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INFLUENCE OF NEONATAL ANDROGENIZATION ON THE EXPRESSION OF α_{2u}-GLOBULIN IN RAT LIVER AND SUBMAXILLARY GLAND

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Summary—We studied the influence of neonatal androgenization on the serum level of α_{2u}-globulin, the level of the corresponding mRNA in liver and submaxillary gland and the concentration of an androgen and oestrogen binding protein in liver cytosol. Male rats gonadectomized on day 15 (after the neonatal surge of androgen secretion) were used as neonatally androgenized animals, their female littermates gonadectomized at the same age served as controls. Using a sensitive radioimmunoassay it could be demonstrated that only very low levels of α_{2u}-globulin are present in adult female control animals. Neonatal androgenization increases these levels some 14-fold. Stimulation with testosterone or dexamethasone results in a relative increase in the serum levels in animals of both sexes. After 11 days of treatment with testosterone propionate or dexamethasone, however, the final α_{2u}-globulin levels are respectively 1.8 times and 8 times higher in neonatally androgenized rats as compared to their female littermates. The concentration of the androgen and oestrogen binding protein in rat liver cytosol displays parallel sex differences in unstimulated animals and parallel changes after treatment with androgens and glucocorticoids. Measurements of α_{2u}-globulin mRNA by dot blot hybridization and by translation in reticulocyte lysate show a good correlation between the serum levels of α_{2u}-globulin and the corresponding mRNA in the liver. The abundance of α_{2u}-globulin mRNA in submaxillary gland is not influenced by neonatal androgens or hormone treatment during adulthood. These data suggest that neonatal androgenization directly or indirectly influences the availability for transcription and/or the transcription rate of α_{2u}-globulin genes in the liver.

INTRODUCTION

α_{2u}-Globulin is the major protein found in the urine of adult male rats. The protein is synthesized by the liver, secreted into the blood and—because of its small size–excreted into the urine [1]. Hepatic α_{2u}-globulin production is under multihormonal control [2]. Androgens, glucocorticoids, thyroxine and growth hormone promote the synthesis of this protein [3–9] whereas oestrogens [10] and a factor secreted by ectopically transplanted pituitary glands [11] suppress α_{2u}-globulin. The secretion of α_{2u}-globulin by the liver is subject to complex developmental control mechanisms: induction is impossible before puberty [12] and most hepatomas lose the ability to produce α_{2u}-globulin [13]. The submaxillary gland also produces some α_{2u}-globulin but this tissue does not modulate α_{2u}-globulin expression in response to the classical hormonal stimuli that control α_{2u}-globulin in rat liver [14]. In previous papers we have demonstrated that exposure to physiological concentrations of androgens during the neonatal period permanently changes the basal level of α_{2u}-globulin in serum from puberty on and enhances the response of α_{2u}-globulin to androgens and glucocorticoids [15–17]. In the present study we further analysed the effects of neonatal androgens on the concentration of α_{2u}-globulin in serum and on the levels of the corresponding mRNA in liver and submaxillary gland.

A 3.5 S protein that binds androgens and oestrogens has been described in liver cytosol of mature male rats [18]. This protein has been named "Cytoplasmic androgen binder" (CAB) by Roy *et al.*[2] and the hypothesis has been advanced that this protein might play a role in the induction of α_{2u}-globulin [2]. Accordingly, we also studied the influence of neonatal androgenization on this binding protein.

EXPERIMENTAL

Chemicals

[^{35}S]Methionine (1050 Ci/mmol), [2,4,6,7-^{3}H]-oestradiol (93 Ci/mmol) and [^{14}C]ovalbumin (12 μCi/mg) were obtained from New England Nuclear. Reticulocyte lysate (nuclease treated) and [^{32}P]dATP (410 Ci/mmol) were purchased from Amersham. *Staphylococcus aureus* cells (Pansorbin) were obtained from Calbiochem-Behring Corporation, U.S.A.

Animals and treatments

Wistar rats were supplied by the breeding center of the Catholic University of Leuven. Animals were housed under standard conditions of temperature, humidity and light and had free access to a standard

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pellet diet and tap water. All animals were gonadectomized on day 15 after birth under anaesthesia with Nembutal. On day 60, male and female rats were divided in three groups of 5 animals. One group received 11 daily injections of testosterone propionate (200 μg in 0.1 ml olive oil s.c.), a second group received dexamethasone (Decadron-shock-pak; Merck and Co. Inc., Rahway, New York, U.S.A.; 20 μg in 0.2 ml 0.9% saline s.c.) and a control group received olive oil only. Blood was taken from the tail vein before the first injection and on day 5 of treatment. On day 11 of treatment the animals were killed by exsanguination under ether anaesthesia. The livers and submandibular glands were removed immediately and were stored in liquid nitrogen. Two gram fragments of the livers of all animals within each experimental group were pooled for RNA extraction.

