Cellular retinol-binding protein messenger RNA levels in normal and retinoid-deficient rats

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Abstract A study was conducted to determine the levels of cellular retinol-binding protein (CRBP) mRNA and protein in various tissues of the rat, to explore relationships between CRBP mRNA and protein levels in different tissues, and to examine the effects of changes in retinol nutritional status on the tissue distribution and levels of CRBP mRNA. Previous studies have shown that tissue CRBP protein levels are reduced in totally retinoid-deficient rats, but are otherwise minimally affected by changes in retinoid status. Three groups of male rats were compared: normal controls, retinoid-deficient, and retinol-repleted deficient rats. CRBP mRNA levels were measured by RNase protection assay and CRBP protein levels by radioimmunoassay in seven tissues. High levels of both CRBP mRNA and CRBP protein were found in the proximal epididymis, kidney, and liver; lower levels were seen in lung, testis, spleen, and small intestine. Tissue CRBP mRNA and protein levels were highly correlated ($P < 0.01$) with each other. Retinoid deficiency did not alter the levels of CRBP mRNA found in the proximal epididymis, kidney, and liver. In contrast, CRBP mRNA levels in the lung, testis, spleen, and small intestine were reduced substantially in retinoid-deficient rats, to values that were only 23% to 50% of the corresponding values in the tissues of control rats. After oral repletion with retinol (4–18 h earlier), CRBP mRNA levels for these latter four tissues were found to have risen to control or near-control levels. The suggestion is raised that retinol repletion may have directly induced the expression of the CRBP gene in these particular tissues. The CRBP II mRNA levels in the small intestine were also measured in the three dietary groups, and the levels in the retinoid-deficient group were higher than those in the control group. The results suggest that the tissue levels of CRBP are regulated mainly by factors that regulate the levels of CRBP mRNA. In some tissues the availability of dietary retinol appears to have a specific effect on CRBP expression. —**Rajan, N., W. S. Blaner, D. R. Soprano, A. Suhara, and D. S. Goodman.** Cellular retinol-binding protein messenger RNA levels in normal and retinoid-deficient rats. *J. Lipid Res.* 1990. **31:** 821–829.

Supplementary key words retinoids • cellular retinol-binding protein mRNA levels • retinoid nutritional status • gene regulation

In higher organisms, retinoids play important roles in cellular growth and differentiation (1) and are necessary for vision (2), reproduction (3), and for the maintenance of health (4). It is clear that retinoids mediate at least some cellular responses by altering the expression of specific genes (5–9). The recent identification of nuclear receptors for retinoic acid (10–12) has provided insights into the possible mechanism by which retinoids affect gene expression.

Within cells, retinoids are normally found associated with specific intracellular retinoid-binding proteins (13, 14). For retinol, two specific intracellular binding proteins have been described and characterized: cellular retinol-binding protein (CRBP) (13, 14) and cellular retinol-binding protein II (CRBP II) (14, 15). CRBP and CRBP II belong to a family of cytosolic proteins whose members bind small hydrophobic ligands, such as retinoids and fatty acids (16).

In previous studies from this laboratory (17, 18), CRBP was found in every rat tissue examined (21 tissues in male, 18 in female rats). This finding, that CRBP is present in all tissues and organs examined, suggests that CRBP may be involved in essential cellular processes throughout the body. The level of CRBP varied widely among different tissues, with the highest levels (for male rats) found in the proximal epididymis, liver, and kidney. The precise cellular functions of CRBP are not well understood. It is likely that CRBP serves as an intracellular transport protein that transports retinol from one locus to another within the cell (19). CRBP may play a role in retinol esterification, particularly in the liver (20, 21). On the other hand, CRBP II is mainly found in the small intestine of adult rats (15), where it is probably involved in the intestinal metabolism of newly absorbed or newly formed retinol (22).

Abbreviations: CRBP, cellular retinol-binding protein; CRBP II, cellular retinol-binding protein II; RBP, retinol-binding protein; HPLC, high performance liquid chromatography; TCA, trichloroacetic acid; CRABP, cellular retinoic acid-binding protein.

