Modulation by Vitamin \(B_6\) of Glucocorticoid Receptor-mediated Gene Expression Requires Transcription Factors in Addition to the Glucocorticoid Receptor*

(Received for publication, January 4, 1993, and in revised form, June 28, 1993)

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We have investigated the mechanism by which vitamin \(B_6\) acts to modulate steroid hormone-mediated gene expression. We show that the level of glucocorticoid-induced gene expression from simple promoters, containing only hormone response elements and a TATA sequence, was not affected by alterations in intracellular vitamin \(B_6\) concentration. However, modulation of hormone-induced gene expression was restored with the inclusion of a binding site for the transcription factor nuclear factor 1 (NF1) within the hormone-responsive promoter: glucocorticoid-induced gene expression was reduced by 44% under conditions of elevated intracellular vitamin \(B_6\) concentration and enhanced by 98% in mild vitamin deficiency. Under these conditions, neither glucocorticoid receptor sedimentation characteristics, receptor activation, nor DNA binding capacity was affected. Quantitatively analogous effects were detected with estrogen-induced gene expression when an NF1 binding site was removed from or introduced into an estrogen-responsive promoter. NF1-mediated constitutive transcription was not affected by alterations in vitamin concentration. The modulatory effect of vitamin did not require strict positioning of or spacing between the glucocorticoid response element and NF1 binding site. Moreover, a heterologous transcriptional activator, composed of the viral Ela transactivation domain and the GAL4 DNA binding domain, does not substitute for NF1 in restoring vitamin \(B_6\) modulation of hormone-induced gene expression. These results suggest that vitamin \(B_6\) modulates steroid hormone-mediated gene expression through its influence on a functional or cooperative interaction between steroid hormone receptors and the transcription factor NF1.

Steroid hormones regulate a variety of physiological processes that are involved in growth, development, and maintenance of homeostasis (1). The biological effects of steroid hormones are achieved through interaction with their intracellular receptor molecules. Although unique receptor proteins exist for each steroid hormone, the steroid hormone receptors, nonetheless all exhibit remarkable similarities in structure, function, and sequence (2-6). Binding of the steroid ligand initiates the process of transformation, through which the steroid hormone receptor acquires affinity for specific DNA sequences, termed hormone response elements. Interaction of the receptors with their corresponding hormone response elements results in the modulation of specific gene expression, through which the physiological effects of steroid hormones are mediated.

Early studies demonstrated that interaction of the steroid hormone receptor with the hormone response element renders a linked heterologous gene hormonally responsive (7, 8). Although the precise mechanism underlying this response has not been defined, it is clear that the hormone effect is manifest as increased transcription (9, 10). Subsequent investigations demonstrate that interaction of the receptor with its response element is only part of the physiological process of steroid hormone action. The promoters of known hormonally regulated genes are complex, with binding sites for transcription factors in addition to the steroid hormone receptor, indicating that other transcriptional regulatory elements are also involved in mediating the response to hormone (11-15). For example, the glucocorticoid-inducible mouse mammary tumor virus (MMTV)\(^1\) long terminal repeat and the estrogen-inducible vitellogenin gene promoter both contain a binding site for the transcription factor nuclear factor 1 (NF1) (16, 17), and mutations of this site influence the ability of these promoters to respond to hormone. Similarly, the promoters for two other glucocorticoid-inducible genes, tyrosine aminotransferase and tryptophan oxygenase, each contains a binding site, a CACCC element, for another transcription factor adjacent to the glucocorticoid response element (GRE) (18-20). Removal of this sequence also interferes with glucocorticoid induction of transcription of these genes. The steroid-inducible ovalbumin gene promoter contains binding sites for both the COUP transcription factor (21) and NF1 (22), both of which are important for both in vivo and in vitro transcription of the gene. Cooperative interactions between steroid hormone receptors and other transcription factors may thus provide another step at which steroid receptor function may be regulated.

