Retinol-binding protein mRNA is induced by estrogen in the kidney but not in the liver

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Abstract Vitamin A is mobilized from the liver and transported in plasma as retinol bound to retinol-binding protein (RBP). In addition to the liver, several extrahepatic tissues including the kidney have been shown to contain RBP mRNA. A study was conducted to explore the role of sex hormones in the regulation of RBP mRNA levels in the kidney compared to those in the liver. Treatment of female rats with a single dose of testosterone or chronic treatment with testosterone had only a slight effect on the steady-state level of RBP mRNA in the kidney and the liver. However, treatment of male rats with estrogen caused an increase in the steady-state level of RBP mRNA in the kidney but not in the liver. A single injection of 17β-estradiol, either 1.0 or 0.1 μg/g body weight, resulted in a rapid rise in the level of RBP mRNA in the kidney which was maximal at 3–6 h (fivefold induction) after treatment. In addition, treatment of ovariectomized female rats with estrogen also resulted in a rapid rise in the accumulated level of RBP mRNA in the kidney while having no influence in the liver. Finally, studies using the antiestrogen drug, hydroxytamoxifen, resulted in blockage of the estrogen-related induction of RBP mRNA in the kidney, suggesting that the induction of RBP mRNA in the kidney by estrogen may be mediated by the nuclear estrogen receptor. Taken together these data suggest that the regulation of RBP mRNA levels in the liver and kidney, at least with respect to estrogen, is different. —**Whitman, M. M., D. C. Harnish, K. J. Soprano, and D. R. Soprano.** Retinol-binding protein mRNA is induced by estrogen in the kidney but not in the liver. *J. Lipid Res.* 1990. **31:** 1483–1490.

Supplementary key words vitamin A • testosterone • hydroxytamoxifen

Retinol-binding protein (RBP) is a 20–21 kDa protein which functions to transport retinol in the plasma (see (1) for a recent review). RBP is secreted into the plasma mainly as the retinol-RBP complex (holo-RBP) (2). In plasma holo-RBP strongly interacts with another protein, transthyretin (TTR), and normally circulates as a 1:1 molar RBP-TTR complex (3–6).

The liver is a major site of RBP synthesis functioning to transport retinol from its storage site in the liver to target tissues (1–6). Recently, several extrahepatic tissues including kidney (7), adipose tissue (8), and visceral yolk sac (9, 10) have been shown to contain RBP mRNA, and visceral yolk sac explants have been shown to synthesize RBP (9). Using in situ hybridization, RBP mRNA in the adult and newborn rat kidney has been localized to the S3 segment of the proximal tubules (8). We have hypothesized that extrahepatic RBP in the kidney and adipose tissue might be involved in the recycling of retinol from these tissues to the liver or to other target tissues (7, 8).

Only limited information has been reported concerning the factors that regulate the synthesis and secretion of RBP by the liver and extrahepatic tissues. Retinol is a factor that specifically influences the secretion of RBP from both hepatocytes (11–14) and the visceral yolk sac (15) without influencing the rate of synthesis of RBP or RBP mRNA levels (7, 15, 16). Additional studies using both hepatoma cells (17) and isolated primary hepatocytes (18) have suggested a role of adrenocortical hormones in the maintenance of RBP production.

Little information is available concerning the role of gonadal hormones in the regulation of RBP synthesis and secretion despite reports that plasma RBP levels do not reach maximum until puberty in both men and women (19, 20). In addition, both plasma vitamin A levels and plasma RBP levels have been shown to fluctuate in a cyclic pattern in women during the menstrual cycle (21) and the plasma concentration of both vitamin A and RBP is elevated in women taking oral contraceptives which contain high levels of estrogen (21–23).

In the present study, we have examined the role of estrogen and testosterone in the regulation of RBP mRNA levels in both the kidney and liver. Our results, reported here, show that RBP mRNA levels are only slightly influenced in either the liver or the kidney by testosterone treatment of adult female rats. However, treatment of both male and female rats with estrogen results in an in-

Abbreviations: RBP, retinol-binding protein; CRBP, cellular retinol-binding protein.

crease in RBP mRNA steady-state levels in the kidney but not the liver. These data suggest that the regulation of extrahepatic RBP mRNA levels, at least with respect to estrogen, is different from the regulation of RBP expression in the liver.

METHODS

Animals

Male adult and female adult Sprague-Dawley (CD) rats weighing 175–200 g were obtained from Charles River Breeding Laboratory, Kingston, NY. Ovariectomized female rats and sham-operated rats were also obtained from Charles River Breeding Laboratory. Ovariectomized rats and sham-operated rats were maintained for 30 days before further experimentation. All animals were maintained on a 12-h light, 12-h dark cycle, with Purina Rodent Chow (Buckshire Corporation, Lansdale, PA) and water ad libitum.

