0031-3998/95/3706-0720$03.00/0
PEDIATRIC RESEARCH

Vol. 37, No. 6, 1995
Printed in U.S.A.

Calbindin-D_{9K} Gene Expression in Rat Chorioallantoic Placenta Is Not Regulated by 1,25-Dihydroxyvitamin D_3

J. D. GLAZIER, E. B. MAWER, AND C. P. SIBLEY

Action Research Placental and Perinatal Unit, Departments of Child Health [J.D.G.] and Medicine [E.B.M.], The University of Manchester, St. Mary's Hospital, Manchester M13 OJH and School of Biological Sciences, University of Manchester, Stopford Building, Manchester M13 9PL, UK [C.P.S.]

ABSTRACT

The aim of this study was to investigate whether 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the active metabolite of vitamin D, regulates the expression of rat placental calbindin-D_{9K} mRNA. One group of rats (−D) was fed a vitamin D-deficient diet before and during pregnancy, whereas a second group (+D) was fed a vitamin D-replete diet over the same period. Animals were killed on d 21 of gestation, and plasma concentrations of $1,25(OH)_2D_3$ for +D and −D animals were significantly ($p < 0.05$) different (260 ± 78 and 39 ± 8 pM in maternal plasma and 122 ± 39 and 42 ± 10 pM in fetal plasma, repectively; mean ± SE, $n = 4–5$). Vitamin D deficiency had no effect on placental weight, fetal weight, fetal ashed weight, fetal calcium accretion, or the maternofetal calcium gradient. Hybridization of RNA from maternal duodena (used as a positive control tissue) and placentas of +D and −D rats with a rat calbindin-D_{9K} cDNA revealed a single 0.6-kb transcript in both tissues. The abundance of this transcript was markedly lower ($p = 0.06$) in the duodena of −D compared with +D rats (mean change −68 ± 9%) but there was no difference between the placentas of the two groups (mean change +13 ± 22%). These changes were significantly ($p < 0.05$) different between the two tissues and the response of each tissue to vitamin D deficiency was significantly different ($p < 0.01$). These data indicate that $1,25(OH)_2D_3$ does not regulate the expression of calbindin-D_{9K} mRNA in rat placenta. (***Pediatr Res* 37: 720–725, 1995**)

Abbreviations

25(OH)D_3, 25-hydroxyvitamin D_3
1,25$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3
+D, vitamin D-replete
−D, vitamin D-deficient
ANOVA, analysis of variance

Calbindin-D_{9K} belongs to a group of intracellular calcium binding proteins that bind calcium with high affinity and is found in highest concentration in calcium-transporting epithelia such as the intestine, kidney, uterus, yolk sac, and chorioallantoic placenta (1). Calbindin-D_{9K} of the rat chorioallantoic placenta is identical to that from the rat intestine (2). The role of calbindin-D_{9K} in these tissues is not fully understood, but it is thought to act as a cytosolic calcium buffer (1) and/or a facilitator of cytosolic calcium diffusion (3, 4). The gestational increase in mRNA for calbindin-D_{9K} in rat chorioallantoic placenta, coincident with the increase in maternofetal calcium flux, suggests that calbindin-D_{9K} may play a rate limiting role in maternofetal calcium transport across this tissue (5). This notion is further supported by the observation that diabetic rat pregnancy lowers both placental net calcium flux and calbindin-D_{9K} mRNA expression compared with insulin-treated controls (6).

Received March 29, 1994; accepted December 27, 1994.
Correspondence: Dr. J. Glazier, Department of Child Health, St. Mary's Hospital, Manchester M13 OJH, UK.
Supported by an Action Research Endowment Fund.

The hormonal factor(s) controlling the expression of the calbindin-D_{9K} gene in rat placenta remain uncertain. In particular, the effect of vitamin D is not clear as the experimental evidence is somewhat conflicting. A role for $1,25(OH)_2D_3$, the biologically active metabolite of vitamin D, is implicated from studies in which the lowering of maternal plasma $1,25(OH)_2D_3$ concentration, induced by thyroparathyroidectomy (7), diabetes (8), or a high cation diet (9), was associated with a reduced placental calbindin-D_{9K} concentration compared with controls.

By contrast, dietary studies in which placental calbindin-D_{9K} concentration was unaltered in rodents fed a vitamin D-deficient diet before and during pregnancy (10, 11) infer vitamin D independency of placental calbindin-D_{9K} synthesis. Furthermore, although maternal administration of $1,25(OH)_2D_3$ to thyroparathyroidectomized rats increased placental calbindin-D_{9K} concentration so that it was similar to that in the control group (7), it had no effect on placental calbindin-D_{9K} concentration in mice fed a high strontium diet (9) and also had no effect on mouse placental calbindin-D_{9K} concentration when given as daily injections over the last third of gestation (12).