We have shown recently that transcriptional activation by steroid hormone receptors is inversely modulated by alterations in the intracellular concentration of pyridoxal 5'-phosphate, the physiologically active form of vitamin \(B_6\) (23, 24). We found reduced levels of hormone-mediated gene expression under conditions of elevated vitamin concentration and enhanced hormone responsiveness under conditions of vitamin deficiency. We have now sought to determine the mechanism through which the vitamin \(B_6\) concentration affects

\(^{*}\) This work was supported by National Institutes of Health Grant DK 32459. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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\(^{1}\) The abbreviations used are: MMTV, mouse mammary tumor virus; NF1, nuclear factor 1; GRE, glucocorticoid response element; CAT, chloramphenicol acetyltransferase; TF, transcription factor.

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transcriptional activation by steroid hormone receptors by examining the effect of intracellular vitamin concentration on receptor-induced gene expression mediated through both complex and simple promoters. Results from these studies demonstrate that the modulation of receptor-induced gene expression by vitamin B6 requires promoter elements in addition to the hormone response element. Cooperation or functional interaction between the hormone receptor and at least one other transcription factor, NF1, is essential for pyridoxal phosphate to influence transcriptional activation by steroid hormone receptors.

EXPERIMENTAL PROCEDURES

Materials—Acetyl-coenzyme A was from Pharmacia LKB Biotechnology Inc. Pyridoxine and 4-deoxypyridoxine were from Sigma. Dexamethasone and 17β-estradiol were from Steraloids. DNA-modifying enzymes were from Life Technologies, Inc. [3H]Chloramphenicol (40-60 μCi/mmol) was obtained from Du Pont-New England Nuclear. [35S]-S-ATP (600 Ci/mmol) was from Amersham Corp. [3H]Dexamethasone (46 Ci/mmol) was from Du Pont-New England Nuclear. Sequense was from U. S. Biochemical Corp. Other reagents were from Fishier Scientific or Sigma.

Recombinant Plasmids—The plasmid pE1bCAT (25), the generous gift of Dr. Pierre Chambon (31), was obtained from Dr. James Lillie. The plasmid pElbCAT, which directs production of the chimaeric GAL4Ela transcriptional activator, was also provided by Dr. James Lillie. The plasmid pGRE2GAL4CAT was obtained from Dr. Keith Yamamoto, contains the MMTV promoter, 5'-CTTCTTTTGGAATTTATCCAAA TCTTATGTT-3' (27), between the proximal GRE and the TATA box in pERECAT. pGRE2GAL4CAT was created by introducing the NF1 oligonucleotide between the vitellogenin fragment and the TATA box in pERECAT. pGRE2GAL4CAT was cloned from the MMTV promoter, 5'-CGGAGTACTGTCCTCCGCTCGAC-3' identified in the text and figure legends prior to determination of CAT activity.

Determination of CAT Activity—CAT activity from HeLa cells transfected with either pGMCS, pGRE2CAT, pGRE3CAT, pGRE2NFlCAT, pNFlGRE2CAT, pERE1CAT, or pERE2NFlCAT was determined as described previously (23, 24) using 500 μg of cell extract/reaction. 100 μg and 50 μg of cell extract were used in the assays from cells transfected with the GAL4-containing or pBLCAT2 constructs, respectively. CAT activity was quantitated by excising the appropriate area from the thin layer chromatography plate and quantitating [3H]chloramphenicol converted to an acetylated form is representative of the level of induced CAT activity.

Cytosol and Sucrose Density Gradient Preparation—HeLa S3 cells were grown as described (23). Eighteen hours prior to harvesting, pyridoxine was added to the medium of designated cells that were approximately 75-80% confluent. The cells were then harvested and incubated with 100 nM [3H]dexamethasone, and activated and non-activated cytoplasmic glucocorticoid receptors were prepared (32). The activated receptors were then incubated without or with a 1,200-base pair fragment of DNA, obtained from an SspI digest of pGRE2NFlCAT, containing the two GREs and the NF1 site. Aliquots of cytosol (0.27 ml) were layered onto 10-25% sucrose density gradients, centrifuged, fractionated, and quantitated as described (33).