Measurement of α_{2u}-globulin

α_{2u}-Globulin was measured in the serum by a specific radioimmunoassay as described previously [19].

RNA extraction and isolation of poly(A)$^+$ RNA

Nucleic acids were extracted as described by Rosen *et al.*[20] with some modifications. Briefly, frozen tissue was homogenized in a Waring blender (1 min at low speed and 30 s at high speed) in the presence of 6 vol of 0.5% sodium dodecyl sulfate (SDS), 0.025 M EDTA, 0.075 M NaCl, pH 8 and 6 vol of buffer-saturated phenol, pH 8.0. The resulting emulsion was chilled for 30 min on ice and was centrifuged at 3.500 ***g*** for 20 min. The aqueous upper phase and protein interphase were reextracted with an equal volume of buffer-saturated phenol containing chloroform (1:1, v/v). Following recentrifugation, the aqueous phase was incubated with proteinase K (20 μg/ml) for 15 min at 37°C. A final phenol–chloroform extraction was then performed and the aqueous phase was made 0.2 M in NaCl. After addition of 2 vol of cold ethanol the solution was allowed to precipitate overnight at −20°C. The precipitate was collected and dissolved in H_2O at a concentration of 1 mg/ml. Three volumes of 4 M sodium-acetate pH 6 were added and after 2 h of incubation at 4°C the RNA was recovered by centrifugation, dissolved in H_2O, made 0.2 M in NaCl and precipitated with ethanol. Poly(A)$^+$ RNA was isolated essentially as described by Aviv and Leder[21]. A 0.1 M NaCl washing step, however, was introduced before the final elution.

RNA translation, immunoprecipitation of α_{2u}-globulin and polyacrylamide gel electrophoresis

Poly(A)$^+$ RNA was translated in rabbit reticulocyte lysate. 3 μg RNA, 60 μl lysate and 100 μCi [^{35}S] methionine were incubated for 90 min at 30°C. The α_{2u}-globulin synthesized *in vitro* was quantitated by immunoprecipitation with a specific anti-α_{2u}-globulin antiserum [19] and *Staphylococcus aureus* cells according to the method of Kessler[22]. The translation products were separated by SDS-polyacrylamide slab gel electrophoresis as described previously [23].

Dot blot hybridization

α_{2u}-Globulin-specific sequences in total RNA or poly(A)$^+$ RNA were analysed by dot blot hybridization using the method described by White and Bancroft[24]. Total RNA was denaturated with formaldehyde. Poly(A)$^+$ RNA preparations were denaturated by heating at 70°C for 5 min followed by cooling in melting ice. The RNA was suspended in 15 × SSC (1 × SSC contains 0.15 M NaCl and 0.015 M sodium citrate pH 7.4). Serial dilutions (1/2) were made in microtiter plates (96-well microtest II, Falcon, Becton Dickinson) using the same buffer and 100 μl aliquots of the RNA dilutions were transferred onto nitrocellulose paper (BA 85, Schleicher and Schüll) with the help of a dot blot template (Minifold, Schleicher and Schüll). Before use the nitrocellulose paper was soaked in 15 × SSC. After application of the RNA the paper was baked at 80°C for 2 h. Prehybridization and hybridization to an excess of nick-translated [25] α_{2u}-globulin cDNA were performed as described by Dobner *et al.*[26] but with omission of glycine in the hybridization mixture. The α_{2u}-globulin probe used consisted of the 530 base-pair EcoRI-Hind III fragment of α_{2u}-globulin cDNA [27]. Approximately 10^6 cpm of the cDNA was allowed to hybridize with each filter for 60 h at 42°C. Filters were exposed to X-ray film (Curix RPII, Agfa-Gevaert) using an intensifying screen (Kyokko, LH II) for 1–4 days at 70°C.

Northern blot hybridization

Northern blot analysis of RNA was performed as described by McMaster and Carmichael[28]. Five μg poly(A)$^+$ RNA was subjected to electrophoresis in 1.5% agarose gels containing 0.01 M NaH_2PO_4 pH 7.0. The RNA was transferred to nitrocellulose filters (BA 85, Schleicher and Schüll) using 20 × SSC. Filters were prehybridized and hybridized to nick-translated α_{2u}-globulin cDNA as described in the previous paragraph.

Measurement of cytoplasmic androgen and oestrogen binding protein of rat liver

The cytoplasmic androgen and oestrogen binding protein of rat liver was studied by sucrose density gradient centrifugation as described by Roy *et al.*[18].

RESULTS

Effects of neonatal androgenization on basal and stimulated levels of α_{2u}-globulin in serum

To study the effects of physiological concentrations of androgens secreted in the neonatal period, male animals were gonadectomized at the age of 15 days.