For studies related to estrogen, male rats were injected subcutaneously with 17β-estradiol (Sigma Chemical Co., St. Louis, MO) dissolved in 70% peanut oil/30% ethanol, either 0.1 μg/g or 1.0 μg/g body weight. Control animals were injected subcutaneously with an equal volume of peanut oil/ethanol only. Animals were killed at various times after injection (0–24 h) and kidneys and livers were obtained for RNA isolation. In another experiment, ovariectomized female rats (30 days after removal of the ovaries) were injected with 17β-estradiol dissolved in 70% peanut oil/30% ethanol, 0.1 μg/g body weight, killed 0, 3, or 6 h later, and tissues were obtained for RNA isolation. Finally, to examine the potential role of the estrogen receptor, male rats were pretreated with a subcutaneous injection of hydroxytamoxifen (a gift from ICI Pharmaceuticals, Macclesfield, United Kingdom) (0.5 μg/g body weight). Two hours later these animals were given a subcutaneous injection of 17β-estradiol, 0.1 μg/g body weight, and killed 6 h later to obtain tissues for RNA isolation. The levels of RBP mRNA in the kidney and liver of these animals were compared to those of control rats receiving either a subcutaneous injection of peanut oil/ethanol alone and killed 6 h later, or 0.5 μg/g body weight hydroxytamoxifen alone and killed 6 h later, or 0.1 μg/g body weight 17β-estradiol alone and killed 6 h later.

For studies related to the acute effects of testosterone, female rats were injected subcutaneously with 2 mg/kg testosterone (Sigma Chemical Co.) dissolved in peanut oil. Control rats were injected with an equal volume of peanut oil alone. Animals were killed at various times after testosterone injection (0–18 h) and tissues were obtained for RNA isolation. In experiments dealing with chronic testosterone treatment, a 75-mg pellet (3 week time-release) of testosterone (Innovative Research of America, Toledo, OH) was subcutaneously implanted at the nape of the neck of each adult female rat. Rats were killed 1, 4, 7, and 14 days later and the tissues were obtained for RNA isolation.

RNA isolation and analysis

Total RNA was prepared from the livers and kidneys of rats by the method of Tushiniski et al. (24). RNA levels were quantitated spectrophotometrically by measuring absorbance at 260 nm and 280 nm; and the integrity of each RNA sample was determined by agarose/formaldehyde gel electrophoresis as described previously (7, 9).

RBP mRNA levels were determined by RNAse protection assay essentially as described previously (25, 26). Antisense strand (hybridizing) RBP cRNA probes were synthesized using the previously reported 548 bp rat RBP cDNA clone (pRRBP) as template (15). As a control for the level of RNA in each sample and for the specificity of the estrogen-related response the level of another transcript, cellular retinol-binding protein (CRBP) was also determined by RNAse protection analysis. The antisense strand (hybridizing) CRBP cRNA probe was synthesized using the 224 bp fragment (position 147 to position 371) of the rat CRBP cDNA clone (pRCRBP) as template (27). All plasmid DNA was isolated by the alkaline lysis method (28). The cRNA probes were synthesized from linearized pRRBP and pRCRBP using either SP6 RNA polymerase or T7 RNA polymerase essentially as described by Melton et al. (29) with the modifications previously reported (15).

Total cellular RNA (3–20 μg depending on tissue and transcript) was used in all solution hybridization assays. Hybridization, RNAse treatment, electrophoresis of samples, and autoradiography were performed as described previously (15). Bands were quantitated by scanning appropriate exposures of the autoradiograms, which gave signals in the linear range of the films with a Hoefer Model 1650 scanning densitometer and Hoefer GS365 densitometer software. The relative level of RBP mRNA found in each sample was calculated relative to the control value arbitrarily set to 1.0.

RESULTS

Estrogen and RBP mRNA levels in male rats

To determine the role that estrogen plays in the regulation of RBP mRNA levels in rats, we injected 17β-estradiol subcutaneously into male rats, either 1.0 μg/g or 0.1 μg/g body weight. Rats were killed at various time points after injection, and total RNA was isolated from the liver and kidney. The level of RBP mRNA was determined by RNAse protection analysis using an antisense RBP cRNA probe. It should be noted that the level of the RBP mRNA in the kidney of the control, untreated Charles River Sprague-Dawley rats used in this study was approximately 0.5–1.0% of that in the liver. This level of kidney