The lowering of maternal $1,25(OH)_2D_3$ concentration by these treatments was associated with a significantly reduced concentration of calbindin-D_{9K} in maternal intestine (7, 9–11), and further evidence suggests that $1,25(OH)_2D_3$ regulates the expression of intestinal calbindin-D_{9K} at both transcriptional and posttranscriptional levels (13, 14). However, no study has addressed whether $1,25(OH)_2D_3$ affects placental calbindin-D_{9K} expression at the mRNA level.

Although the use of the maternal intestinal response as a marker of maternal $1,25(OH)_2D_3$ status in the studies described above may be a reliable indicator of vitamin D deficiency (15), without plasma vitamin D metabolite concentrations the degree of vitamin D deficiency in maternal and fetal compartments cannot be determined. This may be pertinent because rat placenta and fetal kidney have the capacity to synthesize $1,25(OH)_2D_3$ *in vitro* (16), with the former (17) but not the latter (18) source likely to be active *in vivo*.

The aim of this study was therefore to determine whether $1,25(OH)_2D_3$ does regulate calbindin-D_{9K} gene transcription in the rat chorioallantoic placenta. In these experiments, intestinal calbindin-D_{9K} mRNA and maternal and fetal plasma concentrations of $25(OH)D_3$ and $1,25(OH)_2D_3$ were measured to determine vitamin D status. Vitamin D deficiency was induced by dietary means, and fetal calcium accretion was used as an index of net transplacental calcium flux.

METHODS

Animals and diets. All procedures were carried out according to the Animals (Scientific Procedures) Act 1986. Three-week-old Wistar rats from an in-house strain which rapidly develops vitamin D deficiency were fed a diet based on wholemeal flour with no added vitamin D (19) until 8 wk of age. They were then split into two groups of five, both of which were maintained on a synthetic diet based on white flour (19) but modified to be nonrachitogenic (calcium 0.5%, phosphorus 0.5% by weight). The +D group received 1 IU vitamin D (Koch-Light, Colnbrook, Berks, UK) per gram of diet in addition, whereas no further supplement was given to the −D group. Each group was fed their respective diets for 1 wk and then mated and maintained throughout pregnancy on the same diet that they had received during the week before mating.

Sample collection. On d 21 of gestation (d 1 taken to be the day a vaginal plug was found; term is 23 d), animals from both groups were anesthetized by ether inhalation. Maternal blood was collected from the inferior vena cava and fetal blood by axillary incision. Fetal plasma from all fetuses of each rat was pooled for analysis. Fetuses were removed and weighed.

Placentas were weighed and rapidly frozen in liquid nitrogen. Maternal duodenum (9 cm from pylorus) was removed, everted, rinsed in ice-cold saline, blotted, and rapidly frozen in liquid nitrogen. Both duodena and placentas were stored at −80°C before RNA extraction. Rats were killed immediately after completion of sampling.

Vitamin D metabolites. Plasma $25(OH)D_3$ and $1,25(OH)_2D_3$ concentrations were determined after extraction with acetonitrile and preliminary separation by HPLC. Concentrations of the metabolites were estimated by absorbance at 265 nm on a second HPLC separation for $25(OH)D_3$ (20) and by radioimmunoassay using a MAb for $1,25(OH)_2D_3$ (21).

Fetal ashing. Four fetuses from each rat were separately ashed in a muffle furnace at 700°C overnight, ash weight was recorded, and the ash was dissolved in 3 mL 3 M HCl for calcium determination (22).

Calcium determinations. Plasma calcium concentration and fetal ash calcium content were determined by atomic absorption spectrophotometry, after dilution of samples with 1.33% $LaCl_3$. Ionized calcium concentration in whole blood was measured using a calcium selective electrode (ICA1, Radiometer, Crowley, W. Sussex, UK).

RNA extraction and Northern blot analysis. Total RNA was extracted from placentas (three to five pooled from each rat) and maternal duodena of all rats in the +D and −D groups by the method of Chomczynski and Sacchi (23), as described previously (5). Total RNA was fractionated on a 1.2% agarose/formaldehyde gel and then transferred to a nylon filter (5). RNA from tissues of +D and −D rats was always included on the same filter and on some filters, and for both tissues, RNA from more than one rat within the two experimental groups (+D and −D) was included. In some instances RNA from placenta or duodenum of an individual rat was hybridized more than once. Filters were prehybridized, hybridized with ^{32}P-labeled cDNA (24) for rat intestinal calbindin-D_{9K} (25), and then washed and autoradiographed as described previously (5).