Table 1. Influence of neonatal androgenization and treatment with testosterone propionate or dexamethasone on body and organ weights

	n	Body (g)	Adrenal (mg)	Uterus (mg)	Prostate (mg)	Seminal vesicles (mg)
Male rats						
Control	5	250 ± 11	109 ± 6	—	ND	ND
Testosterone propionate	5	271 ± 10	73 ± 5	—	218 ± 9	291 ± 12
Dexamethasone	5	211 ± 9	40 ± 3	—	ND	ND
Female rats						
Control	4	208 ± 12	78 ± 6	40 ± 4	—	6
Testosterone propionate	5	212 ± 9	67 ± 3	132 ± 5	—	—
Dexamethasone	5	172 ± 7	30 ± 8	29 ± 6	—	—

Male and female rats were gonadectomized on day 15 after birth. From day 60 on the animals were treated either with testosterone propionate (200 μg/day), dexamethasone (20 μg/day) or olive oil. Body and organ weights were recorded on day 71 when the animals were killed. Results represent the mean ± SE of the indicated number of animals. The seminal vesicles and prostate in oil or dexamethasone treated animals were barely detectable. Their weight was not determined (ND).

Female littermates ovariectomized at the same age served as non-androgenized controls. The influence of neonatal androgenization on basal and androgen- or glucocorticoid-induced levels of α_{2u}-globulin was studied from day 60 on. At autopsy the efficiency of the endocrine treatments was controlled visually and by measurements of body and organ weights (Table 1). Body weights were higher in neonatally androgenized animals irrespective of any further hormonal treatment, confirming earlier observations [17]. In preliminary experiments (not shown) we demonstrated that 20 μg of dexamethasone and 200 μg of testosterone propionate are maximally effective doses for the induction of α_{2u}-globulin (Vandoren and Verhoeven, unpublished results). It may be seen that this dose of dexamethasone decreases adrenal and body weight whereas testosterone markedly stimulates the prostate and seminal vesicles in male animals and uterus weight in female rats.

On day 60, α_{2u}-globulin can clearly be detected in the serum of female rats (Table 2). Neonatally androgenized (male) animals, however, display α_{2u}-globulin levels that are some 14 times higher. Androgen treatment increases α_{2u}-globulin in gonadectomized animals of both sexes, the relative increase is even greater in female animals than in male rats but the final level after 11 days of treatment is 1.8 times higher in neonatally androgenized rats. Dexamethasone also stimulates α_{2u}-globulin in both sexes but the increase tends to level off between day 5 and 11 of treatment. Moreover, after 11 daily injections the attained serum level is 8 times higher in male animals than in female ones.

Effects of neonatal androgenization on the androgen and oestrogen binding protein in liver cytosol

The influence of the endocrine manipulations mentioned in the previous paragraph on the androgen and oestrogen binding protein in liver cytosol (CAB) was also investigated. Representative results are shown in Fig. 1. It may be noted that the changes in CAB in liver cytosol parallel those of α_{2u}-globulin in serum. Neonatally androgenized male rats display higher levels than female controls. Androgen treatment during adulthood increases the binding in animals of both sexes. Administration of dexamethasone also markedly stimulates oestradiol binding to CAB but the response of male animals to this glucocorticoid is distinctly higher than that of female animals. Comparable results were obtained after incubation of liver cytosol with [^{3}H]5α-dihydro-

Table 2. Influence of neonatal androgenization on α_{2u}-globulin levels in the serum of gonadectomized male and female rats

		α_{2u}-Globulin (mg/l)		
	n	Day 60	Day 65	Day 71
Male rats				
Control	5	2.28 ± 0.72[a]	3.27 ± 0.68[a]	3.24 ± 0.49[a]
Testosterone propionate	5	4.82 ± 1.32[a]	*14.50 ± 1.72[b]	22.84 ± 0.65[b]
Dexamethasone	5	3.06 ± 0.94[a]	*8.69 ± 1.60[b]	9.38 ± 1.86[c]
Female rats				
Control	4	0.23 ± 0.08[a]	0.25 ± 0.14[a]	0.33 ± 0.12[a]
Testosterone propionate	5	0.28 ± 0.05[a]	*3.28 ± 0.73[b]	*12.36 ± 0.57[b]
Dexamethasone	5	0.22 ± 0.03[a]	*0.84 ± 0.19[c]	1.23 ± 0.56[c]

Male and female animals were gonadectomized on day 15 after birth. From day 60 on male (neonatally androgenized) as well as female (not neonatally androgenized) animals were treated either with testosterone propionate (200 μg/day), dexamethasone (20 μg/day) or olive oil. Blood was taken on day 60, 65 and 71. α_{2u}-Globulin was determined in the serum by a specific RIA. Results represent the mean ± SE of the indicated number of animals. The effect of treatment in male and female animals was evaluated on day 60, 65 and 71 by oneway analysis of variance following logarithmic transformation of the data. Values followed by the same letter do not differ significantly ($P < 0.05$). Within each treatment group the effect of treatment as a function of time was analysed in the same way. Data preceded by an asterisk differ significantly ($P < 0.05$) from the immediately preceding measurement.