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Very little information is available about the factors that regulate the levels of CRBP in different issues. The level of CRBP in a given tissue appears to be highly characteristic of that particular tissue (17, 18). Reduced tissue CRBP levels were found in totally retinoid-deficient rats (17). However, when rats were maintained on a retinol-deficient retinoic acid-supplemented diet, only the CRBP level of the proximal epididymis was found to differ significantly from the corresponding control level (18). Rats fed excess retinol (10 times control levels) showed no changes in CRBP levels in any tissue examined (17). Thus, even in the presence of marked changes in retinoid nutritional status, the tissue levels of CRBP are highly regulated and maintained in rats (17, 18).

In order to obtain information about the regulation of the synthesis and metabolism of CRBP, we have undertaken to explore the levels and nutritional regulation of CRBP mRNA in various rat tissues. We now report detailed studies on: *a*) the levels of CRBP mRNA in various rat tissues; *b*) the relationships that exist between the levels of CRBP mRNA and CRBP protein in different rat tissues; and *c*) the effects of changes in nutritional retinol status on the tissue distribution and levels of CRBP mRNA. These studies employed a sensitive RNase protection assay to determine absolute CRBP mRNA levels, and a specific radioimmunoassay for CRBP. Data are also reported on the effects of retinol status on the levels of CRBP II mRNA in the small intestine.

EXPERIMENTAL PROCEDURES

Animals, diets, and tissue processing

Weanling male Sprague-Dawley rats were obtained from Charles River Laboratories, (Wilmington, MA). All rats were housed individually in a virus- and pathogen-free environment, and had free access to food and water. Upon arrival, the rats were randomly divided into two groups: control and retinoid-deficient. Both groups of rats were fed the same nutritionally complete retinoid-deficient, semipurified diet (23). For the control group, the diet was supplemented with 2.4 mg retinol (as retinyl acetate) per kg of diet (23); no retinoid supplementation was provided to the retinoid-deficient group.

On day 70 or 71 after starting on the diets, the serum retinol levels of the rats maintained on the retinoid-deficient diet were measured. If the serum retinol level was below 3 μg/dl, then the rat was considered to be retinoid-deficient and was killed the next day for the study. Within the control diet group rats with similar body weights were paired; within the retinoid-deficient group rats with similar body weights and serum retinol levels were paired. One rat from each pair was used for tissue CRBP mRNA measurements and the other rat from each pair was used for tissue CRBP protein determinations. Four such pairs of rats from each diet group were killed and used for CRBP mRNA and protein determinations. For CRBP mRNA determinations, the rats (one from each pair) were anesthetized with isoflurane, decapitated, and the tissues were removed and immediately homogenized with a Polytron Homogenizer (Brinkmann Instruments, Westbury, NY) for RNA isolation (see below for details). For CRBP protein determinations, the rats (the other rat from each pair) were anesthetized with isoflurane, and the tissues were collected and processed for radioimmunoassay, as described previously (17). Seven tissues were removed from each rat for analysis; these were the liver, kidneys, lungs, spleen, testes, proximal epididymis, and the upper 10 cm of the small intestine. At the time of killing, serum and a piece of liver were taken from each animal for determination of serum and liver retinol and retinol-binding protein (RBP) levels.

Some rats from the retinoid-deficient diet group (serum retinol levels < 3 μg/dl) were repleted with retinol (150 μg) given orally (as a bolus of retinyl acetate in 0.25 ml peanut oil) 2, 4, 6, or 18 h prior to killing. After repletion, the rats were given free access to the control (retinol-containing) diet. Like the diet groups described above, the retinol-repleted rats were paired so that animals with both similar body weights and initial serum retinol levels were used for parallel CRBP mRNA and CRBP protein determinations (carried out in different rats in each pair). For the repletion studies, one pair of retinol-repleted rats was killed 2, 6, and 18 h after repletion, and three pairs of rats were killed 4 h after repletion. The tissues were collected and processed as described above for the control and retinoid-deficient animals.

Isolation of rat CRBP cDNA

The tissue levels of CRBP mRNA were determined by RNase protection assay. In order to maximize the sensitivity and specificity of the assay, we first isolated a rat CRBP cDNA clone using the previously described human CRBP cDNA clone (24) as a probe. A library of rat adult testis cDNA inserted into lambda gt 11 was purchased from Clontech Laboratories, Inc., (Palo Alto, CA). Approximately 1.5×10^5 plaques from the library were screened by colony hybridization (25) with human CRBP cDNA labeled with [^{32}P]dCTP to a specific activity of 5×10^5 cpm/μg by the oligolabeling technique (26). Conditions used for prehybridization, hybridization, and washing were similar to procedures described earlier (27). Of the 12 plaques that showed positive hybridization, three were chosen for purification and subsequent analysis. The largest insert size from these three clones was 570 bp in length. This 570 bp EcoRI insert was isolated and ligated to EcoRI-digested pGEM 3 (Promega Biotec, Madison, WI) and sequenced by the dideoxy chain ter-

mination method (28) using SP6 and T7 primers (Promega Biotec). The nucleotide sequence of this clone (prCRBP) was the same as that reported by Sherman, Lloyd, and Chytil (29) and extends from position 147 to the poly A tail.