RESULTS

Glucocorticoid-induced expression of reporter genes linked to the MMTV promoter is mediated at the level of initiation of gene transcription and is dependent on the interaction of the receptor with GREs contained in the MMTV promoter (7, 9, 10). To determine if alterations in glucocorticoid receptor binding to GRE sites in DNA represented the mechanism through which the vitamin B6 acted to influence glucocorticoid-induced gene expression, we constructed CAT reporter plasmids whose promoters were significantly less complex than the MMTV promoter used previously (23, 24). These plasmids, pGRE2CAT and pGRE3CAT, have promoters comprised of two and three copies, respectively, of the GRE consensus sequence derived from the glucocorticoid-inducible tyrosine aminotransferase gene (26). A binding site for the ubiquitous transcription factor TFIID is located immediately upstream of the CAT gene to direct accurate transcription initiation. When transfected into HeLa cells, both of the "minimal promoter"-driven vectors supported glucocorticoid-induced, receptor-mediated CAT gene expression. Table I shows that the magnitude of induction with these two vectors was quantitatively similar to that obtained with CAT gene expression and was induced through the complex MMTV promoter in pGMCS. The parent vector pE1bCAT, which does not contain receptor binding sites, was not responsive to glucocorticoid treatment. The similarity in hormone responsiveness through the three different promoters demonstrated that the glucocorticoid receptor was fully capable of binding to the GREs contained in the two minimal promoters and inducing transcription of the heterologous target gene despite

| Table I |
| --- |
| **Glucocorticoid-regulated gene expression from complex and simple promoters** |
| Plasmid | Control | Dexamethasone | Induction |
| --- | --- | --- | --- |
| pGMCS | 0.8 ± 0.27 | 15.6 ± 1.3 | 19.5 |
| pGRE2CAT | 1.14 ± 0.32 | 23.6 ± 7.8 | 20.9 |
| pGRE3CAT | 0.63 ± 0.2 | 11.9 ± 3.5 | 18.9 |
| pE1bCAT | <0.1 | <0.1 | --- |

HeLa cells were transfected with each of the plasmids as indicated. Forty-eight hours after transfection, the cells were exposed to 100 nM dexamethasone or vehicle for 8 h prior to determination of CAT activity. The data shown represent the average from at least four independent determinations from a minimum of two experiments.
the absence of binding sites for other ancillary transcription factors.

We then asked if the receptor-dependent transcription induced by each of the constructs was susceptible to modulation by vitamin B₆. After transfection of these three reporter vectors into HeLa cells, the intracellular pyridoxal phosphate concentration was either increased or reduced as described previously (23). The data in Fig. 1 show that treatment with dexamethasone in control medium results in an approximate 12-fold induction in CAT activity. Under vitamin conditions that approximately double the intracellular pyridoxal phosphate concentration, the level of glucocorticoid receptor-induced CAT gene expression through the complex MMTV promoter (pGMCS) was only 5-fold, a reduction to 41% of that observed with dexamethasone treatment in unaltered medium. Conversely, mild vitamin deficiency resulted in an approximate 46% enhancement, represented by the 27-fold induction, in the level of receptor-induced gene expression. In contrast, the level of receptor-induced gene expression derived from the vectors containing the simplified promoters, pGRE2CAT or pGRE3CAT, was not affected by these same alterations in vitamin concentration. These studies suggest that two functions of the glucocorticoid receptor, its capacity to interact with DNA in vivo, and its ability to activate transcription, were not affected by alterations in vitamin B₆ concentration. Interestingly, neither basal (glucocorticoid-independent) CAT expression nor constitutive expression, from transcription, were not affected by alterations in vitamin B₆ concentration.

These differences in vitamin effects on transcription mediated through the complex versus the simple promoters suggested that some additional step(s) must be involved in induction of gene expression from the complex promoter. Corwingley et al. (27) have demonstrated that in addition to the GREs, two other regions of the MMTV promoter are protected in whole cells as a result of exposure to glucocorticoid. These regions encompass binding sites for TFIID, the TATA sequence-binding protein (−41 to +1 relative to the transcription start site), and the transcription factor NF1 (−82 to −56).

Binding of proteins to these regions in vivo does not occur in the absence of glucocorticoid treatment and suggests that the receptor may be involved in recruitment of transcription factors. Thus we examined the possibility that the effect of vitamin B₆ concentration might be mediated at the level of receptor recruitment of, or cooperation with, other transcription factors. Since the data in Fig. 1 show that the level of gene expression from promoters containing the TFIID binding site in combination with the GREs (pGRE2CAT and pGRE3CAT) was not affected by changes in vitamin B₆ concentration we next considered that the transcriptional modulation could be mediated through NF1.