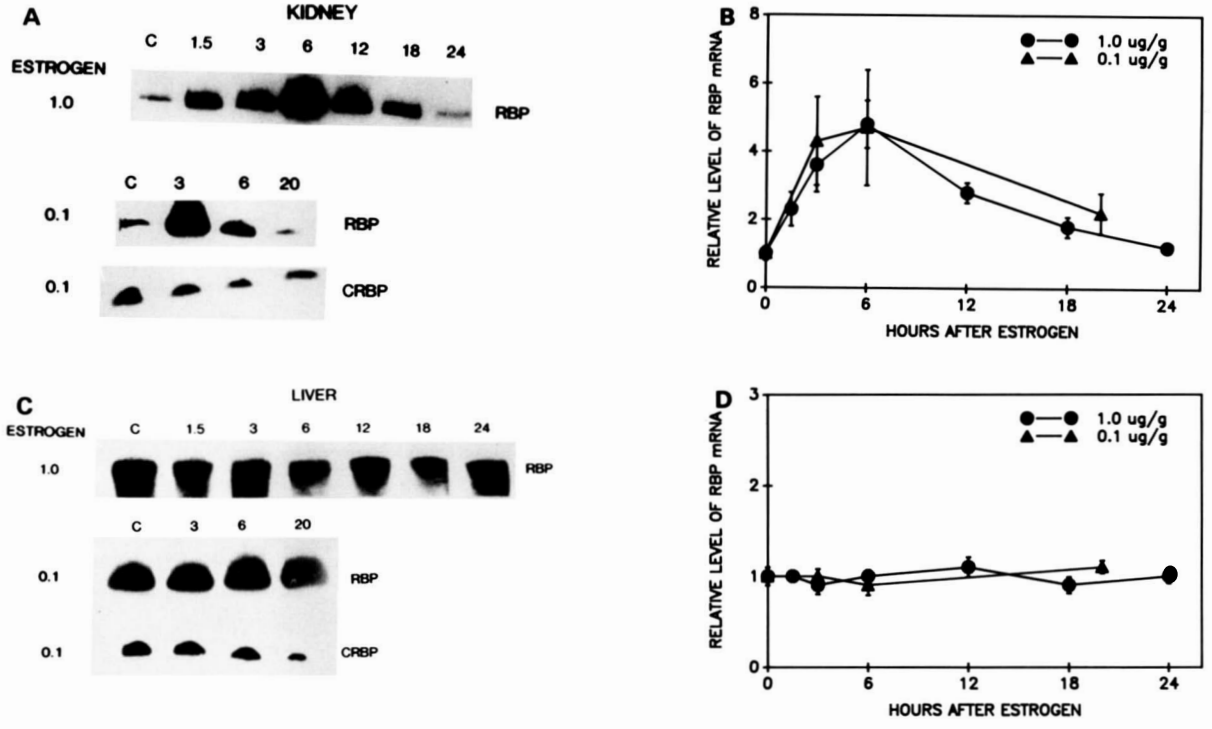

Fig. 1. The effect of β-estradiol on the level of RBP mRNA in the kidney and liver of male rats. Male Charles River Sprague-Dawley rats were injected subcutaneously with 17β-estradiol either 1.0 μg or 0.1 μg/g body weight. Rats (three animals per time point and treatment) were killed at the indicated times (0–24 h after injection) and total RNA was isolated from the kidney and the liver. The levels of RBP mRNA and CRBP mRNA were determined by RNAse protection analysis and quantitated by densitometric scanning. Panels A and C are representative autoradiograms of RBP mRNA and CRBP mRNA levels in the kidney (A) and the liver (C). Panels B and D are graphic representations of the relative level of RBP mRNA in the kidney (B) and the liver (D). The relative level of RBP mRNA was calculated with the control value arbitrarily set to 1.0. Values are mean ± SEM.

RBP mRNA is lower than that we previously reported for Camm Sprague-Dawley rats (7). In studying a number of strains of rats purchased from different animal suppliers we have observed that the level of kidney RBP mRNA varies depending on the strain and the supplier while the level of liver RBP mRNA appears to be quite similar (D.R. Soprano, unpublished observation).

Fig. 1A is a representative autoradiogram that shows the steady state level of RBP mRNA in the kidney of rats at various times after treatment with estrogen. An increase in the level of RBP mRNA in the kidney was seen as early as 1.5 h after injection with 17β-estradiol and maximum induction occurred between 3 and 6 h after estrogen treatment. The level of RBP mRNA in the kidney of rats declined after 6 h of estrogen treatment such that by 24 h the level was comparable to that of the control animals. Treatment of rats with 17β-estradiol, 0.01 μg/g body weight, resulted in a small increase in RBP mRNA steady state level in the kidney at 3 h and 6 h after treatment (data not shown). Fig. 1B shows the quantitation of the RBP mRNA hybridizing band resulting from analysis of several independent experiments. Each time point represents the mean of at least three animals. As shown in Fig. 1B, there was very little variation in the level of kidney mRNA in the untreated animals; however, there was a considerable amount of variation in the level of kidney RBP mRNA in the estrogen-treated animals particularly at the time points of maximum induction (3 and 6 h). The maximum increase in the level of RBP mRNA averaged approximately fivefold after 6 h of estrogen treatment.

To determine whether an increase in the amount of RBP mRNA also occurs in the liver as well as in the kidney, the amount of RBP mRNA in the liver was determined in liver samples obtained from the same animals. Figs. 1C and 1D show the results of this experiment. The amount of RBP mRNA in the liver exhibited very little variability and no consistent change was observed when the rats were treated with 17β-estradiol, either 1.0 μg/g or 0.1 μg/g body weight. Hence, estrogen treatment of male rats results in an increase in the level of RBP mRNA in the kidney without influencing the level of RBP mRNA in the liver.

As a control for the level of mRNA in liver and kidney samples and the specificity of the induction of RBP mRNA levels in the kidney, the amout of another transcript involved in retinol metabolism, cellular retinol-binding protein (CRBP), was also determined. As shown in Figs. 1A and 1C the level of CRBP mRNA was relative-

ly constant in the kidney and in the liver of rats treated with estrogen. Hence, the increased level of RBP mRNA in the kidney does not appear to be due to a nonspecific induction of overall transcription in the kidney by estrogen.

Estrogen and RBP mRNA levels in female rats

We next wished to determine whether the level of RBP mRNA in the kidney was also modulated by estrogen in female rats. Female rats were ovariectomized and then maintained for 30 days to insure that the estrogen was depleted in these animals. Upon examination of all animals at the time of killing, we could detect no ovarian tissue and the uterus was greatly reduced in size indicating that the animals were depleted of estrogen. After 30 days, some animals were killed and the level of RBP mRNA was determined in the liver and kidney and compared to control, sham-operated animals. Other ovariectomized rats were repleted with 17β-estradiol, 0.1 μg/g body weight, and killed after 3 and 6 h.