RNase protection assay for CRBP mRNA levels

Total RNA was prepared from the various rat tissues by the method of Tushinski et al. (30) and was quantified spectrophotometrically by measuring the absorbance at 260 nm. For RNase protection analysis of CRBP mRNA levels, a ^{32}P-labeled antisense (hybridizing) CRBP cRNA probe was synthesized as described by Melton et al. (31) from 2 μg of prCRBP linearized with Pvu II. An accurately measured amount of total RNA was hybridized with the ^{32}P-labeled probe at 55°C for 12–16 h. Unhybridized (unprotected) RNA including excess probe was digested with a mixture of RNase A and RNase T1 according to published procedures (32–34). After RNase digestion, the undigested hybridized material was treated according to the procedures described by Williams et al. (35), with slight modifications. Briefly, 100 μg of salmon sperm DNA was added to each sample following RNase digestion and the samples were precipitated by the addition of trichloroacetic acid (TCA) to a final concentration of 10% (w/v). Following TCA addition, the samples were kept on ice for 15 min and then filtered through GF/C glass filters (Whatman) to trap precipitated protected hybrids. The filters were then washed with 25 ml of 10% TCA containing 0.5% (w/v) sodium pyrophosphate. The washed filters were dried and the amount of RNase-resistant hybrids was determined by liquid scintillation counting. All RNA samples from a given tissue (across all diet groups) were assayed in a single assay.

Absolute CRBP mRNA values

A single sample of liver total RNA (comprising pooled RNA from several control rats) was used as a reference standard for each RNase protection assay performed in this study. First, the absolute level of CRBP mRNA in this reference standard of liver total RNA was determined. This was done by comparing the amount of ^{32}P-labeled CRBP cRNA antisense probe precipitated by a given amount of standard liver total RNA, with the amount of the same ^{32}P-labeled probe precipitated by a known amount of pure sense strand CRBP in a single RNase protection assay. For this purpose, the sense strand CRBP cRNA was synthesized from prCRBP linearized with BamH I and was isolated and quantified essentially as described by Toscani et al. (33). Aliquots of the sense strand of CRBP cRNA were subjected to electrophoresis in a 5% polyacrylamide/7 M urea gel and compared with known concentrations of RNA molecular weight standards (Bethesda Research Laboratories, Gaithersburg, MD). The gel was stained with ethidium bromide and photographed on a UV-transilluminator. The appropriate bands were quantified by scanning the negative of the photograph with a densitometer.

A series of samples, comprising 0, 0.625, 1.25, 2.5, 5, 10, and 20 pg of in vitro synthesized sense strand CRBP cRNA were incubated with an excess of the ^{32}P-labeled antisense CRBP cRNA probe. At the same time, samples of 8, 16, 32, 64, and 128 μg of the reference standard liver total RNA were similarly incubated with an excess of the same ^{32}P-labeled antisense CRBP probe. Both sets of samples (containing either the sense CRBP cRNA or the reference standard liver total RNA) were hybridized, RNase-digested, TCA-precipitated, and quantified as described above. Thus, two standard curves shown in **Fig. 1** were obtained. It can be seen that hybridization was linear for both sets of samples over the entire range of added RNA. Panel A of Fig. 1 shows how much ^{32}P radioactivity (representing TCA-precipitable RNase-resistant hybrids) was observed with each given absolute amount of in vitro synthesized CRBP cRNA. Panel B of Fig. 1 shows how much ^{32}P radioactivity was observed with each given amount of the reference standard liver total RNA. By comparison of the standard curves (lines) in panels A and B, the content of CRBP mRNA in the reference standard liver total RNA preparation could be expressed as pg CRBP mRNA per μg total liver RNA. By including known amounts of the reference standard in each RNase protection assay, the amount of CRBP mRNA in each tissue sample of total RNA could then be estimated and expressed as pg CRBP mRNA per μg total RNA.