Filters were subsequently stripped and reprobed with β-actin cDNA which served as a control to correct for variations in sample loadings (5). Preliminary studies showed that β-actin mRNA levels in duodenum and placenta, respectively, were not significantly different between the +D and −D groups (data not shown), in agreement with others (14).

Quantitation of transcript density peak area was performed by spectrodensitometry (Shimadzu Scientific Instruments, Kyoto, Japan), and preliminary studies showed that hybridization signals were within the linear range of the film used. Relative calbindin-D_{9K} mRNA abundance is reported as density values for calbindin-D_{9K} mRNA normalized to density for β-actin mRNA.

Statistics. For plasma vitamin D metabolites and calcium determinations, n is the number of pregnant rats. For some animals, insufficient plasma was available for determination. For placental weight, fetal weight, fetal ash weight, and for fetal calcium content, respectively, an average value was determined for each pregnant rat, and n is the number of pregnant rats.

For Northern blot analyses, the relative calbindin-D_{9K} mRNA abundance (calbindin-D_{9K}/β-actin mRNA ratio) for each tissue from individual rats was determined and used for statistical comparison, and n is the number of hybridizations performed. To compare the difference in the relative calbindin-D_{9K} mRNA abundance (calbindin-D_{9K}/β-actin mRNA ratio) between the +D and −D groups for both tissues, concurrent hybridizations were compared, and where more than one rat within the +D and −D groups was included on the same filter, a mean calbindin-D_{9K}/β-actin mRNA ratio value for the +D and −D rats on that filter was used for statistical comparison and n is the number of filters hybridized.

Data are presented as mean ± SE. Statistical comparisons have been made using the Mann-Whitney, Wilcoxon signed rank, *t* test (unpaired or paired as appropriate), or ANOVA.

RESULTS

The vitamin D-deficient status of rats in the −D group was confirmed by the significant reduction in $25(OH)D_3$ and $1,25(OH)_2D_3$ concentration, respectively, in both maternal and fetal plasma (Table 1).

Placental weight, fetal weight, fetal ash weight, ashed fetal calcium content, and total and ionized calcium concentrations in maternal and fetal plasma were similar in the +D and −D groups (Table 2). Total and ionized calcium concentrations in fetal plasma were significantly higher than maternal in both +D and −D groups, and this maternofetal difference was unaffected by vitamin D deficiency (Table 2).

Northern hybridization studies identified a single calbindin-D_{9K} transcript corresponding to 0.6 kb in both maternal duodenum and placenta (Fig. 1*A*). The relatively greater abundance of mRNA for calbindin-D_{9K} in duodenum compared with placenta was reflected by the shorter exposure time for the former (Fig. 1) and is consistent with the 6-fold higher tissue calbindin-D_{9K} concentration in the former at this gestational age (26). The abundance of mRNA for calbindin-D_{9K} in the duodenum was markedly lower in a vitamin D-deficient rat than in a vitamin D-replete rat, but there was no difference in calbindin-D_{9K} mRNA abundance between the placentas of these two same animals (Fig. 1*A*). β-Actin transcripts of 2.1 kb were identified in both tissues, with higher expression in placenta compared with duodenum, as indicated by the shorter exposure time for the former (Fig. 1*B*).

Figure 2 shows all hybridization signals and calbindin-D_{9K}/β-actin mRNA density ratios for both groups of rats. In every hybridization performed, calbindin-D_{9K} mRNA abundance (normalized to β-actin) in duodenum was lower in the −D group compared with the +D group (Fig. 2*A*), this diminution ranging from −38 to −87%, such that mRNA abundance was 68 ± 9% ($n = 5$) lower in the −D group (significant at the 6% level; Wilcoxon signed rank test). However, in placenta there was no consistent trend in the difference between calbindin-D_{9K} mRNA abundance of the −D group compared with the +D group (Fig. 2*B*), values ranging from −14 to +52%, the overall change being +13 ± 22% ($n = 4$; not significant). The change in placenta was significantly different from that in duodenum ($p < 0.05$; Mann-Whitney test), and the response to vitamin D deficiency was significantly different between the two tissues ($p < 0.01$, ANOVA; Fig. 3).