Fig. 2A is a representative autoradiogram showing the level of RBP mRNA in the kidney of ovariectomized and sham-operated control female rats. We observed an approximately twofold increase in the amount of RBP mRNA in the kidney of the ovariectomized compared to the sham-operated rats. To determine whether this twofold increase in the amount of RBP mRNA in the ovariectomized rats was specific for RBP, the level of CRBP mRNA was measured in the same samples. We observed a similar twofold increase in the level of CRBP mRNA in the kidney samples obtained from the ovariectomized rats compared to the sham-operated animals.

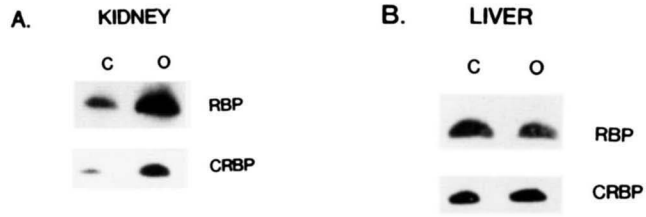

Fig. 2. The effect of ovariectomy on the level of RBP mRNA in the kidney and liver of female rats. Female Charles River Sprague-Dawley rats were surgically ovariectomized (O). Thirty days after the removal of the ovaries, these rats along with sham-operated control animals (C) were killed (three animals per treatment). Total RNA was isolated from both the kidney and the liver. The levels of RBP mRNA and CRBP mRNA in the kidney (panel A) and the liver (panel B) were determined by RNAse protection analysis and the autoradiograms were quantitated by densitometric scanning.

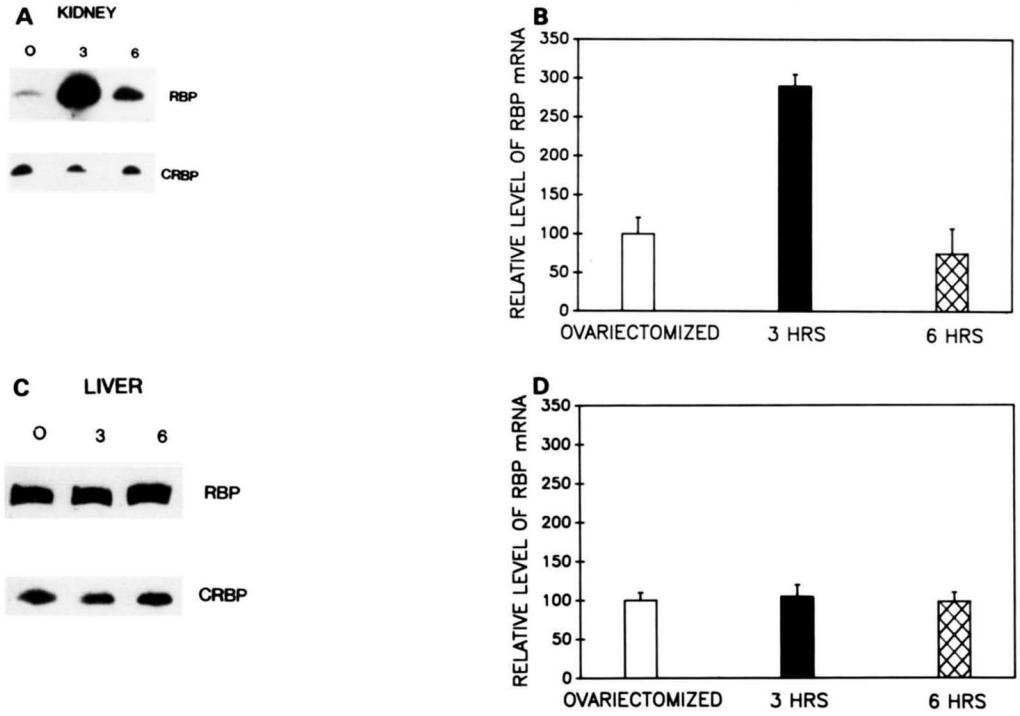

Fig. 3. The effect of β-estradiol on the level of RBP mRNA in the kidney and liver of ovariectomized female rats. Female Charles River Sprague-Dawley rats were ovariectomized. Thirty days after ovariectomy, the rats were injected subcutaneously with 17β-estradiol, 0.1 μg/g body weight. Rats (three animals per time point) were killed 3 and 6 h after injection and total RNA was isolated from the kidney and liver. The levels of RBP mRNA and CRBP mRNA were determined by RNAse protection analysis and quantitated by densitometric scanning. Panels A and C are representative autoradiograms of RBP mRNA and CRBP mRNA levels in the kidney (A) and liver (C). Panels B and D are graphic representations of the relative level of RBP mRNA in the kidney (B) and the liver (D). The relative level of RBP mRNA was calculated with the control value arbitrarily set to 100. Values are mean ± SEM.

We, therefore, could detect no consistent, specific difference in the steady-state level of RBP mRNA in the kidneys of the ovariectomized and the sham-operated animals. Likewise, Fig. 2B shows that ovariectomy also had no effect on RBP mRNA levels in the livers of these rats.