The absolute level of CRBP mRNA in the reference standard of liver total RNA was also measured by a second method. Different amounts of reference standard were hybridized with a ^{32}P-labeled antisense CRBP

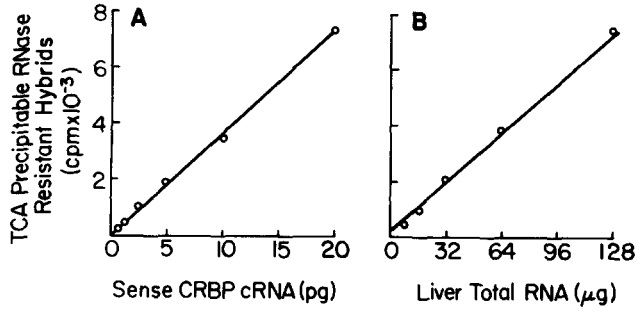

Fig. 1. Determination of the level of CRBP mRNA in the reference standard preparation of liver total RNA. Excess ^{32}P-labeled antisense CRBP was hybridized with: panel A, sense strand CRBP cRNA obtained from linearized prCRBP; panel B, reference standard liver total RNA. A sample of this preparation of liver total RNA was employed as a reference standard for each RNase protection assay performed in this study. By comparing the data in panel A with those in panel B, the absolute amount (in pg) of CRBP mRNA present per μg of liver total RNA was calculated.

cRNA probe of known specific activity (4.9×10^8 cpm/μg) and the RNase-resistant hybrids were collected and counted in scintillation fluid as described above. Given the known specific radioactivity of the probe, the counts obtained from the TCA-precipitable RNase-resistant hybrids could be converted to an absolute amount of pg of mRNA. The absolute values obtained for the CRBP mRNA from the reference standard by the two methods were in close agreement; hence, the mean value was used for further computations.

The values of CRBP mRNA reported here are approximately 20% less than the true values present in the various tissues. This is so because the size of CRBP mRNA is approximately 700 nucleotides (29), whereas the tissue CRBP mRNA values reported here were obtained with a probe of approximately 550 nucleotides in length. The present values were not corrected for the true size of CRBP mRNA, because this size is not precisely known.

CRBP II mRNA levels

Rat CRBP II cDNA subcloned in pGEM 2 (prCRBP II) was kindly provided by Drs. Marc S. Levin and Jeffrey I. Gordon (Washington University School of Medicine, St. Louis, MO). CRBP II mRNA levels were measured by RNase protection assay using ^{32}P-labeled CRBP II cRNA antisense probe (specific activity, 2.7×10^8 cpm/μg) made from BamH 1-linearized prCRBP II. Accurately known amounts of small intestine total RNA from all diet groups were hybridized with labeled CRBP II cRNA antisense probe and the RNase resistant hybrids were processed and quantified as described above for CRBP mRNA. The absolute CRBP II mRNA levels were estimated from the known specific radioactivity of the probe.

Radioimmunoassay of tissue levels of CRBP

CRBP protein levels were measured by a sensitive and specific radioimmunoassay described previously (17).

Serum retinol, liver retinoid, and RBP levels

Serum retinol levels were measured by high performance liquid chromatography (HPLC), as described by Bieri, Tolliver, and Catignani (36). Liver retinoid levels were also measured by HPLC (37, 38). RBP levels were measured by radioimmunoassay as reported previously (39).

Statistical methods

The possible existence of diet-dependent differences in the measured tissue levels of CRBP mRNA and protein between diet groups was explored by *t*-tests (40). Possible correlations between CRBP mRNA and protein levels were examined by linear regression analysis (40).

RESULTS

Retinoid nutritional status of experimental animals

The retinoid status of the animals was assessed by their retinol and RBP levels in the liver and serum at the time of killing. **Table 1** shows the mean liver and serum retinol and RBP levels for the rats, at the time of killing, in the different diet groups. As seen in Table 1, the control diet, containing 2.4 mg of retinol per kg of diet, was sufficient to maintain the animals in normal vitamin A nutritional status. The rats maintained on the retinoid-deficient diet were truly retinoid-deficient, as judged by the very low levels of retinol in the serum and liver, and the low serum and high liver levels of RBP (41, 42). The rats in both diet groups gained weight steadily for 45–50 days after the start of the dietary regimen. After this time the retinoid-deficient group reached a weight plateau, whereas the control group rats continued to gain weight steadily. For the retinol-repleted diet group, serum retinol and RBP levels were found to be similar to control levels for each of the time intervals examined.