Table 1. *Maternal (M) and fetal (F) plasma concentrations of vitamin D metabolites in rats fed a +D or −D diet*

Group		$25(OH)D_3$ (nM)	$1,25(OH)_2D_3$ (pM)
+D	M	15.3 ± 0.3 ($n = 3$)	260 ± 78 ($n = 4$)
	F	38.8 ± 5.5 ($n = 4$)	122 ± 39 ($n = 5$)
−D	M	3.3 ± 1.5[a] ($n = 4$)	39 ± 8[b] ($n = 5$)
	F	3.8 ± 0.5[b] ($n = 4$)	42 ± 10[b] ($n = 5$)

[a] $p = 0.057$ vs +D group (Mann-Whitney).
[b] $p < 0.05$ vs +D group (Mann-Whitney).

Table 2. *Placental weight, fetal weight, fetal ash weight, ashed fetal calcium content and maternal (M) and fetal (F) plasma calcium, and ionized calcium concentrations in rats fed a +D or −D diet*

		+D	−D
Placental weight (g)		0.32 ± 0.01 ($n = 5$)	0.35 ± 0.01 ($n = 5$)
Fetal weight (g)		2.74 ± 0.06 ($n = 5$)	2.85 ± 0.10 ($n = 5$)
Ashed fetal weight (g)		0.044 ± 0.001 ($n = 5$)	0.046 ± 0.002 ($n = 5$)
Ashed fetal calcium (mmol)		0.16 ± 0.004 ($n = 5$)	0.16 ± 0.007 ($n = 5$)
Plasma calcium (mM)	M	2.12 ± 0.05 ($n = 5$)	2.04 ± 0.04 ($n = 5$)
	F	2.47 ± 0.15[a] ($n = 4$)	2.62 ± 0.03[a] ($n = 5$)
Blood ionised calcium (mM)	M	1.14 ± 0.08 ($n = 4$)	1.09 ± 0.03 ($n = 3$)
	F	1.60 ± 0.02[b] ($n = 5$)	1.59 ± 0.04[b] ($n = 4$)

[a] $p < 0.05$ vs maternal (*t* test).
[b] $p < 0.005$ vs maternal (*t* test).

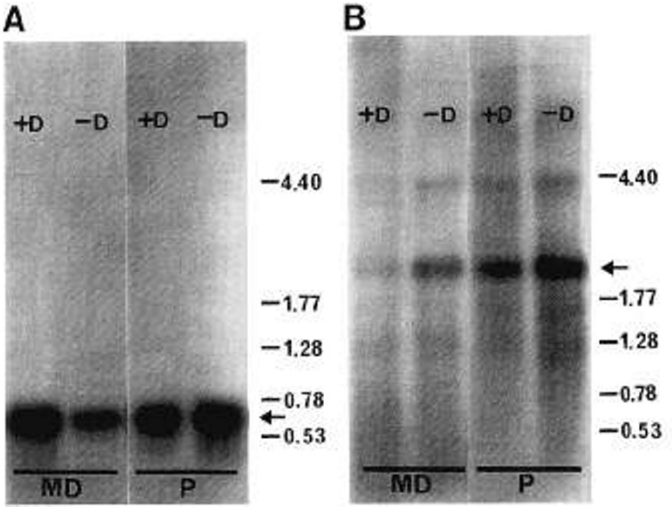

Figure 1. Northern analysis of calbindin-D_{9K} and β-actin mRNA in maternal duodenum (*MD*) and placenta (*P*) of two individual rats, one fed a +*D* and one fed a −*D* diet. Maternal plasma concentration of $1,25(OH)_2D_3$ was 169 and 42 pM, respectively, and fetal plasma concentration of $1,25(OH)_2D_3$ was 117 and 31 pM, respectively, for these individual +*D* and −*D* rats. Total RNA (15 μg/lane) was hybridized with cDNA for: (*A*) rat calbindin-D_{9K} followed by autoradiography for 0.75 h (MD) or 4.5 h (P) and then (*B*) β-actin followed by autoradiography for 18.5 h (MD) or 6 h (P). Position of RNA size markers (in kb) (GIBCO, Uxbridge, Middlesex, UK) is shown on right. *Arrows* indicate transcripts at 0.6 kb in (*A*) and 2.1 kb in (*B*). Data are representative of six and seven hybridizations for MD and P, respectively.

