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Vitamin B_6 Modulates Transcriptional Activation by Multiple Members of the Steroid Hormone Receptor Superfamily*

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Recent studies have shown that vitamin B_6 modulates transcriptional activation by the human glucocorticoid receptor in HeLa S_3 cells. We have now examined the possibility that vitamin B_6 might similarly influence transcriptional activation by the glucocorticoid receptor in other cell types, as well as gene expression mediated by other members of the steroid hormone receptor superfamily. We show that elevated vitamin B_6 concentrations suppress by 40–65% the level of transcription mediated through the endogenous murine L cell glucocorticoid receptor, as well as the human receptor transfected into E8.2 and T47D cells. In contrast, glucocorticoid receptor-mediated transcription was enhanced 60–110% in mild vitamin deficiency. The level of hormone-independent constitutive gene expression was not affected by these same alterations in vitamin B_6 concentration. These studies indicated that the transcriptional modulatory effects of the vitamin were neither restricted to specific cell types nor limited to the human form of the glucocorticoid receptor. We next determined if hormone-induced transcription by several other steroid receptors (androgen, progesterone, and estrogen receptors) was analogously affected by alterations in vitamin B_6 concentration. Analysis of gene expression mediated through the mouse mammary tumor virus promoter revealed that transcriptional activation of both the androgen and progesterone receptors was reduced by 35–40% under conditions of elevated vitamin B_6 and enhanced by 60–90% in deficiency, again under conditions where constitutive expression was unaffected. Using a different promoter, the estrogen-regulated vitellogenin promoter, we found that transcriptional activation of the estrogen receptor was simlarly affected. Estrogen-induced gene expression was reduced by 30% under conditions of elevated intracellular vitamin B_6 and enhanced by 85% in vitamin deficiency. Thus, vitamin B_6 modulates transcriptional activation by multiple classes of steroid hormone receptors. The similarities in vitamin B_6 effects on transcription mediated through different promoters, the mouse mammary tumor virus and vitellogenin promoters, suggest that this vitamin may modulate the expression of a diverse array of hormonally responsive genes. These observations together support the hypothesis that vitamin B_6 represents a physiological modulator of steroid hormone action.

The steroid hormone receptors belong to a superfamily of transcription factors that regulate the expression of specific target genes, and, in so doing, have profound effects on many physiological processes, including growth, development, homeostasis, reproduction, and behavior (1). Despite the diverse array of physiological events modulated by different members of this superfamily, receptors for the steroid hormones glucocorticoid, estrogen, androgen, and progesterone exhibit many structural and functional similarities. For example, early studies employing limited proteolytic digestion of steroid receptor preparations first suggested that these molecules are structurally similar (2); subsequent cDNA cloning and DNA sequence analysis has demonstrated not only that the steroid hormone receptors share a common modular organizalion, with specific domains encompassing the ligand and DNA-binding domains, but also that considerable sequence homology exists among the receptor molecules (3–6). These receptors are also functionally analogous in mediating their biological effects, and they all proceed through common mechanistic steps, including binding of steroid ligand, recognition of specific DNA sequences, and modulation of target gene expression (5, 7–9).

The capacity of target cells or tissues to respond to hormonal stimulation is influenced by a variety of conditions, including the intracellular concentration of receptors (10, 11), the state of differentiation or development of the cell or tissue (12, 13), and the stage of the cell cycle (14). Hormone responsiveness can also be affected by other factors, for example, serum components (15), mitogenic agents (16), intracellular second messengers (15, 17), and nutritional compounds, such as butyric acid (18–20) and the vitamin A derivative retinoic acid (21–23). In addition, data from several laboratories has suggested that another nutritional compound, vitamin B_6, may influence steroid hormone action. In early studies with the progesterone receptor, pyridoxal-5′-phosphate, the physiologically active form of vitamin B_6, was observed to inhibit the interaction of this receptor with ATP-Sepharose (24). With the caveat that binding to ATP-Sepharose only mimics binding to DNA, this provocative observation first suggested a link between vitamin B_6 and steroid hormone receptors. Subsequent studies with the progesterone, estrogen, androgen, and glucocorticoid receptors demonstrated that pyridoxal phosphate influences not only the *in vitro* DNA binding capacity (25–29), but also other common properties of steroid hormone receptors, including molecular conformation or subunit complexity (26, 27, 30) and subcellular localization (31–35). While these effects suggested that this vitamin influences

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the structure and function of steroid hormone receptors, it was not clear if these effects were representative of *in vivo* physiological actions of the vitamin.