Although ovariectomy did not specifically change the level of RBP mRNA in the kidney of female rats, we observed an increase in the steady state level of RBP mRNA in the kidney (**Fig. 3A**) but not the liver (Fig. 3C) of ovariectomized rats 3 h after treatment with 0.1 μg/g body weight 17β-estradiol. For each experimental time point, the hybridizing band representing RBP mRNA from at least three individual animals was quantitated. These data are shown in Figs. 3B and 3D. Estradiol treatment of the ovariectomized female rats resulted in an approximately threefold increase in the steady state level of RBP mRNA in the kidney while no difference was observed in the livers of these animals. Again, as a control to demonstrate the specificity of the elevation of RBP mRNA in the kidney, CRBP mRNA levels were also determined in the kidney and the liver. CRBP mRNA levels were not influenced by estrogen treatment in either the kidney or the liver of the ovariectomized animals (Figs. 3A and 3C). Therefore, the increase in the level of RBP mRNA in the kidney of estrogen-treated ovariectomized female rats is most likely a specific response.

Hydroxytamoxifen and RBP mRNA levels in the kidney

In order to begin to address the question of whether the increase in the RBP mRNA in the kidney is mediated by the nuclear estrogen receptor, we examined the effect of the synthetic anti-estrogen, hydroxytamoxifen. Hydroxytamoxifen is a weak estrogen that binds tightly to the nuclear estrogen receptor and serves as a specific antagonist of estrogen binding (30). **Fig. 4** is a representative autoradiogram that shows that analysis of RBP mRNA levels in

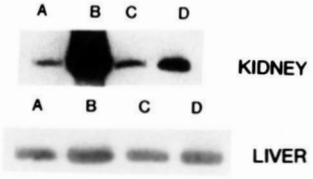

Fig. 4. The effect of hydroxytamoxifen on estrogen induction of RBP mRNA in the kidney. Males Charles River Sprague-Dawley rats were treated in the following manner: A, no treatment; B, subcutaneous injection (0.1 μg/g body weight) of 17β-estradiol and killed 6 h later; C, subcutaneous injection (0.5 μg/g body weight) of hydroxytamoxifen and killed 6 h later; D, 2 h pretreatment with a subcutaneous injection of hydroxytamoxifen (0.5 μg/g body weight) followed by a subcutaneous injection of 17β-estradiol (0.1 μg/g body weight) and killed 6 h later. At the end of each of the treatments, the rats were killed and total RNA was isolated from the kidney. The level of RBP mRNA was determined by RNAse protection analysis.

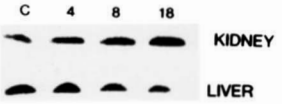

Fig. 5. The effect of a single dose of testosterone on the level of RBP mRNA in the kidney and liver of female rats. Female Charles River Sprague-Dawley rats were injected subcutaneously with testosterone (2 mg/kg body weight). Rats (three animals per time point) were killed at the indicated times (0–18) after injection and total RNA was isolated from the kidney and liver. The level of RBP mRNA was determined by RNAse protection analysis and quantitated by densitometric scanning.

the kidneys isolated from rats treated with hydroxytamoxifen alone, estrogen alone, and hydroxytamoxifen pretreatment followed by estrogen. When animals were treated with 17β-estradiol alone and killed 6 h later (lane B), we observed an increase in RBP mRNA levels similar to those described previously (Fig. 1A), and treatment of animals with hydroxytamoxifen alone (lane C) resulted in no change in the level of RBP mRNA in the kidney. However, when the animals were pretreated with hydroxytamoxifen followed by treatment with 17β-estradiol (lane D), we observed a considerable reduction in the estrogen-related increase in RBP mRNA level in the kidney (compare lanes B and D). This suggests that the increase in the level of RBP mRNA in the kidney may be mediated by the nuclear estrogen receptor.

Effect of testosterone on RBP mRNA levels

In addition, we wished to determine whether testosterone might also play a role in the regulation of RBP mRNA levels in either the kidney or the liver. Adult female animals were injected subcutaneously with 2 mg testosterone/rat and killed 4, 8 or 18 h later. **Fig. 5** is a representative autoradiogram of the steady-state levels of RBP mRNA in the kidney and liver of female rats treated with testosterone. We observed no reproducible change in the level of RBP mRNA in either the kidney or the liver of the testosterone-treated animals compared to the control animals.

Since several days of treatment of testosterone are often required to observe an alteration in the level of a specific transcript in the kidney (31–33), we surgically implanted 75-mg pellets (3 week time-release) of testosterone subcutaneously in the nape of the neck of rats and measured the RBP mRNA levels in the kidney and liver after 1–14 days. As seen in the representative autoradiogram in **Fig. 6**, chronic treatment of female rats with testosterone resulted in a slight increase in the level of RBP mRNA in the kidney with time and no consistent effect on the level of RBP mRNA in the liver.