To this end, an oligonucleotide bearing the sequence to which NF1 binds, derived from the MMTV promoter (30), was inserted into the simple pGRE2CAT promoter between the proximal GRE and the TATA sequence to create pGRE2NF1CAT. The same oligonucleotide cloned upstream of the TATA sequence in the absence of the GREs, to generate pNFlCAT, permitted analysis of vitamin effects on the transcription factor NF1 alone. These vectors were individually transfected into HeLa cells, and the intracellular pyridoxal phosphate concentration was altered prior to treatment with dexamethasone. The data in Fig. 2 show that glucocorticoid treatment in unaltered medium results in an approximate 44-fold induction in the level of receptor-mediated CAT gene expression derived from the vector containing binding sites for both the glucocorticoid receptor and NF1, (pGRE2NF1CAT). However, this induction was decreased to 24.7-fold, a 44% reduction, under conditions of elevated intracellular vitamin concentration, and enhanced by 95%, to 87.2-fold, under vitamin deficiency. These data are analogous to the effects of vitamin concentration on gene expression induced through the complex MMTV promoter (Fig. 1, pGMCS), suggesting that the transcription factor NF1 may be important in the transcriptional modulatory response to vitamin B₆.

Interestingly, gene expression mediated by the NF1 sequence in the absence of the GREs, pNFlCAT, was unaffected by modulations in intracellular vitamin B₆ concentrations (Fig. 2, right panel). These data demonstrate that vitamin B₆ does not mediate its transcriptional modulatory effects through the NF1 protein alone.

**Fig. 1. Effect of vitamin B₆ concentration on the level of glucocorticoid-induced gene expression from complex and simple promoters.** HeLa cells were transfected with pGMCS, pBLCAT2, pGRE2CAT, or pGRE3CAT as indicated. Sixteen hours after transfection, the culture media were supplemented with either pyridoxine (PYR), 4-deoxypyridoxine (4-DEOXY), or left unaltered (CONTROL). After incubation under these conditions for 48 h, cells were exposed to 100 nM dexamethasone (DEX) or vehicle control (CON) for 8 h and then harvested and assayed for CAT activity. The data shown are from one experiment, representative of at least three independent experiments. TK, thymidine kinase.
FIG. 2. Transcription factor NF1 is important for modulation of glucocorticoid-induced gene expression. HeLa cells were transfected with (left panel) pGRE2NF1CAT or (right panel) pNF1CAT. Sixteen hours after transfection, the culture media were supplemented with either pyridoxine (PYR), 4-deoxypyridoxine (4-DEOXY), or left unaltered (CONTROL). After 48 h under these conditions, cells were exposed to 100 nM dexamethasone (DEX) or vehicle control (CON) for 8 h and then harvested and assayed for CAT activity. The data shown are from one experiment, representative of at least three independent experiments.

Previous studies conducted under in vitro conditions indicated that pyridoxal phosphate could influence both glucocorticoid receptor structure and binding to DNA (34 and references therein). Therefore, we examined glucocorticoid receptor activation and DNA binding under the conditions in which physiological changes in vitamin B6 concentration occur within cells. The upper panel in Fig. 3 shows that in response to an increase in intracellular concentration of pyridoxal phosphate, there is no influence on the sedimentation profiles of both activated and nonactivated glucocorticoid receptors. In the upper panel the faster-sedimenting peak (fractions 10–15) represents unactivated glucocorticoid receptors, and fractions 15–20 reflect the population of activated glucocorticoid receptors. These receptor preparations were incubated with a DNA fragment derived from pGRE2NF1CAT which encompasses the GRE dimer and NF1 binding site. The lower panel of Fig. 3 shows that equivalent amounts of DNA-glucocorticoid receptor complexes (fractions 2–9) were formed with both preparations. These data indicate that changes in vitamin B6 concentration in vivo do not alter glucocorticoid receptor activation state or its capacity to interact with DNA.