RNase protection assay

A sensitive and specific RNase protection assay, described under Experimental Procedures, was used to determine the tissue levels of CRBP mRNA. Tissues with quite low levels of CRBP mRNA, such as small intestine and spleen, can be assayed with precision with this assay. For each tissue studied, a range of added total RNA was

TABLE 1. Mean retinol and RBP levels in serum and liver[a]

			Retinol-Repleted			
			Hours of Retinol Repletion			
	Control	Retinoid-Deficient	2	4	6	18
Body weight (g)	364 ± 22	331 ± 41	322	336 ± 42	322	366
Serum retinol (μg/dl)	58.1 ± 7.9	1.9 ± 0.8	69.1	60.4 ± 12.0	51.0	51.0
Serum RBP (μg/ml)	36.0 ± 0.8	12.2 ± 0.4	43.8	48.7 ± 12.3	33.0	29.3
Liver retinol (μg/g)	261 ± 64	0.4 ± 0.3	31.3	19.9 ± 3.0	31.7	31.0
Liver RBP (μg/g)	17.8 ± 3.8	89.0 ± 19.9	59.8	18.1 ± 5.9	16.0	13.2

[a]Values represent means ± SD determined for eight experimental animals each from the control and the retinoid-deficient groups; six animals from the 4-h retinol-repleted group; and two animals each from the 2-, 6-, and 18-h retinol-repleted group.

identified within which a linear hybridization curve (such as the line shown in Fig. 1, panel B, for liver) was observed. All assays for each tissue used amounts of RNA that were within the linear range of that assay for that tissue. The level of CRBP mRNA observed for a given sample of tissue RNA was highly reproducible from assay to assay. The within-assay coefficient of variation for the CRBP RNase protection assay was found to be 4.5%, and the between-assay coefficient of variation was 8.8%. Thus, the assay reported here provides a highly sensitive and quantitatively precise method for measuring tissue CRBP mRNA levels.

In this study we have expressed CRBP mRNA levels in terms of pg CRBP mRNA/μg total RNA and not as pg CRBP mRNA/g tissue (43). This was done to eliminate the possible concern that RNA recoveries from different animals and tissues, and among different diet groups, may be variable. In fact, the quantitative recoveries of total RNA per g tissue across all diet groups were generally similar and did not differ significantly for any of the tissues studied.

CRBP mRNA and protein levels and their correlation

The levels of CRBP mRNA found in all tissues examined, across all diet groups, are shown in **Table 2**. The mean tissue CRBP mRNA levels for control rats ranged from 0.57 pg CRBP mRNA/μg total RNA in the proximal epididymis to 0.014 pg CRBP mRNA/μg total RNA in the small intestine. Proximal epididymis, kidney, and liver had high levels of CRBP mRNA, whereas lung, testis, spleen, and small intestine had much lower levels of CRBP mRNA.

CRBP protein levels for the same seven tissues from animals in all diet groups are shown in **Table 3**. The CRBP levels determined for control tissues agree well with those reported in previous studies from this laboratory (17, 18). The highest levels of CRBP were found in the proximal epididymis and the lowest levels in the small intestine and spleen. Within the control group of rats, the tissue CRBP protein levels roughly followed the same order as the mRNA levels. Thus, a strong and highly significant ($P<0.01$) correlation between the tissue CRBP mRNA and CRBP protein levels was observed (**Fig. 2**, panel A). No relationship was observed between CRBP mRNA and protein levels within tissues on the two diets.

Effects of nutritional retinol status

Nutritional retinol status was found to affect the CRBP mRNA levels in some tissues but not in others. As seen in Table 2, the CRBP mRNA levels in the liver, kidney, and proximal epididymis were not affected by retinoid nutritional status. In contrast, in the lung, testis, spleen, and small intestine, the CRBP mRNA levels of the retinoid-deficient rats were reduced substantially, to 38%, 23%, 43%, and 50%, respectively, of the levels found in the corresponding tissues of the control rats. The mean tissue CRBP protein levels of the retinoid-deficient rats were lower than the corresponding control levels (Table 3), but the difference was statistically significant ($P<0.05$) only for testis. Within the retinoid-deficient diet group, as seen in Fig. 2B, CRBP mRNA and protein levels were also found to be highly correlated ($P<0.01$). Although the slope of the regression line for this correlation (Fig. 2B) was less than the slope for the correlation in control rats (Fig. 2A), this difference was not statistically significant.