DISCUSSION

The present study was carried out to determine whether calbindin-D_{9K} mRNA expression is regulated by vitamin D in

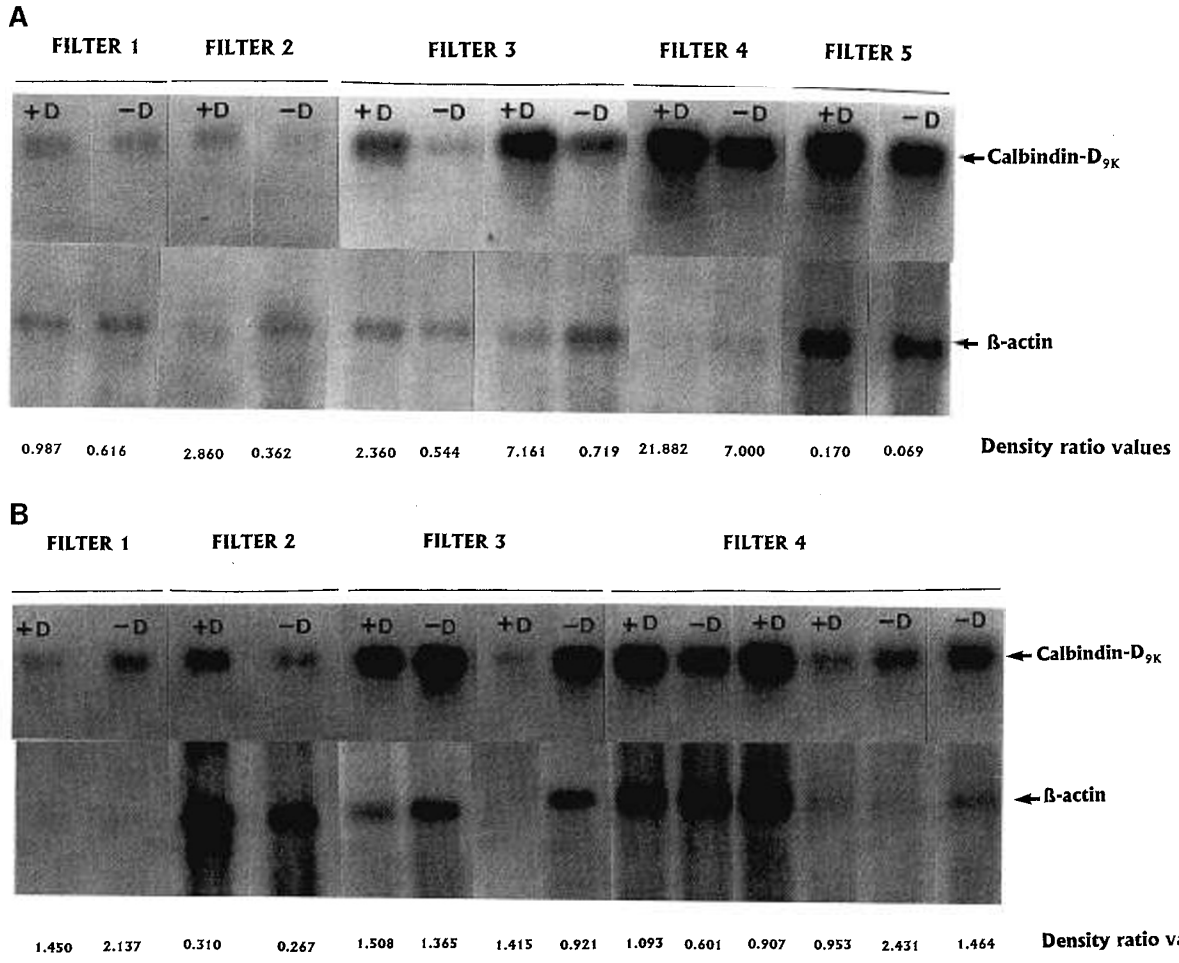

Figure 2. Northern hybridization of RNA from maternal duodenum (*A*) or placenta (*B*) of +D and −D rats. Filters were hybridized with calbindin-D_{9K} cDNA and then reprobed with β-actin cDNA. Not all filters were hybridized concurrently. Calbindin-D_{9K}/β-actin mRNA density ratios are given beneath each lane. Data are from five rats in each group.

the rat placenta. The data shows that our dietary regime induced pronounced vitamin D deficiency.

The markedly lower 25(OH)D_3 and 1,25$(OH)_2D_3$ plasma concentrations in the −D group were associated with a lower relative abundance of mRNA for calbindin-D_{9K} in maternal intestine, but not in placenta compared with the +D group. The data therefore demonstrate that the active metabolites of vitamin D do not regulate the expression of calbindin-D_{9K} mRNA in the latter tissue, despite the co-localization of vitamin D receptors (27) and calbindin-D_{9K} mRNA (28) within the labyrinth layer of rat placenta. The present findings support previous observations from dietary studies in which cytosolic calbindin-D_{9K} protein concentration was reduced in maternal intestine but unaltered in placenta in response to vitamin D deficiency (10, 11). Furthermore, they suggest that the reduction in placental calbindin-D_{9K} concentration associated with thyroparathyroidectomy (7), diabetes (8), and a high cation diet (9) is not directly related to a lowered maternal 1,25$(OH)_2D_3$ concentration.