Since activation of the estrogen receptor is also modulated by changes in intracellular vitamin B6 concentration (28) we next determined if the modulatory effect of the vitamin on these steroid-responsive promoters also exhibited a dependence on NF1. Because the estrogen receptor activates transcription through a different DNA sequence, the estrogen response element, we examined the effect of vitamin concentration on estrogen-induced gene expression mediated through an estrogen-responsive promoter either containing (pERENF1CAT) or lacking (pERECAF) an NF1 binding site in its natural context (35). To assess estrogen inducibility, we transfected these vectors into HeLa cells which are devoid of estrogen receptors but have been shown by others and us (24, 31) to be capable of estrogen-induced transcriptional responses after cotransfection with estrogen receptor expression vectors. After exposure of transfected cells to pyridoxine or 4-deoxypyridoxine to alter intracellular pyridoxal phosphate concentration, cells were treated with 17β-estradiol prior to CAT activity determination. Interestingly, the level of estrogen-induced CAT activity derived from pERECAF was not affected by alternations in pyridoxal phosphate concentration, as shown in Fig. 4, left panel. In contrast, however, the level of estrogen-induced CAT activity derived from pERENF1CAT was significantly modulated by alterations in vitamin concentration. Under conditions of elevated vitamin concentration, only an 8.3-fold induction in CAT activity was observed, which reflects a 41% reduction in response. Vitamin deficiency resulted in a 21.6-fold induction in the lower panel the faster-sedimenting peak (fractions 10–15) represents unactivated glucocorticoid receptors, and fractions 15–20 reflect the population of activated glucocorticoid receptors. These receptor preparations were incubated with a DNA fragment derived from pGRE2NF1CAT which encompasses the GRE dimer and NF1 binding site. The lower panel of Fig. 3 shows that equivalent amounts of DNA-glucocorticoid receptor complexes (fractions 2–9) were formed with both preparations. These data indicate that changes in vitamin B6 concentration in vivo do not alter glucocorticoid receptor activation state or its capacity to interact with DNA.

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3 J. A. Cidlowski, unpublished data.
or a 54% enhancement over that observed in control medium. Basal CAT activity, in the estrogen-unstimulated cells, was not affected by these changes in vitamin B₆ concentration. These observations suggest that the requirement for NF1 in modulation of steroid-induced gene expression by vitamin B₆ was not restricted to glucocorticoid-inducible promoters.

These experiments with pGRE2NF1CAT and pERENF1CAT (which have different spacing between the GRE and NF1 binding) also suggest that the proximity or position of the receptor binding sites and the NF1 binding site may not be crucial for modulation by vitamin of receptor-mediated gene expression. To investigate further the role of transcription factor binding site orientation and spacing, we reversed the positions of the glucocorticoid receptor and NF1 binding sites, to generate pNF1GRE2CAT, and then performed experiments to determine if the level of glucocorticoid-induced gene expression would be affected by alterations in vitamin B₆ concentration. As shown in the left panel of Fig. 5, when the NF1 binding site was on the 5’ side of the GREs, a 56-fold induction in CAT activity was observed in controls. The level of glucocorticoid-induced CAT gene expression was reduced by 57% under conditions of elevated vitamin concentration, where only 23.7-fold induction was observed, and enhanced by 190% (164-fold induction) in vitamin deficiency. These results were analogous to those in which the NF1 binding site was located on the 3’ side of the GREs (pGRE2NF1CAT, right panel), where hormone-induced CAT activity was reduced by 53% in vitamin elevation and enhanced by 130% in vitamin deficiency. We conclude from these observations, and those with the differently spaced estrogen-responsive promoters, that vitamin B₆ modulation of hormone-induced gene expression does not depend on strictly conserved spacing between or orientation of the binding sites for the receptor and NF1. It was not especially surprising that orientation was not crucial since other hormone-regulated promoters have transcription factor binding sites positioned on both the 5’ and 3’ sides of the receptor binding sites, and spacing between the hormone response elements and the other transcription factor binding sites is tremendously variable (16–20, 22, 27, 35–38).