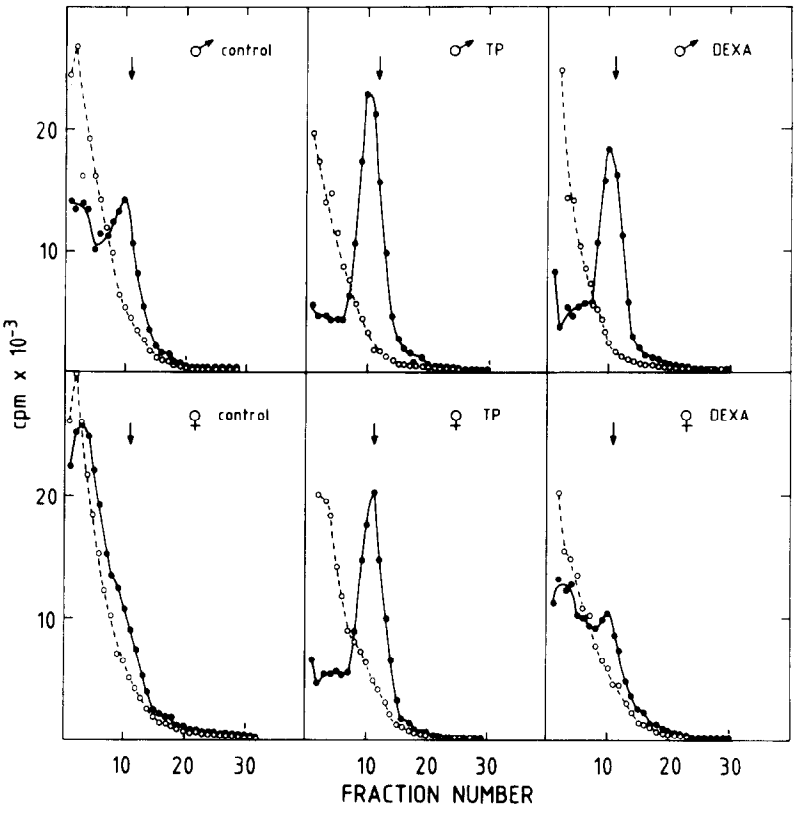

Fig. 1. Influence of neonatal androgens on the androgen and oestrogen binding protein in liver cytosol. Male and female rats were gonadectomized on day 15 after birth. From day 60 on male (neonatally androgenized) as well as female (not neonatally androgenized) animals were treated either with olive oil (control), testosterone propionate (TP: 200 μg/day) or dexamethasone (DEXA: 20 μg/day). On day 71 the animals were killed. The livers were stored in liquid nitrogen and the binding of [^{3}H]oestradiol to the cytosolic androgen and oestrogen binding protein was analysed as described by Roy *et al.*[18]. Liver cytosol was incubated with 40 nM [^{3}H]17β-oestradiol in the presence (open symbols) or absence (closed symbols) of 12 μM unlabelled oestradiol for 40 min at 0°C. 0.05 ml aliquots of the samples were applied on 5–15% sucrose gradients. Gradients were spun for 22 h at 220,000 *g*. Fractions are numbered from the top to the bottom of the tube. The position of [^{14}C]ovalbumin is indicated by an arrow.

testosterone but the binding peaks were consistently lower. No specific binding could be demonstrated in the 3.5 S region after incubation of the cytosols with [^{3}H]dexamethasone (not shown).

Effects of neonatal androgenization on basal and stimulated levels of α_{2u}-globulin mRNA in liver and submaxillary gland

To investigate whether the observed differences in the serum concentration of α_{2u}-globulin are related to changes in the rate of α_{2u}-globulin synthesis we studied the abundance of α_{2u}-globulin RNA in total liver RNA extract (not shown) and poly(A)$^+$ RNA derived from the different experimental groups using dot blot hybridization (Fig. 2). These data show that there exists a good correlation between the concentration of α_{2u}-globulin in serum (Table 2) and the amount of α_{2u}-globulin RNA. Quantitative analysis of the hybridization data was performed by scanning of the autoradiograms in a Titertek apparatus (Flow Laboratories, Irvine, U.K.). Normalization of the measurements to those observed in androgen treated males (arbitrarily taken as 100%) yields values of 25 and 39% for oil treated and dexamethasone treated males respectively. The corresponding figures for oil treated, androgen treated and dexamethasone treated females were 2.7, 52 and 12% respectively. The very low levels of α_{2u}-globulin RNA observed in female control animals were apparently real. They disappeared after treatment with RNAse. In addition poly(A)$^+$ RNA from prostate, a tissue that does not produce α_{2u}-globulin, showed no hybridization at all. The observed effects of perinatal and adult hormones are limited to the liver. In fact dot blot analysis of total RNA of submaxillary gland shows no effects of neonatal androgenization or adult hormone treatment (Fig. 3). Northern blot analysis of the hepatic poly(A)$^+$ RNA preparations (Fig. 4) reveals no differences in the size of the α_{2u}-globulin mRNA in the different treatment groups. A major band can be observed in the 1.6 kb region.

