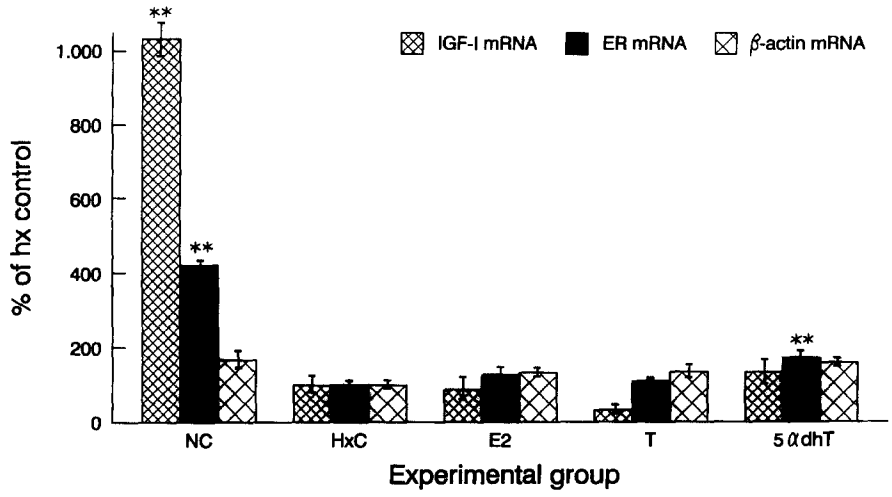

Fig. 6. The effect of estradiol (E2), testosterone (T) and 5α-dihydrotestosterone (5αdhT) on the hepatic mRNA levels of IGF-I, ER and β-actin in hypophysectomized (hx) rats. Total nucleic acids (TNA) were analyzed for the presence of specific IGF-I mRNA, ER mRNA and β-actin mRNA using a solution hybridization technique. Hypophysectomized rats were used as controls and their mRNA contents set to 100%. Values are expressed as means ± 1 SEM. **$P < 0.01$ compared to hx control. $n = 4$ rats in the treatment groups, $n = 6$ in normal and hx controls.

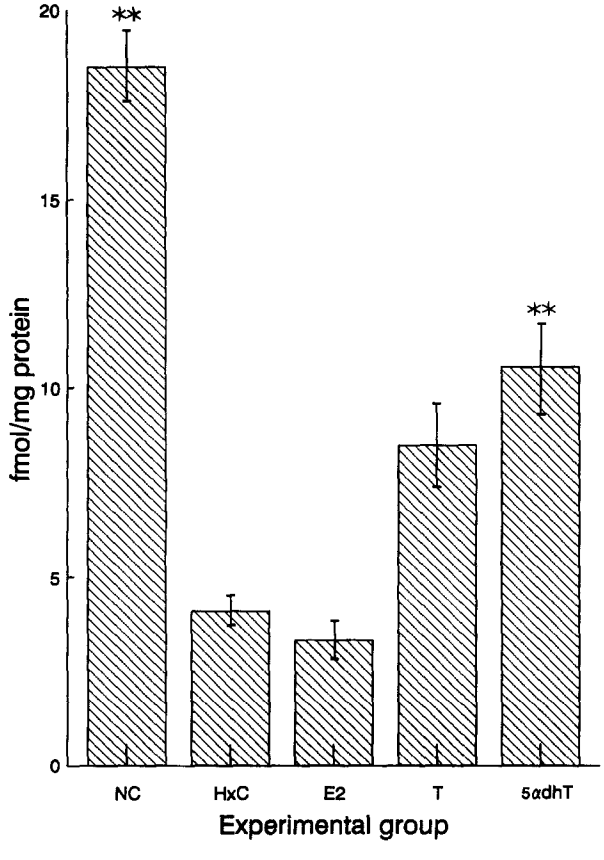

Fig. 7. The effect of estradiol (E2), testosterone (T) and 5α-dihydrotestosterone (5αdhT) on the hepatic ER level in hypophysectomized (hx) rats. The receptor content was analyzed by the dextran coated charcoal method. Values are expressed as means ± 1 SEM. **$P < 0.01$ compared to hx control. $n = 4$ rats in the treatment groups, $n = 6$ in normal and hx controls.

reproduce many of the effects of estrogen on the murine female reproductive tract and may partially mediate estrogen-induced growth. EGF mimicked the effects of estrogen on enhanced nuclear localization of the ER. Their results suggest that EGF may induce effects similar to those of estrogens in the mouse uterus by an interaction between the EGF signalling pathway and the classical ER via a non-ligand dependent activation of ER. A similar mechanism could exist for the regulation of IGF-I according to which testosterone triggers the initial formation of IGF-I which by a non-ligand dependent mechanism activates the ER which in turn stimulate IGF-I expression via an interaction with EREs. According to this hypothesis the downregulation of ER, which is seen after ligand-dependent activation, would not take place if the activation was by an IGF-I mediated phosphorylation, and thus a constant induction of IGF-I would be maintained in a fashion similar to that shown in this paper.

In this study we also monitored the estrogen receptor. In the uterus the cytosolic receptor concentration increased after hypophysectomy, although the ER mRNA level was unaffected. The increase is relative and depends on a reduction of the cellular protein content. Treatment with estradiol or testosterone decreased the cytosolic receptor amount to 25% of the control value. 5α-Dihydrotestosterone on the other hand, did not affect the receptor level. The difference between the two androgens could be due to the ability of testosterone to be aromatized to estrogen, and subsequent downregulation of the receptor.

In the liver hypophysectomy had dramatic effects on the monitored mRNA levels. IGF-I and ER mRNA both

drastically decreased compared to normal control rats. 5α-Dihydrotestosterone increased ER mRNA to 50% of the normal control level.

The ER level in the liver was also reduced after hypophysectomy. This effect could be corrected to some extent, just as for ER mRNA, by 5α-dihydrotestosterone treatment. The level was restored to 50% of the normal, although GH was not available. GH has been shown to regulate ER and ER mRNA both *in vivo* [14, 37] and *in vitro* [38].

Henrikson *et al.* [39] claims that the most extensive induction of a uterine mRNA reported to date is that of c-*fos* which increased 30- to 40-fold during the 3 h following estradiol administration. In this study we show equally high induction of IGF-I mRNA in hypophysectomized rats 1 week after androgen administration.

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