Our data also show that vitamin D deficiency did not impair fetal growth or mineralization, or alter the maternofetal plasma or whole blood ionized calcium concentration difference. This confirms previous observations that net placental calcium transport in the rat is not regulated by vitamin D (29, 30). These data therefore provide further support for the hypothesis that change, or as here, lack of change in calbindin-D_{9K} mRNA expression is always coincident with any change in maternofetal calcium flux across the placenta (5, 6).

Steroid hormones regulate gene expression by the interaction of the receptor-steroid complex with specific DNA sequences, hormone-responsive elements, located in or near the responsive genes (reviewed in Ref. 31). The identification of two different hormone-responsive elements in the rat calbindin-D_{9K} gene, a 1,25$(OH)_2D_3$-responsive element (32), and an estrogen-responsive element (33) may confer hormone-specific transcriptional regulation of this gene in different tissues. Indeed, the expression of the calbindin-D_{9K} gene in rat intestine is regulated by 1,25$(OH)_2D_3$ and not by estrogen (34, 35) whereas in rat uterus it appears to be regulated by estrogen (33, 35, 36) and not by 1,25$(OH)_2D_3$ (34). In rat lung neither hormone appears to regulate the expression of this gene (35).

The regulation of the calbindin-D_{9K} gene in any one tissue may be multifactorial as suggested by a dietary study demonstrating that the stimulation by 1,25$(OH)_2D_3$ of duodenal calbindin-D_{9K} biosynthesis appears to be dependent on both prior vitamin D and calcium status (37), and by *in vitro* studies confirming that both calcium and 1,25$(OH)_2D_3$ stimulate calbindin-D_{9K} gene expression in rat enterocytes (38). This may

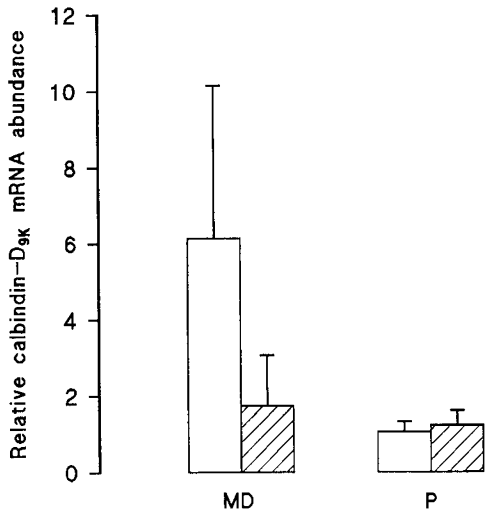

Figure 3. Effect of vitamin D deficiency on relative calbindin-D_{9K} mRNA abundance in maternal duodenum (*MD*) and placenta (*P*) from +D 1(□) and −D ( ) groups. Total RNA was hybridized and autoradiographed as described in Figure 1 followed by densitometric quantitation of transcript peak area. Relative calbindin-D_{9K} mRNA abundance is shown as calbindin-D_{9K}/β-actin mRNA density ratios. Mean ± SE are shown with *n* (number of hybridizations) being 6 and 7 for MD and P, respectively.

account for the apparently disparate observations in nonpregnant rats, in which intestinal calbindin-D_{9K} concentration appeared to be significantly lower after dietary-induced vitamin D deficiency in some studies (15, 39, 40) but not in others (10, 41). In this context, placental calbindin-D_{9K} expression is unlikely to be directly modulated by maternal calcemia as both hypocalcemia (42) and hypercalcemia (9) elicited a marked decrease in placental calbindin-D_{9K} concentration (8, 9).

The coordinated induction of calbindin-D_{9K} expression in uterus, placenta, and yolk sac, during the last third of gestation in the rat (43), suggests that the calbindin-D_{9K} gene in these three closely apposed tissues may be regulated by a common hormone, possibly estrogen. In summary, the factors which control steady state calbindin-D_{9K} mRNA expression in rat placenta have yet to be elucidated, and may be tissue-specific, but do not include $1,25(OH)_2D_3$.

Acknowledgments. The authors are grateful to Dr. V. Hillier for statistical advice. We would also like to thank Dr. M. Thomasset for donating the rat calbindin-D_{9K} cDNA probe used in this study.