To study the amount of *functional* α_{2u}-globulin mRNA, poly(A)$^+$ RNA preparations from the livers of the different experimental groups were analysed by

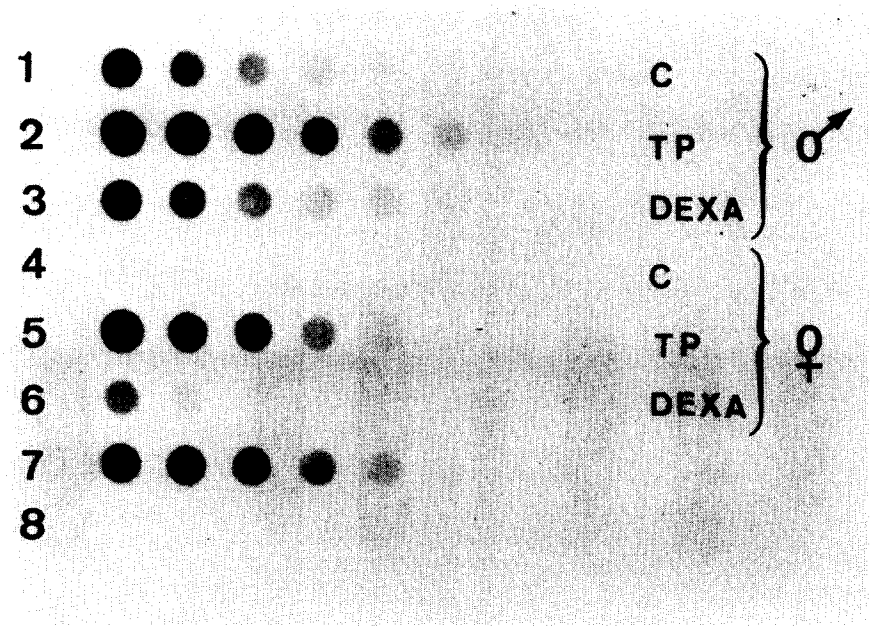

Fig. 2. Influence of neonatal androgenization on α_{2u}-globulin RNA abundance in liver. The animals were treated as explained in Fig. 1 and poly(A)$^+$ RNA was prepared as described in the Experimental section. The abundance of α_{2u}-globulin RNA was examined by dot blot analysis using serial dilutions (1/2) of the RNA preparations. The treatments of the experimental groups (lanes 1–6) have been indicated on the right (C: control; TP: testosterone propionate; DEXA: dexamethaxone). Lane 7 contains a reference liver poly(A)$^+$ RNA preparation. Lane 8 contains prostatic poly(A)$^+$ RNA. The highest amount of RNA applied to the filters is 0.1 μg for lane 1–7 and 5 μg for lane 8.

in vitro translation and immunoprecipitation with and anti-α_{2u}-globulin antiserum. The gel profiles displayed in Fig. 5 demonstrate marked differences in the amount of α_{2u}-globulin synthesis in the distinct experimental groups. In addition it may be noted that treatment with androgens in adulthood provokes important changes in the synthesis of a number of other proteins. A protein with a mol.wt somewhat lower than 30,000 disappears and some 2 proteins with a mol.wt of approx 30,000 increase in concen-