DISCUSSION

This study was designed to explore the effect of estrogen and testosterone on the steady-state level of RBP

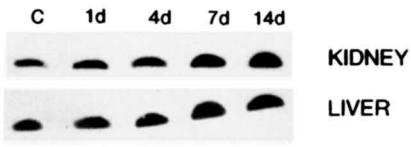

Fig. 6. Effect of chronic treatement of female rats with testosterone on RBP mRNA levels in the kidney and liver. A 75-mg pellet (3 week time-release) of testosterone was surgically implanted subcutaneously at the nape of the neck of female Charles River Sprague-Dawley rats. Rats (three animals per time point) were killed at various times (0–14 days later) and total RNA was isolated from the kidney and liver. The level of RBP mRNA was determined by RNAse protection analysis and quantitated by densitometric scanning.

mRNA in the kidney and liver of rats. These data demonstrate that testosterone has, potentially, a slight effect on the steady-state level of RBP mRNA in the kidney. However, estradiol treatment results in a rapid increase in the steady-state level of kidney RBP mRNA in both male and ovariectomized female rats. In contrast, estradiol had no apparent effect on RBP mRNA level in the livers of these animals. Thus, it appears that the regulation of RBP mRNA levels in the kidney, at least with respect to estrogen, is different from the regulation in the liver. In addition, the anti-estrogen drug, hydroxytamoxifen, appears to block the estrogen-related increase in RBP mRNA in the kidney. This suggests that the mechanism of estrogen induction in the kidney is mediated by the nuclear estrogen receptor.

It has been well established that estrogen induces the level of expression of a number of mammalian mRNAs including several rat uterine genes (34), ornithine aminotransferase in rat kidney (35), and prolactin in the rat pituitary (36). In each of these examples, the kinetics of response to estrogen varies. The rapid response observed here with kidney RBP mRNA is similar to that of ornithine aminotransferase in the kidney (35) and prolactin in the pituitary (36).

The literature contains numerous reports that suggest a relationship between estrogen and plasma RBP and vitamin A levels in mammals. Vahlquist et al. (19) and Michaelsson et al. (20) have demonstrated that adult plasma levels of RBP are not reached until puberty. This is in contrast to other serum proteins such as transferrin and transthyretin where adult levels are achieved by 2 yr of age (19). Gal, Parkinsson, and Graft (37) and Laurence and Sobol (38) have demonstrated changes in plasma vitamin A levels in women during the menstrual cycle. A similar variation in plasma RBP levels during the menstrual cycle has also been reported by Vahlquist, Johnsson, and Nygren (21). This variation in plasma RBP level appears to be correlated with the peak levels of estradiol in women. Finally, there are a number of reports that correlate the use of high estrogen oral contraceptives and slightly elevated plasma RBP and plasma vitamin A levels (21–23). Taken together these data suggest a relationship between estrogen and plasma RBP levels; however, they do not address the mechanism of how estrogen might be causing this effect. The data presented in this paper demonstrate that one possible way that the plasma RBP levels, at least in part, may be elevated by estrogen is by an increase in kidney RBP mRNA levels and potentially RBP synthesis in the kidney.

It is very interesting that estrogen treatment increased RBP mRNA levels in the kidney but not in the liver. The lack of response of the RBP gene in the liver to estrogen treatment is not surprising in light of the report by Dixon and Goodman (18) that estrogen had no influence on RBP production in primary hepatocytes in culture. RBP is one of a number of genes that respond to estrogen treatment in one organ and do not respond in the liver. For example, insulin-like growth factor I is induced in the uterus by estrogen treatment while estrogen treatment has no significant effect on hepatic insulin-like growth factor I gene expression (39). Ornithine aminotransferase is also transcriptionally regulated in the kidney by estrogen treatment while the liver enzyme is not influenced by estrogen treatment (35). The lack of response of RBP in the liver to estrogen treatment could be related to an inability of the liver to respond to estrogen, although estrogen receptors have been reported in the liver (40–42) and very low density lipoprotein levels have been demonstrated to be increased by estrogen treatment (43). However, it is also possible that the lack of response of the liver RBP gene to estrogen is related to some additional tissue-specific liver transcription factor(s).

Our data suggest that estrogen is not required to maintain the basal level of RBP mRNA in the kidney. Both ovariectomy and treatment with hydroxytamoxifen alone did not appear to change RBP mRNA levels. Hence, basal expression of RBP does not appear to be dependent on estrogen; however, under physiological circumstances such as at the time of peak estradiol during the menstrual cycle or when high estrogen-containing oral contraceptives are ingested, RBP mRNA levels may be elevated in the kidney.

While our analysis has measured only the steady-state RBP mRNA levels, reports by McKearin et al. (44) and McKearin and Shapiro (45) have shown that at least in *Xenopus* the RBP gene is transcriptionally regulated in the liver by estrogen treatment. Because of the rapid rise in RBP mRNA levels in the rat kidney after estradiol treatment (as early as 1.5 h and maximal at 6 h) and the potential role of the nuclear estrogen receptor suggested by the hydroxytamoxifen experiment, it is possible that the elevation in kidney mRNA levels is due, at least in part, to increased transcription of the RBP gene. However, further experiments that directly measure transcription of the RBP gene by run-on transcription assays will be necessary to definitively demonstrate this.

We wish to thank ICI Pharmaceuticals for the generous gift of the hydroxytamoxifen. This work was supported by National Institutes of Health Grants DK 41089 and BRSG SO7 RR05417. D.C.H. was supported in part as a predoctoral fellow by National Institutes of Health Training Grant T32-DKO7162 and a Federal Work Study Award.