A limited study was carried out to examine the effects of retinol repletion of retinoid-deficient rats on the tissue

TABLE 2. Tissue CRBP mRNA levels in control, retinoid-deficient, and retinol-repleted rats

Tissue	Control	Retinoid-Deficient	Retinol-Repleted, Hours of Retinol Repletion 2	4	6	18
	CRBP mRNA/total RNA, (pg × 10^2/μg)					
Epididymis, proximal	57.0 ± 1.8(4)[a]	58.0 ± 10.0(4)	54.8	47.5 ± 13.0(3)	65.1	66.7
Liver	21.6 ± 4.6(4)	18.6 ± 1.8(4)	18.6	13.6 ± 1.5(3)	21.9	21.3
Kidney	28.9 ± 5.0(3)	29.1 ± 4.6(4)	26.3	27.6 ± 5.2(3)	22.8	33.6
Lung[b]	7.2 ± 2.2(4)	2.7 ± 0.8(4)	1.5	4.7 ± 1.8(3)	4.1	6.2
Testis[b]	5.6 ± 0.8(4)	1.3 ± 0.3(4)	2.0	5.1(2)[c]		5.9
Spleen[b]	2.3 ± 0.3(3)	1.0 ± 0.3(3)	0.9	1.9(2)[c]	1.9	2.2
Small intestine	1.4 ± 0.5(3)	0.7 ± 0.1(4)	1.4	1.4(2)[c]	2.0	2.0

[a]The mean tissue levels of CRBP mRNA were determined for the number of rats given in parentheses. The absence of a number indicates that one rat was used for the determination and where n = 3 or 4 the values are reported as mean ± SD.

[b]Significant ($P < 0.01$) differences in CRBP mRNA levels were found between the control and retinoid-deficient groups.

[c]One of the three samples was lost, hence n = 2.

TABLE 3. Tissue CRBP levels in control, retinoid-deficient, and retinol-repleted rats[a]

Tissue	Control	Retinoid-Deficient	Retinol-Repleted			
			Hours of Retinol Repletion			
			2	4	6	18
	μg CRBP/g wet tissue					
Epididymis, proximal	53.4 ± 23.4[b]	36.2 ± 13.2	32.3	41.7 ± 11.2	36.3	52.3
Liver	46.2 ± 13.1	35.1 ± 15.8	46.8	37.9 ± 4.4	38.8	42.6
Kidney	36.3 ± 9.6	25.5 ± 13.9	33.1	33.3 ± 4.8	27.3	32.2
Lung	17.4 ± 4.0	12.3 ± 5.7	13.5	10.9 ± 0.7	6.1	11.6
Testis[c]	17.0 ± 6.0	8.3 ± 3.2	10.9	6.3 ± 1.5	3.4	12.2
Spleen	7.7 ± 2.9	6.3 ± 1.6	8.1	4.6 ± 1.0	2.7	7.9
Small intestine	8.8 ± 2.3	5.3 ± 3.4	8.0	4.5 ± 0.9	7.8	7.1

[a]The mean tissue levels of CRBP were determined from four rats for the control and retinoid-deficient groups, three rats for the 4-h retinol-repleted group, and one rat for 2-, 6-, and 18-h repleted group.
[b]Where n = 3 or 4 the values are reported as mean ± SD.
[c]Significant ($P < 0.05$) differences in CRBP levels were found between control and retinoid-deficient groups.

levels of CRBP mRNA; the results are shown in Table 2. Administration of 150 μg retinol in peanut oil reversed the effects of retinoid depletion in every one of the four tissues (lung, testis, spleen, and small intestine) that had shown marked reductions in CRBP mRNA levels in retinoid-deficient rats. In each of these four tissues, increased CRBP mRNA levels were seen by 4 h after oral repletion and the CRBP mRNA levels had fully returned to control values by 18 h. This latter conclusion is preliminary, since only single animals were studied at the 18-h time point. For purposes of statistical analysis, the CRBP mRNA levels found in each tissue for the 4-, 6-, and 18-h retinol-repleted rats were pooled and compared with the corresponding tissue levels seen in the retinoid-deficient rats. The increases in CRBP mRNA levels in each of the four tissues (lung, testis, spleen, and small intestine) were statistically highly significant ($P<0.01$).