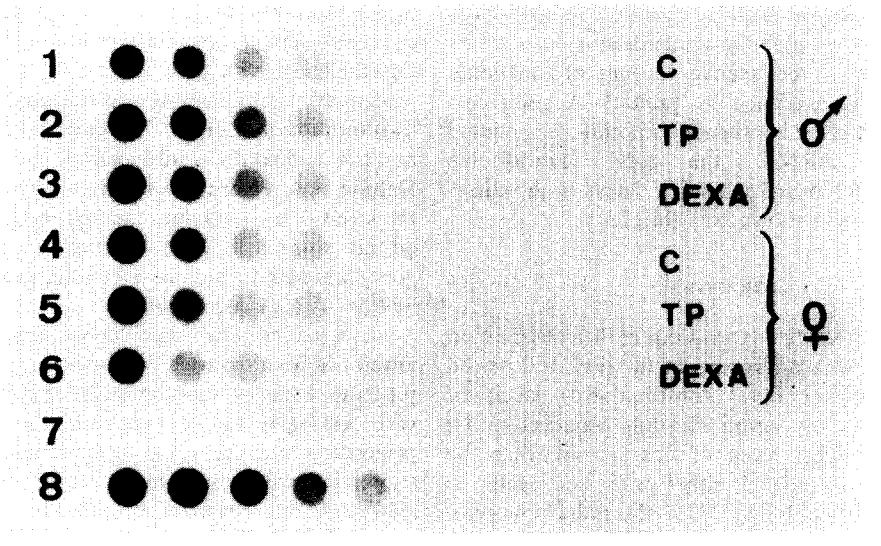

Fig. 3. Dot blot analysis of α_{2u}-globulin RNA abundance in submaxillary gland. The animals were treated as explained in Fig. 1. Total RNA was extracted as described in the Experimental section and dot blot analysis for α_{2u}-globulin RNA was performed as described in Fig. 2. Lane 7 shows the same RNA extract as that presented in lane 2 after treatment with RNase A (4 μg/ml) and T_1 (200 U/ml) for 30 min at 37°C. Lane 8 contains a reference liver poly(A)$^+$ RNA preparation. The highest amount of RNA applied to the filters is 5 μg for lanes 1–7 and 0.05 μg for lane 8.

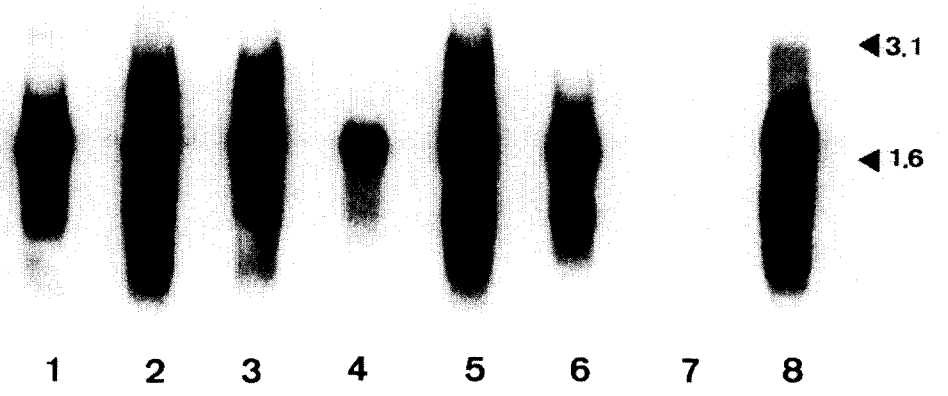

Fig. 4. Electrophoretic properties of liver RNA (Northern Blot). Northern Blot analysis of the poly(A)$^+$ RNA preparations of the experimental groups described in Fig. 1 was performed as explained in the Experimental section. The poly(A)$^+$ RNA in lane 1, 2 and 3 was derived from the livers of male animals treated with oil, testosterone priopionate and dexamethasone respectively. Poly(A)$^+$ RNA preparations in lane 4, 5 and 6 were derived from the livers of female animals treated with oil, testosterone propionate and dexamethasone respectively. Lane 7 contains prostatic poly(A)$^+$ RNA. Lane 8 contains a reference poly(A)$^+$ RNA preparation derived from adult male rats. Size markers were from bacterial ribosomal RNA concurrently subjected to electrophoresis. Exposure time was 36 h.

tration. These proteins are probably identical to those observed by Roy *et al.*[2]. A quantitative analysis of the amount of α_{2u}-globulin synthesized *in vitro*, which is a measure of the relative amount of functional α_{2u}-globulin is presented in Table 3. A good correlation is observed between the amount of functional α_{2u}-globulin mRNA, the total hybridizable α_{2u}-globulin RNA and the serum levels of the corresponding protein shown in Table 2.

DISCUSSION

The present study confirms our earlier observation that contact with androgens in the perinatal period results in higher basal (unstimulated) levels of α_{2u}-globulin in the serum of adult gonadectomized rats and in higher levels of this protein when the animals are stimulated either with androgens or with glucocorticoids [16]. With the radial immunodiffusion technique used in previous work we were unable to demonstrate α_{2u}-globulin in the serum of gonadectomized female animals [16]. Moreover, after administration of dexamethasone to such animals virtually no response was observed [15, 16]. The specific and sensitive radioimmunoassay used in the present study unambiguously shows that low levels of α_{2u}-globulin are present in the serum of gonadectomized female rats. Furthermore it can be noted that the relative increase in the concentration of this protein after administration of dexamethasone is at least as important in female animals as that observed in their neonatally androgenized male littermates. Despite this remarkable relative increase, however, the levels of α_{2u}-globulin observed in female animals do not approach those observed in unstimulated gonadectomized male rats even after prolonged stimulation with glucocorticoids.