Since several transcription factors have been implicated in steroid hormone action, we wished to determine if the modulatory effects of vitamin B₆ were observed under conditions in which the glucocorticoid receptor was allowed to interact with another transcription factor. Thus we constructed a reporter vector whose promoter contained binding sites for the glucocorticoid receptor and the Ela which is not endogenous to HeLa cells but can cooperate with a number of transcription factors (39–41). The Ela protein has no DNA binding domain, but it can be converted into a DNA-binding protein by linking its transactivation domain to the DNA binding domain of a known DNA-binding protein (25). In our experiments we used the well characterized chimaeric construct in which the transcriptional activation domain of the Ela protein is fused to the DNA binding region of the yeast GAL4 protein (25). This chimaeric protein, (GAL4Ela) efficiently activates transcription in mammalian cells when bound to promoters containing the GAL4 binding site (25). We replaced the NF1 binding site in pGRE2NF1CAT with an oligonucleotide comprising the GAL4 binding site to generate pGRE2GAL4CAT. This plasmid, when transfected alone into HeLa cells, responds to glucocorticoid treatment with a 25.4-fold induction in CAT activity, as shown in Table II (CON versus DEX, control medium). Moreover, in the absence of the GAL4Ela transactivator, neither basal nor glucocorticoid-induced CAT activity from pGRE2GAL4CAT was affected by alterations in intracellular vitamin B₆. Thus simply altering the location of the receptor binding sites within the promoter, by insertion of this oligonucleotide, was not sufficient to restore transcriptional modulation by vitamin.

When HeLa cells were cotransfected with the CAT reporter containing the GAL4 promoter element, pGRE2GAL4CAT, and the expression vector which directs expression of the GAL4/Ela chimaeric transactivator protein, pGAL4Ela, we observed that the level of basal (hormone-free) transcription was elevated in comparison with cells not transfected with the transactivator (Table II). Basal CAT activity was increased from 0.41 to 5.75%. This 13-fold increase in basal activity is presumably derived from enhanced transcriptional activity from the Ela transactivator. This finding demonstrated that this chimaeric transactivator protein was func-

![Fig. 4. Transcription factor NF1 is important for modulation of estrogen-induced gene expression. HeLa cells were transfected with (left panel) pERE CAT and pHEO or (right panel) pERENF1CAT and pHEO. Sixteen hours after transfection, the culture media were supplemented with either pyridoxine (PYR), 4-deoxypyridoxine (4-DEOXY), or left unaltered (CONTROL). After 48 h under these conditions, cells were exposed to 5 nM 17β-estradiol (E₂) or vehicle control (CON) for 24 h and then harvested and assayed for CAT activity. The data shown are from one experiment, representative of at least three independent experiments. ERE, estrogen response element.](image-url)
FIG. 5. Position-independent effects of NF1 on modulation of glucocorticoid-induced gene expression. HeLa cells were transfected with (left panel) pNF1GRE2CAT or (right panel) pGRE2NF1CAT. Sixteen hours after transfection, the cell culture media were supplemented with pyridoxine (PYR), 4-deoxypyridoxine (4-DEOXY), or left unaltered (CONTROL). After 48 h under these conditions, cells were exposed to 100 nM dexamethasone (DEX) or vehicle control (CON) for 8 h and then harvested and assayed for CAT activity. The data shown are from one experiment, representative of at least three independent experiments.

**Table II**

**Influence of vitamin B₆ on glucocorticoid-regulated gene expression from synthetic promoters**

The ElaGAL₄ transcriptional activator cannot replace NF1 in restoring modulation of glucocorticoid-induced gene expression. HeLa cells were transfected with pGRE2GAL4CAT or pGRE2GAL4-CAT and pElaGAL₄. Sixteen hours after transfection, the cell culture media were supplemented with pyridoxine (PYR), 4-deoxypyridoxine (4-DEOXY), or left unaltered (CON). After 48 h under these conditions, cells were exposed to 100 nM dexamethasone (+DEX) or vehicle control (+CON) for 8 h prior to CAT activity assay. The data shown are from one experiment, reflective of three independent determinations.

| Condition | Plasmids | pGRE2GAL4CAT* | pElaGAL₄* |
|-----------|----------|---------------|-----------|
|           | Induction| Induction     |
| CON       |          |               |
| −DEX      | 0.41*    | 5.75          |
| +DEX      | 10.40    | 25.4          | 33.90     | 5.9 |
| PYR       |          |               |
| −DEX      | 0.47     | 5.30          |
| +DEX      | 12.00    | 25.5          | 34.50     | 6.5 |
| 4-DEOXY   |          |               |
| −DEX      | 0.37     | 5.50          |
| +DEX      | 10.20    | 27.6          | 33.80     | 6.1 |

*CAT activity is expressed as percent substrate conversion.