CRBP II mRNA levels

CRBP II mRNA levels in the small intestine were determined for the rats from each of the three dietary groups. In the small intestine of four control rats the CRBP II mRNA level was found to be 0.60 ± 0.12 pg CRBP II mRNA/μg total RNA (mean ± SD). The small intestine CRBP II mRNA level found in three retinoid-deficient rats was 0.85 ± 0.07 pg CRBP II mRNA/μg total RNA (significantly different from control values, with $P<0.05$). Four hours after retinol repletion of retinoid-deficient rats, the CRBP II mRNA level found in the small intestine of three rats was 0.75 ± 0.2 pg CRBP II mRNA/μg total RNA. CRBP II mRNA levels 2 and 18 h after retinol repletion (as determined for one rat at each time interval) were, respectively, 0.84 and 0.90 pg CRBP II mRNA/μg total RNA.

DISCUSSION

Many studies have been carried out to explore the distribution and localization of CRBP in different tissues and in different cell types within tissues (13, 14, 17, 44–47). Previous studies from our laboratory have shown that CRBP is present in all tissues examined and that the levels of CRBP are not markedly affected by major

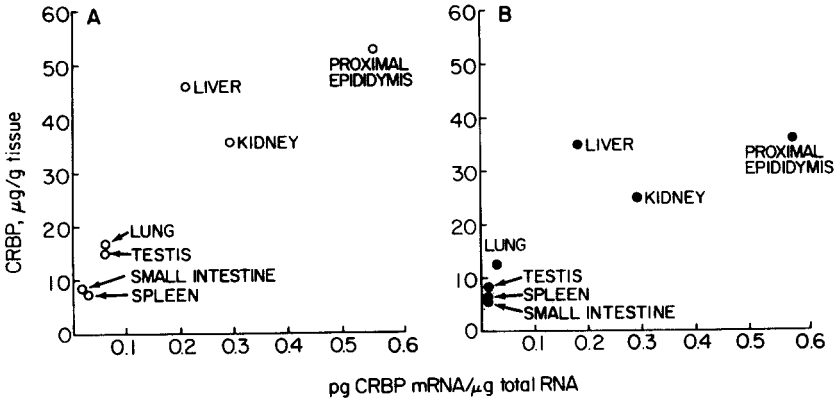

Fig. 2. Relationships between mean tissue CRBP mRNA and CRBP protein levels in control rats (panel A) and in retinoid-deficient rats (panel B). The regression analyses showed highly significant correlations between these sets of data (r = 0.90 in A and 0.85 in B, with $P<0.01$ for both).

changes in retinoid nutritional status (17, 18). Only totally retinoid-deficient rats showed reduced tissue levels of CRBP. Thus, it would appear that the levels of CRBP are highly regulated and maintained throughout the body, although little information is available concerning this regulatory process. The present study was designed to obtain information about the regulation of CRBP metabolism and gene expression, by exploring the relationship between CRBP mRNA and protein levels in various rat tissues, and the effects of the availability of dietary retinol on CRBP mRNA levels.

This report represents the first quantitative study of the levels of CRBP mRNA in different tissues and organs. Within both the control and retinoid-deficient groups, the tissue levels of CRBP mRNA and of CRBP protein were found to be highly correlated ($P<0.01$). If the tissue level of CRBP mRNA reflects the rate of CRBP synthesis within that tissue, then this highly significant correlation suggests that the tissue differences in CRBP protein levels arise mainly from tissue-specific differences in the rates of CRBP synthesis. Thus, tissue levels of CRBP would appear to be controlled mainly at the level of CRBP synthesis, through factors that regulate the tissue levels of CRBP mRNA. As noted previously, no relationship was observed between CRBP mRNA and protein levels within tissues on the two diets.

In the present study, the availability of dietary retinol had an effect on CRBP mRNA levels in some tissues but not in others. The availability of dietary retinol did not affect the levels of CRBP mRNA in the three tissues with the highest levels, namely the proximal epididymis, liver, and kidney. In contrast, in retinoid-deficient rats, the levels of CRBP mRNA found in the lung, testis, spleen, and small intestine were substantially reduced to levels that were only 23% to 50% of the corresponding values in the tissues of control rats. Upon oral repletion with retinol in peanut oil, CRBP mRNA levels increased in these four tissues, reaching control levels by 4–18 h after repletion. Because of the limited number of animals studied in the repletion group, a more precise statement about the time course of the effects of repletion cannot be made at this time. Thus, only preliminary conclusions would be possible, since only single animals were studied at the 6-h and 18-h time points. It should also be noted that the repletion study did not specifically exclude the unlikely possibility that some of the observed effects may be due to the peanut oil vehicle and not to the retinol.