Increased responsiveness upon secondary stimualtion has been reported for other steroid responsive proteins. Primary stimulation of the chick oviduct with oestrogens for instance results in an enhanced response of the egg-white proteins upon secondary stimulation with oestrogens or progestogens [29]. The effects of androgens on α_{2u}-globulin, however, are remarkable in at least two ways. (1). The "imprinting" effect of androgens is limited to the perinatal period. Administration of androgens to gonadectomized female rats or neonatally gonadectomized male animals during adult life does not have a similar effect on basal and stimulated levels of

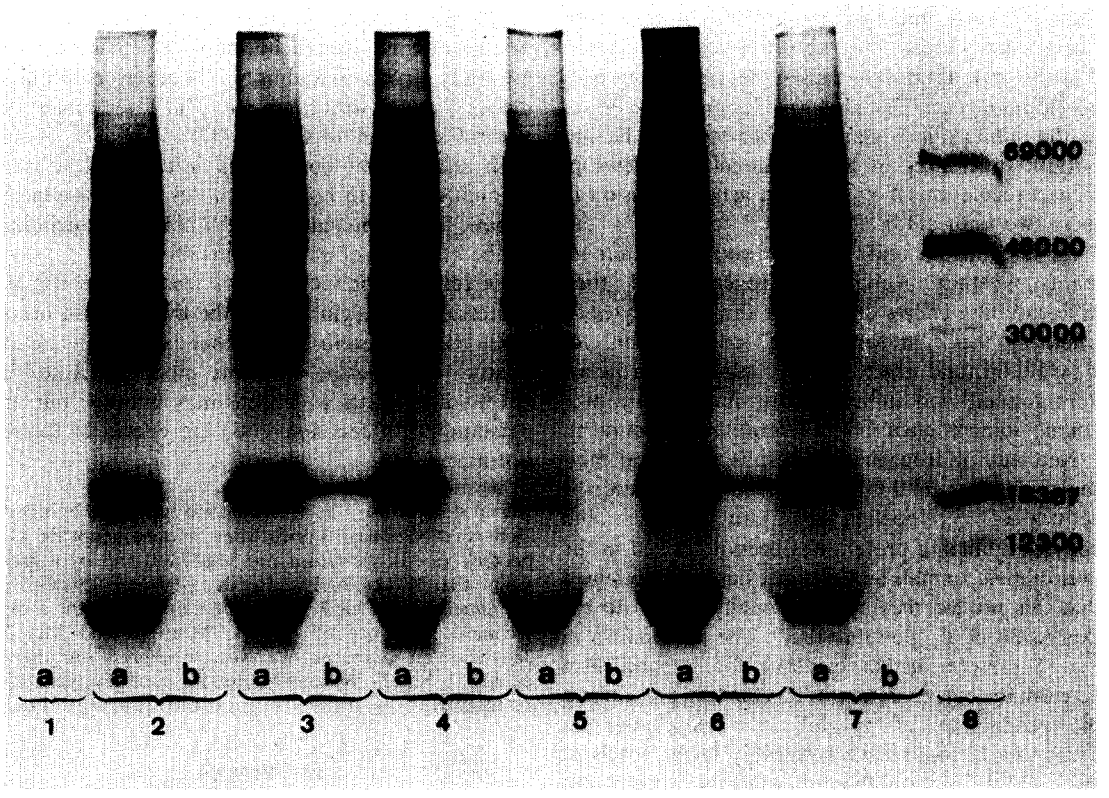

Fig. 5. Influence of neonatal androgenization on α_{2u}-globulin functional mRNA in rat liver. The animals were treated as explained in Fig. 1. Poly(A)$^+$ RNA was prepared and translated in rabbit reticulocyte lysate as described in the Experimental section. Total (lanes a) and immunoprecipitated proteins (lanes b), using an anti-α_{2u}-globulin antiserum, were analysed by polyacrylamide gel electrophoresis and autoradiography. The origin of the poly(A)$^+$ RNA preparations studied in the different lanes is as follows: lane 1: no RNA; lane 2, 3, 4: male animals treated with oil, testosterone propionate or dexamethasone respectively; lanes 5, 6, 7: female animals treated with oil, testosterone propionate or dexamethasone; lane 8: reference proteins (albumin, ovalbumin, carbonic anhydrase, lactoglobulin A, cytochrome c).

α_{2u}-globulin [16]. (2). The effects of perinatal androgens come to expression after a latent period extending throughout prepubertal life [16]. With respect to these two characteristics α_{2u}-globulin resembles a number of other parameters shown to be influenced by neonatal androgenization [30].