**DISCUSSION**

The level of glucocorticoid-induced gene expression through the complex MMTV promoter is influenced by intracellular pyridoxal phosphate concentration. Using reporter vectors whose promoters are simple, containing only glucocorticoid receptor binding sites and the ubiquitous TFIID binding sequence, we demonstrate that the glucocorticoid receptor can activate transcription efficiently in the absence of other transcription factor binding sites. However, the level of glucocorticoid-induced gene expression derived from the simplified promoters was not influenced by alterations in pyridoxal phosphate. This was in sharp contrast to the effects of vitamin B₆ on receptor-induced gene expression through the MMTV promoter. Thus, binding of receptor to DNA and transcriptional activation account for only part of the physiological process of glucocorticoid hormone action, and other steps, which do not occur at the simplified promoters, must be involved in receptor-mediated transcription from the MMTV promoter. The data presented here suggest that the effect of vitamin B₆ on receptor-induced gene expression is mediated at these other steps. The importance of additional or ancillary transcription factors in mediating hormone responsive gene expression has been clearly shown (16–20, 22, 27, 35–38). Our experiments demonstrated the importance of the transcription factor NF1 in mediating the effects of vitamin B₆ on receptor-induced gene expression. These data support the idea described above that steroid receptors alone may generate only part of the overall process of steroid hormone action. It is important to note although that our limited analysis shows no strict position or orientation requirements for NF1/GR cooperation, further studies are necessary to assess the issue of spacing.

It will also be important to elucidate the role of other transcription factors in modulation of glucocorticoid receptor-induced gene expression by alterations in intracellular vitamin B₆ concentration. For example, Bruggemeier *et al.* (37) suggest that the transcription factor OTF1 may be involved in glucocorticoid-mediated transcription through the MMTV promoter. Further, Zorbas *et al.* (42) have reported recently that a protein component of HeLa cell nuclear extracts, in addition to NF1, also interacts with a DNA sequence from
the α-globin gene promoter which is homologous in sequence to the oligonucleotide encompassing the NFI binding site which we have used here. They have not identified this protein component, and it is not known if this factor also binds to the MMTV promoter sequence or interacts restrictively with the globin promoter. Nevertheless, these observations support the interesting possibility that other transcription factors, and not exclusively NFI, might be involved in modulation by vitamin B₆ of hormone action.

Zaret and Yamamoto (43) have suggested that glucocorticoid treatment of cells induces receptor-mediated alterations in the structure of MMTV DNA. Subsequent work of Cordin-
gley et al. (27) demonstrated that as a result of hormone treatment and glucocorticoid receptor binding, regions of MMTV DNA encompassing binding sites for other transcription factors were protected from exonuclease digestion, indicating the receptor-induced recruitment of additional transcription factors onto target gene promoters. Thus, pyridoxal phosphate may act to influence the ability of the receptor to alter chromatin structure and allow access of other transcription factors. Alternately, or perhaps additionally, vitamin B₆ may influence receptor-induced transcription factor assembly onto target gene promoters.

Another speculative mechanism through which vitamin B₆ may act is a direct interaction of the glucocorticoid receptor with the transcription factor AP1, the FOS-JUN complex, at the vitellogenin gene promoter is mediated through the contact and glucocorticoid receptor binding, regions of MMTV DNA encompassing binding sites for other transcription factors were protected from exonuclease digestion, indicating the receptor-induced recruitment of additional transcription factors onto the MMTV promoter. Thus, pyridoxal phosphate may act to influence the ability of the receptor to alter chromatin structure and allow access of other transcription factors. Alternately, or perhaps additionally, vitamin B₆ may influence receptor-induced transcription factor assembly onto target gene promoters.

through a dietary compound may represent a conserved mechanism for regulation of steroid hormone action.

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