Manuscript received 22 January 1990 and in revised form 4 April 1990.

REFERENCES

1. Goodman, D. S. 1984. Plasma retinol-binding protein. *In* The Retinoids. M. B. Sporn, A. B. Roberts, and D. S. Goodman, editors. Academic Press, Orlando, FL. Vol. 2: 41–88.
2. Smith, F. R., and D. S. Goodman. 1971. The effects of diseases of the liver, thyroid and kidneys on the transport of vitamin A in human plasma. *J. Clin. Invest.* **50:** 2426–2436.
3. Kanai, M., A. Raz, and D. S. Goodman. 1968. Retinol-binding protein: the transport protein for vitamin A in human plasma. *J. Clin. Invest.* **47:** 2025–2044.
4. Muto, Y., and D. S. Goodman. 1972. Vitamin A transport in rat plasma: isolation and characterization of retinol-binding protein. *J. Biol. Chem.* **247:** 2533–2541.
5. Van Jaarsveld, P. P., H. Edelhoch, D. S. Goodman, and J. Robbins. 1973. The interaction of human plasma retinol-binding protein with prealbumin. *J. Biol. Chem.* **248:** 4698– 4705.
6. Kopelman, M., U. Cogan, S. Mokady, and M. Shinitzky. 1976. The interaction between retinol-binding proteins and prealbumins studied by fluorescence polarization. *Biochim. Biophys. Acta.* **439:** 449–460.
7. Soprano, D. R., K. J., Soprano, and D. S. Goodman. 1986. Retinol-binding protein messenger RNA levels in the liver and extrahepatic tissues of the rat. *J. Lipid Res.* **27:** 166–171.
8. Makover, A., D. R. Soprano, M. L. Wyatt, and D. S. Goodman. 1989. Localization of retinol-binding protein messenger RNA in the rat kidney and in perinephric fat tissue. *J. Lipid Res.* **30:** 171–180.
9. Soprano, D. R., K. J. Soprano, and D. S. Goodman. 1986. Retinol-binding protein and transthyretin mRNA levels in visceral yolk sac and liver during fetal development in the rat. *Proc. Natl. Acad. Sci. USA.* **83:** 7330–7334.
10. Makover, A., D. R. Soprano, M. L. Wyatt, and D. S. Goodman. 1989. An in situ-hybridization study of the localization of retinol-binding protein and transthyretin messenger RNAs during fetal development in the rat. *Differentiation.* **40:** 17–25.
11. Muto, Y., J. E. Smith, P. O. Milch, and D. S. Goodman. 1972. Regulation of retinol-binding protein metabolism by vitamin A status in the rat. *J. Biol. Chem.* **247:** 2542–2550.
12. Smith, J. E., Y. Muto, P. O. Milch, and D. S. Goodman. 1973. The effect of chylomicron vitamin A on the metabolism of retinol-binding protein in the rat. *J. Biol. Chem.* **248:** 1544–1549.
13. Smith, J. E., C. Borek, M. A. Gawinowicz, and D. S. Goodman. 1985. Structure-function relationships of retinoids in their effects on retinol-binding protein metabolism in cultured H_4IIEC_3 liver cells. *Arch. Biochem. Biophys.* **238:** 1–9.
14. Dixon, J. L., and D. S. Goodman. 1987. Studies on the metabolism of retinol-binding protein by primary hepatocytes from retinol-deficient rats. *J. Cell. Physiol.* **130:** 14–20.
15. Soprano, D. R., M. L. Wyatt, J. L. Dixon, K. J. Soprano, and D. S. Goodman. 1988. Retinol-binding protein synthesis and secretion by the rat visceral yolk sac. Effect of retinol status. *J. Biol. Chem.* **263:** 2934–2938.
16. Soprano, D. R., J. E. Smith, and D. S. Goodman. 1982. Effect of retinol on retinol-binding protein biosynthesis rate and translatable messenger RNA levels in rat liver. *J. Biol. Chem.* **257:** 7693–7697.
17. Smith, J. E., C. Borek, and D. S. Goodman. 1978. Regulation of retinol-binding protein metabolism in cultured rat liver cell lines. *Cell.* **15:** 865–873.
18. Dixon, J. L., and D. S. Goodman. 1987. Effect of nutritional and hormonal factors on the metabolism by glucocorticoid hormones in primary cultures of rat hepatocytes. *J. Cell. Physiol.* **130:** 6–13.
19. Vahlquist, A., L. Rask, P. A. Peterson, and T. Berg. 1975. The concentration of retinol-binding protein, prealbumin, and transferrin in the sera of newly delivered mothers and children of various ages. *Scand. J. Clin. Lab. Invest.* **35:** 569–575.
20. Michaelsson, G., A. Vahlquist, T. Juhlin, T. Mellbin, and L. Bratt. 1976. Zinc and vitamin A: serum concentration of zinc and retinol-binding protein (RBP) in healthy adolescents. *Scand. J. Clin. Lab. Invest.* **36:** 827–832.
21. Vahlquist, A., A. Johnsson, and K. G. Nygren. 1979. Vitamin A transporting plasma proteins and female sex hormones. *Am. J. Clin. Nutr.* **32:** 1433–1438.
22. Yeung, D. L. 1974. Effects of oral contraceptives on vitamin A metabolism in the human and the rat. *Am. J. Clin. Nutr.* **27:** 125–129.
23. Prasad, A. S., K. Y. Lei, D. Oberleas, K. S. Moghissi, and J. S. Stryker. 1975. Effect of oral contraceptive agents on nutrients. II. Vitamins. *Am. J. Clin. Nutr.* **28:** 1433–1438.
24. Tushiniski, R. J., P. M. Sussman, L. Y. Yu, and F. L. Bancroft. 1977. Pregrowth hormone messenger RNA: glucocorticoid induction and identification in rat pituitary cells. *Proc. Natl. Acad. Sci. USA.* **74:** 2357–2361.
25. Toscani, A., D. R. Soprano, S. C. Cosenza, T. A. Owen, and K. J. Soprano. 1987. Normalization of multiple RNA samples using an in vitro-synthesized external standard cRNA. *Anal. Biochem.* **165:** 309–319.
26. Owen, T. A., S. C. Cosenza, D. R. Soprano, and K. J. Soprano. 1987. Time of c-fos and c-myc expression in human diploid fibroblasts stimulated to proliferate after prolonged periods in quiescence. *J. Biol. Chem.* **262:** 15111–15117.
27. Rajan, N., W. S. Blaner, D. R. Soprano, A. Suhara, and D. S. Goodman. 1990. Cellular retinol-binding protein messenger RNA levels in normal and retinoid-deficient rats. *J. Lipid Res.* **31:** 821–829.
28. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 312–319.
29. Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green. 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing bacteriophage SP6 promoter. *Nucleic Acids Res.* **12:** 7035–7056.
30. Eckert, R. L., and B. S. Katzenellenbogen. 1982. Physical properties of estrogen receptor complexes in MCF-7 human breast cancer cell. *J. Biol. Chem.* **257:** 8840–8846.
31. Berger, F. G., P. Szymanski, E. Read, and G. Watson. 1984. Androgen-regulated ornithine decarboxylase mRNAs of mouse. *J. Biol. Chem.* **259:** 7941–7946.
32. Berger, F. G., K. W. Gross, and G. Watson. 1981. Isolation and characterization of a DNA sequence complementary to an androgen-inducible messenger RNA from mouse kidney. *J. Biol. Chem.* **256:** 7006–7013.