Since α_{2u}-globulin is the best-characterized single protein influenced by neonatal androgenization it may represent a valuable tool for further studies on the molecular mechanisms underlying this process and underlying steroid-induced differentation in general. The experiments summarized in Figs 2 and 5 show that the mentioned changes in the basal level of α_{2u}-globulin in serum and in its response to androgens and glucocorticoids are paralleled by quantitatively comparable alterations in the amount of the corre-

Table 3. Influence of neonatal androgenization and treatment with testosterone propionate or dexamethasone in adulthood on the amount of functional α_{2u}-globulin mRNA

		cpm after immunoprecipitation		α_{2u}-Globulin	
Treatment	Total cpm	α_{2u}-Globulin antiserum	Non-immune antiserum	Percent	Normalized
Male control	452,713	14,229	3562	2.35	37
Male TP	474,067	33,901	3974	6.31	100
Male DEXA	509,487	19,323	3787	3.04	48
Female control	390,920	3637	3050	0.15	2
Female TP	341,593	19,285	2688	4.85	77
Female DEXA	336,213	5564	1993	1.06	17

Rats were treated and poly(A)$^+$ RNA was prepared as described in the Experimental section. The RNA was translated in rabbit reticulocyte lysate. Values represent total TCA-precipitable radioactivity (as [^{35}S]methionine) or immunoprecipitated radioactivity using a specific anti α_{2u}-globulin or non-immune rabbit serum, present in 20 μl reticulocyte lysate system. The percentage of the total protein synthesis that is α_{2u}-globulin and the percentage α_{2u}-globulin synthesis normalized to that observed in testosterone propionate treated male animals are also indicated.

sponding total and functional RNA in the liver. These data suggest that neonatal androgenization directly or indirectly increases the transcription of α_{2u}-globulin genes. The absence of comparable effects in the submaxillary gland further stresses the important differences in the hormonal and developmental regulation of α_{2u}-globulin gene expression in these two tissues [14].

A fundamental and still unresolved question remains whether neonatal androgens exert their differentiating effects directly on the hepatocytes or whether they influence the transcription of α_{2u}-globulin (and other similarly regulated proteins) during adulthood indirectly, for instance by provoking some change in the hormonal environment.

Neonatal androgenization results in several molecular changes in the liver that might enhance the activity of α_{2u}-globulin genes. An androgen and oestrogen binding protein has been described in the liver of male animals and evidence has been presented that this protein may be functionally related to the expression of α_{2u}-globulin [18, 2]. The data shown in Fig. 1 present further evidence for a parallelism between the concentration of this binding protein and the production of α_{2u}-globulin. Neonatally androgenized animals have noticeably higher levels of this binding protein than their female littermates, confirming the observations of Sloop *et al.*[31]. Moreover, the concentration of the binding protein increases after treatment with androgens and glucocorticoids and—as for α_{2u}-globulin—the increase is more important in neonatally androgenized animals. The exact biochemical role of this binding protein (if any) in the hepatic synthesis of α_{2u}-globulin, however, remains to be established. Very recently it has been demonstrated that the concentration of the 8S-hepatic androgen receptor is increased by neonatal androgenization [32]. Although this finding might present an attractive explanation for the increased androgen responsiveness, it does not explain the increased response to glucocorticoids. Finally, recent experiments suggest that a rearrangement of the chromatin configuration within and around the α_{2u}-globulin genes via perinatal "imprinting" by androgens might be responsible for endowing the differential androgen responsiveness in male and female rats [2].

On the other hand, there is considerable evidence that neonatal androgens might influence the liver "indirectly" by suppressing the secretion of a hypophyseal "feminizing factor" [33, 34]. Based on experiments showing that implantation of an uncoupled pituitary under the renal capsule suppresses α_{2u}-globulin secretion and that this effect is reversed by an inhibitor of prolactin secretion we have suggested that prolactin might be this feminizing factor. Recently, Gustafsson *et al.*[15, 36] have presented evidence that growth hormone might be the feminizing factor in a number of systems influenced by neonatal androgens. MUP, the major urinary protein of the mouse is stimulated by the pulsatile secretion or injection of growth hormone which is achieved naturally in males and is inhibited by continuous delivery of growth hormone which resembles the natural situation in females [37]. Moreover, it has been shown that continuous infusion of a large amount of growth hormone causes a decrease in the hepatic androgen and oestrogen binding protein and in the synthesis of α_{2u}-globulin [38].

Direct and indirect control mechanisms for the expression of α_{2u}-globulin in the liver are not necessarily mutually exclusive. Further experiments on the relative contribution of these mechanisms to the effects of neonatal androgens may improve our understanding of the differentiating effects of steroid hormones.

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