Several alternative hypotheses could be proposed to explain the observed differences between these two groups of tissues. A likely explanation is the possibility that the observed differences between the two groups of tissues reflected differences in tissue retinoid levels in the animals studied. Because of our desire to study retinoid-deficient but clinically healthy rats, it was not possible to use animals whose serum retinol levels had dropped to zero. Thus, in this study, the mean serum retinol level for the retinoid-deficient group was 1.9 μg/dl (as compared to 58.1 μg/dl for the control group) and the animals from the retinoid-deficient diet group reached a weight-gain plateau approximately 3 weeks prior to killing. This low serum retinol level and weight-gain plateau are standard indices of retinoid deficiency (17, 18, 41), and in our experience indicate the final stage at which the retinoid-deficient animal can still be considered healthy. In the present study, tissue retinol levels were measured only for the liver. Livers from retinoid-deficient rats were found to have very low, but detectable, levels of retinol (see Table 1). It is possible that, like the liver, some of the remaining tissues (specifically, the proximal epididymis and kidney), while highly depleted, were also not fully retinoid-deficient. Thus, the reduction of CRBP mRNA levels may reflect the response of the tissue to the actual level of retinol within the tissue. Considering the rise of CRBP mRNA levels seen in the lung, small intestine, spleen, and testis 4–18 h after retinol repletion, it seems likely that low levels of retinol present in the proximal epididymis, liver, and kidney of the deficient rats might have accounted for the absence of a drop in CRBP mRNA levels in these tissues. Alternatively, it is possible that the observed differences in CRBP expression reflect tissue-specific differences in the role played by retinol in the regulation of the CRBP gene. Future studies will be needed to address these and other possibilities.

In the adult rat, CRBP II protein and CRBP II mRNA both have been localized to the small intestine (15, 48) where this protein is thought to be involved in the uptake and metabolism of dietary retinoid. CRBP II and CRBP are highly homologous proteins (49) and are members of the same gene family (50). For control rats, we observed CRBP II mRNA levels in the small intestine to be approximately 43-times greater than those of CRBP mRNA. In contrast to CRBP mRNA, retinoid-deficient rats showed no decrease, and in fact showed a 42% increase in CRBP II mRNA levels in the small intestine. Thus, it would appear that the genes for these two homologous proteins respond differently to the availability of their ligand, retinol.

Retinoids are known to exert acute effects on gene expression. Transglutaminase mRNA levels in myeloid cells become elevated in response to retinoids in a manner that is independent of protein synthesis (8). The rate of epidermal growth factor receptor gene transcription by normal rat kidney cells is reported to be increased in the presence of retinoids (51). Retinoids are also known to suppress the expression of certain genes, such as c-myc (52).

Some data are available on the effects of retinoids on CRBP gene expression. It has been reported that the administration of retinol (29) and retinoic acid (53) to

retinoid-deficient rats increased CRBP mRNA levels in the lungs, and that the addition of retinol to cultured rat Sertoli cells elevated CRBP mRNA levels (54, 55). In a recent report from our laboratory (9), the induction of CRBP mRNA and CRBP protein was demonstrated in P19 embryonal carcinoma cells stimulated to differentiate in vitro by addition of retinoic acid. CRBP mRNA was induced with 10^{-9} M retinoic acid within 3 h (the first time point examined), and this induction was independent of concurrent protein synthesis (i.e., not inhibited by cycloheximide). As part of this study (9), cellular retinoic acid-binding protein (CRABP) mRNA induction was also studied and was found to occur at a later time after addition of retinoic acid, and to be dependent on concurrent protein synthesis.

The results reported here, with intact rats, are consistent with our earlier cell culture study (9). Thus, in the present study, rises in CRBP mRNA were seen in lung, testis, spleen, and small intestine 4–18 h after oral administration of retinol in peanut oil. As indicated above, future more extensive studies will be needed to precisely determine the time course of this response. The combined in vitro (9) and in vivo (present study) data suggest that retinoids may act directly in cells to influence CRBP gene expression. As suggested previously (9) CRBP may be an early response gene induced by retinoids.

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