REFERENCES

1. Christakos S, Gabrielides C, Rhoten WB 1989 Vitamin D-dependent calcium binding proteins: chemistry, distribution, functional considerations, and molecular biology. Endocr Rev 10:3–26
2. MacManus JP, Watson DC, Yaguchi M 1986 The purification and complete amino acid sequence of the 9000-M_r Ca^{2+}-binding protein from rat placenta. Biochem J 235:585–595
3. Bronner F 1988 Vitamin D-dependent active calcium transport: the role of CaBP. Calcif Tissue Int 43:133–137
4. Feher JJ, Fullmer CS, Fritzsch GK 1989 Comparison of the enhanced steady-state diffusion of calcium by calbindin-D9K and calmodulin: possible importance in intestinal calcium absorption. Cell Calcium 10:189–203
5. Glazier JD, Atkinson DE, Thornburg KL, Sharpe PT, Edwards D, Boyd RDH, Sibley CP 1992 Gestational changes in Ca^{2+} transport across rat placenta and mRNA for calbindin$_{9K}$ and Ca^{2+}-ATPase. Am J Physiol 263:R930–R935
6. Husain SM, Birdsey TJ, Glazier JD, Mughal MZ, Garland HO, Sibley CP 1994 Effect of diabetes mellitus on maternofetal flux of Ca and Mg and calbindin$_{9K}$ mRNA expression in rat placenta. Pediatr Res 35:376–381
7. Garel J-M, Delorme AC, Marche P, Nguyen TM, Garabedian M 1981 Thyroparathyroidectomized pregnant rats: effects on calcium-binding proteins of maternal duodenum and of fetoplacental unit. Endocrinology 109:284–289
8. Verhaeghe J, Thomasset M, Bréhier A, Van Assche FA, Bouillon R 1988 $1,25(OH)_2D_3$ and Ca-binding protein in fetal rats: relationship to the maternal vitamin D status. Am J Physiol 254:E505–E512
9. Bruns MEH, Wallshein V, Bruns DE 1982 Regulation of calcium-binding protein in mouse placenta and intestine. Am J Physiol 242:E47–E52
10. Marche P, Delorme A, Cuisinier-Gleizes P 1978 Intestinal and placental calcium-binding proteins in vitamin D-deprived or -supplemented rats. Life Sci 23:2555–2562
11. Bruns ME 1983 Vitamin D action during pregnancy: the induction of calcium binding protein in intestine, placenta and yolk sac. In: Holick MF, Gray TK, Anast CS (eds) Perinatal Calcium and Phosphorus Metabolism. Elsevier Science, pp 183–195
12. Bruns ME, Kleeman E, Bruns DE 1986 Vitamin D-dependent calcium-binding protein of mouse yolk sac. J Biol Chem 261:7485–7490
13. Dupret JM, Brun P, Thomasset M 1986 *In vivo* effects of transcriptonal and translational inhibitors on duodenal vitamin D-dependent calcium binding protein messenger ribonucleic acid stimulation by 1,25-dihydroxycholecalciferol. Endocrinology 119:2476–2483
14. Dupret J-M, Brun P, Perret C, Lomri N, Thomasset M, Cuisinier-Gleizes P 1987 Transcriptional and post-transcriptional regulation of vitamin D-dependent calcium-binding protein gene expression in the rat duodenum by 1,25-dihydroxycholecalciferol. J Biol Chem 262:16553–16557
15. Bronner F, Freund T 1975 Intestinal CaBP: a new quantitative index of vitamin D deficiency in the rat. Am J Physiol 229:689–694
16. Paulson SK, DeLuca HF 1986 Vitamin D metabolism during pregnancy. Bone 7:331–336
17. Lester GE, Gray TK, Lorenc RS 1978 Evidence for maternal and fetal differences in vitamin D metabolism. Proc Soc Exp Biol Med 159:303–307
18. Weisman Y, Sapir R, Harell A, Edelstein S 1976 Maternal-perinatal interrelationships of vitamin D metabolism in rats. Biochim Biophys Acta 428:388–395
19. Lumb GA, Mawer EB, Stanbury SW 1971 The apparent vitamin D resistance of chronic renal failure. A study of the physiology of vitamin D in man. Am J Med 50:421–441
20. Mawer EB, Hann J, Berry JL, Davies M 1985 Vitamin D metabolism in patients intoxicated with ergocalciferol. Clin Sci 68:135–141
21. Mawer EB, Berry JL, Cundall JP, Still PE, White A 1990 A sensitive radioimmunoassay that is equipotent for ercalcitriol and calcitriol (1,25-dihydroxyvitamin D2 and D3). Clin Chim Acta 190:199–210
22. Chalon S, Garel J-M 1983 1,25-Dihydroxyvitamin D_3 injections into rat fetuses: effects on fetal plasma calcium, plasma phosphate and mineral content. Reprod Nutr Dev 23:567–573
23. Chomczynski P, Sacchi N 1987 Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159
24. Feinberg AP, Vogelstein B 1984 A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266–267
25. Desplan C, Thomasset M, Moukhtar M 1983 Synthesis, molecular cloning, and restriction analysis of DNA complementary to vitamin D-dependent calcium-binding protein mRNA from rat duodenum. J Biol Chem 258:2762–2765
26. Bruns MEH, Fausto A, Avioli LV 1977 Immunochemical identity between rat vitamin D-dependent calcium-binding protein in duodenal mucosa and a calcium-binding protein in placenta tissue In: Wasserman RH, Corradino RA, Carafoli E, Kretsinger RH, MacLennan DH, Siegel FL (eds) Calcium-Binding Proteins and Calcium Function. North-Holland, Amsterdam, pp 350–352
27. Stumpf WE, Sar M, Narbaitz R, Huang S, DeLuca HF 1983 Autoradiographical localization of 1,25-dihydroxyvitamin D_3 in rat placenta and yolk sac. Horm Res 18:215–220
28. Warembourg M, Perret C, Thomasset M 1986 Distribution of vitamin D-dependent calcium-binding protein messenger ribonucleic acid in rat placenta and duodenum. Endocrinology 119:176–184
29. Halloran BP, DeLuca HF 1981 Effect of vitamin D deficiency on skeletal development during early growth in the rat. Arch Biochem Biophys 209:7–14
30. Brommage R, DeLuca HF 1984 Placental transport of calcium and phosphorus is not regulated by vitamin D. Am J Physiol 246:F526–F529
31. Beato M 1989 Gene regulation by steroid hormones. Cell 56:335–344
32. Darwish HM, DeLuca HF 1992 Identification of a 1,25-dihydroxyvitamin D_3-response element in the 5′-flanking region of the rat calbindin D-9k gene. Proc Natl Acad Sci USA 89:603–607
33. Darwish H, Krisinger J, Furlow JD, Smith C, Murdoch FE, DeLuca HF 1991 An estrogen-responsive element mediates the transcriptional regulation of calbindin D-9K gene in rat uterus. J Biol Chem 266:551–558
34. Delorme AC, Danan JL, Acker MG, Ripoche MA, Mathieu H 1983 In rat uterus 17β-estradiol stimulates a calcium-binding protein similar to the duodenal vitamin D-dependent calcium-binding protein. Endocrinology 113:1340–1347
35. Dupret J-M, L'Horset F, Perret C, Bernaudin J-F, Thomasset M 1992 Calbindin-D9K gene expression in the lung of the rat. Absence of regulation by 1,25-dihydroxyvitamin D_3 and estrogen. Endocrinology 131:2643–2648
36. L'Horset F, Perret C, Brehier A, Thomasset M 1990 17β-estradiol stimulates the calbindin-D9k (CaBP9k) gene expression at the transcriptional and posttranscriptional levels in rat uterus. Endocrinology 127:2891–2897
37. Buckley M, Bronner F 1980 Calcium-binding protein biosynthesis in the rat: regulation by calcium and 1,25-dihydroxyvitamin D_3. Arch Biochem Biophys 202:235–241