Recent work from our laboratory has demonstrated that the primary biological manifestation of glucocorticoid hormone action, the regulation of target gene expression, is affected by modulation of intracellular pyridoxal phosphate concentration (36). Specifically, the level of glucocorticoid-induced receptor-mediated gene expression is reduced under conditions of vitamin elevation, and enhanced in mild vitamin deficiency, indicating that vitamin B_6 acts physiologically to modulate glucocorticoid hormone action. The structural and functional similarities between the glucocorticoid and other steroid hormone receptors, together with the earlier reports of analogous effects of vitamin B_6 on other steroid hormone receptors, suggested that the vitamin may modulate transcriptional activation by other steroid hormone receptors. We have therefore examined the effect of pyridoxal phosphate on the regulation of target gene expression by the estrogen, androgen, and progesterone receptors. Results from these investigations demonstrated that alterations in pyridoxal phosphate concentration influence the capacity of these different receptors to induce expression of target genes under physiological conditions. In addition, data presented here show that the modulatory effects of the vitamin on transcriptional activation by the glucocorticoid receptor are not limited to the human form of the glucocorticoid receptor, nor are the modulatory effects mediated through a factor restricted to a specific cell type. These observations together support the hypothesis that pyridoxal phosphate acts specifically to regulate the biological activity of several members of the steroid receptor superfamily. We propose that vitamin B_6 is a physiological modulator of steroid hormone action.

MATERIALS AND METHODS

Reagents—Acetyl coenzyme A was from Pharmacia LKB Biotechnology. Pyridoxine, 4-deoxypyridoxine, and glutamine were from Sigma. Dexamethasone, progesterone, and 17β-estradiol were from Steraloids. R1881 and [^{14}C]chloramphenicol (40–60 mCi/mmol) were from DuPont-New England Nuclear. Other reagents were from Fisher Scientific.

Recombinant Plasmids—Plasmid pGMCS (37, 38), containing the chloramphenicol acetyltransferase (CAT)[1] coding sequence downstream of the mouse mammary tumor virus (MMTV) promoter and glucocorticoid regulatory elements, was provided by Drs. Roger Miesfeld and Keith Yamamoto (UCSF). pBLCAT2 (39), obtained from Drs. Bruno Luckow and Gunther Schutz (Institute for Tumor and Cell Biology, Heidelberg, Germany), contains the thymidine kinase promoter driving expression of the CAT coding sequence. pHEO (40), the human estrogen receptor expression vector, was provided by Dr. Pierre Chambon (Institut de Chimie Biologique). The human glucocorticoid receptor expression vector pRShGR (41) was provided by Dr. Ronald Evans (Salk Institute). The estrogen-responsive reporter plasmid pERENF1CAT was constructed by inserting the −331/−87-base pair fragment from the vitellogenin A2 gene promoter (42) and an oligonucleotide comprising a binding site for the transcription factor NF1 (derived from the MMTV long terminal repeat sequence (43)) into the vector pE1bCAT (44); pE1bCAT, the gift of Dr. James Lillie (Harvard University), contains an oligonucleotide bearing the adenovirus E1b TATA sequence upstream of the CAT coding sequence.