33. Felder, M. R., G. Watson, M. O. Huff, and J. D. Ceci. 1988. Mechanism of induction of mouse kidney alcohol dehydrogenase by androgen. Androgen-induced stimulation of transcription of the Adh-1 gene. *J. Biol. Chem.* **263:** 14531–14537.
34. Frankel, F. R., C-Y. J. Hsu, J. C. Myers, E. Lin, C. R. Lyttle, B. Komm, and K. Mohn. 1988. Regulation of Alpha 2 (I), Alpha 1 (III) and Alpha 2 (V) collagen mRNAs by estradiol in the immature rat uterus. *DNA.* **7:** 347–354.
35. Mueckler, M. M., S. Moran, and H. C. Pitot. 1984. Transcriptional control of ornithine aminotransferase synthesis in rat kidney by estrogen and thyroid hormone. *J. Biol. Chem.* **259:** 2302–2305.
36. Maurer, R. A. 1982. Estradiol regulates the transcription of the prolactin gene. *J. Biol. Chem.* **257:** 2133–2136.
37. Gal, J., C. Parkinsson, and I. Graft. 1971. Effects of oral contraceptives on human plasma vitamin-A levels. *Br. Med. J.* **2:** 436–438.
38. Laurence, P. A., and A. E. Sobol. 1953. Changes in serum vitamin A levels during the human menstrual cycle. *J. Clin. Endocrinol. Metab.* **13:** 1192–1200.
39. Murphy, W. J., and H. G. Friesen. 1988. Differential effects of estrogen and growth hormone on uterine and hepatic insulin-like growth factor gene I expression in ovariectomized and hypophysectomized rat. *Endocrinology.* **122:** 325–332.
40. Powell-Jones, W., W. P. Davies, and K. Griffiths. 1976. Specific binding of [^{3}H]oestradiol by cytoplasmic protein components of female rat liver. *J. Endocrinol.* **69:** 167–170.
41. Eisenfeld, A. J., R. F. Aten, M. J. Weinberger, G. Haselbacher, K. Halpern, and L. Krakoff. 1976. Estrogen receptor in the mammalian liver. *Science.* **191:** 862–865.
42. Chamness, G. C., M. E. Costlow, and W. L. McGuire. 1975. Estrogen receptor in rat liver and its dependence on prolactin. *Steroids.* **26:** 363–371.
43. Thompson, C., P. M. Hudson, and G. W. Lucier. 1983. Correlation of hepatic estrogen receptor concentrations and estrogen-mediated elevation of very low density lipoproteins. *Endocrinology.* **112:** 1389–1397.
44. McKearin, D. M., M. C. Barton, M. J. Keller, and D. J. Shapiro. 1987. Estrogen induces transcription of the *Xenopus laevis* serum retinol-binding protein gene. *J. Biol. Chem.* **262:** 4939–4942.
45. McKearin, D. M., and D. J. Shapiro. 1988. Persistent estrogen induction of hepatic *Xenopus laevis* serum retinol binding protein mRNA. *J. Biol. Chem.* **263:** 3261–3265.