38. Brehier A, Thomasset M 1990 Stimulation of calbindin-D9K (CaBP9K) gene expression by calcium and 1,25-dihydroxycholecalciferol in fetal rat duodenal organ culture. Endocrinology 127:580–587
39. Thomasset M, Parkes CO, Cuisinier-Gleizes P 1982 Rat calcium-binding proteins: distribution, development, and vitamin D dependence. Am J Physiol 243:E483–E488
40. Perret C, Desplan C, Thomasset M 1985 Cholecalcin (a 9-kDa cholecalciferol-induced calcium-binding protein) messenger RNA. Distribution and induction by calcitriol in the rat digestive tract. Eur J Biochem 150:211–217
41. Thomasset M, Tenenhouse A 1988 Vitamin D dependence of calbindin D 9K and calbindin D 28K synthesis in various rat tissues. In: Vitamin D. Molecular, Cellular and Clinical Endocrinology. Walter de Gruyter & Co., Berlin, pp 516–517
42. Verhaeghe J, Bouillon R, Nyomba BL, Lissens W, Van Assche FA 1986 Vitamin D and bone mineral homeostasis during pregnancy in the diabetic BB rat. Endocrinology 118:1019–1025
43. Mathieu CL, Burnett SH, Mills SE, Overpeck, JG, Bruns DE, Bruns ME 1989 Gestational changes in calbindin-D_{9k} in rat uterus, yolk sac, and placenta: implications for maternal-fetal calcium transport and uterine muscle function. Proc Natl Acad Sci USA 86:3433–3437