Cell Culture—HeLa S_3 cells were grown as monolayer cultures as previously described (36). E8.2 cells (45), the generous gift of Dr. Paul Housley (University of South Carolina Medical School), were cultured in Dulbecco's modified essential medium supplemented with 3% calf serum and 2 mM glutamine. T47D cells (46), kindly provided by Drs. Kathryn Horwitz (University of Colorado Health Sciences Center) and Mark Graham (University of North Carolina, Chapel Hill), were cultured in Eagle's minimal essential medium supplemented with 5% fetal calf serum, 5 ng/ml insulin, and 2 mM glutamine. Murine L929 fibroblasts (47), generously provided by Dr. Daniel Linzer (Northwestern University), were cultured in Eagle's minimal essential medium supplemented with 10% calf serum and 2 mM glutamine. Pyridoxine and 4-deoxypyridoxine were dissolved in water and diluted into the culture media to the indicated final concentrations. Dexamethasone was dissolved in water; progesterone, 17β-estradiol, and R1881 were dissolved in ethanol. Steroids were added to culture media at the concentrations indicated in the figure legends.

Cell Transfections—HeLa cells were transfected by calcium phosphate precipitation as previously described (36), using 5 μg of CAT reporter plasmid per 100-mm culture dish. For estrogen receptor studies, HeLa cells were co-transfected with 10 μg of pHEO/dish. The E8.2, T47D, and L cells were identically transfected using 5 μg of CAT reporter plasmid/100-mm dish. Where indicated, E8.2 and T47D cells were co-transfected with 0.5 μg of pRShGR/100-mm dish. Sixteen hours after transfection, the cell culture media were supplemented with pyridoxine, 4-deoxypyridoxine, or vehicle (H_2O) to modulate intracellular pyridoxal phosphate concentration. After incubation for 48 h under these conditions, cells were exposed to the appropriate hormone under conditions described in the figure legends prior to determination of CAT activity.

Determination of CAT Activity—CAT activity from transfected HeLa cells was determined as previously described (36), using 500 μg of cell extract/reaction. CAT activity from transfected E8.2 cells was identically determined, using 200 μg of cell extract in a 5-h reaction. Transfected T47D cells were analyzed for CAT activity with 300 μg of cell extract in a 6-h reaction. CAT activity from transfected L cells was determined with 100 μg of cell extract in a 2-h reaction. CAT activity was quantitated by excising the appropriate area from the thin layer chromatography plate and quantitating ^{14}C by scintillation counting; the portion of total [^{14}C]chloramphenicol converted to an acetylated form is representative of the level of induced CAT activity. The level of CAT activity measured in hormone-treated cells grown in the control or unaltered medium is assigned a value of 1.0; the amount of CAT activity measured in hormone-treated cells grown in the presence of either pyridoxine or 4-deoxypyridoxine is expressed as a fraction of the amount of CAT activity from cells grown in unaltered medium, *i.e.* as a fraction of 1.0. For experiments in which multiple cell types were used, CAT activity is determined and expressed for each individual cell type and comparisons are not made across different cell types. The level of CAT activity detected in hormone-free cells was barely detectable (less than 1% conversion) and not influenced by different vitamin states; thus, these values are not shown in the figures.

RESULTS

We previously demonstrated that the capacity of the endogenous human glucocorticoid receptor in HeLa S_3 cells to mediate gene expression through the glucocorticoid-responsive MMTV promoter is affected by intracellular pyridoxal phosphate concentration (36). Specifically, supplementation of the culture medium with pyridoxine, which we (36) and others (32, 33, 48–50) have shown elevates intracellular pyridoxal phosphate concentration, results in suppression of glucocorticoid receptor-induced gene expression. In contrast, modest intracellular pyridoxal phosphate deficiency, achieved by treatment of cells with the pyridoxal phosphate synthesis inhibitor 4-deoxypyridoxine (51), produces an enhancement in the level of receptor-induced target gene expression. These effects are observed under conditions where glucocorticoid receptor number and ligand binding capacity are not affected, and the level of glucocorticoid-independent constitutive gene expression is unaltered (36). To determine if the modulation of glucocorticoid-stimulated gene expression by intracellular pyridoxal phosphate concentration is restricted to the human form of glucocorticoid receptor, we examined the effects of pyridoxine or 4-deoxypyridoxine supplementation on the capacity of the murine glucocorticoid receptor to induce target gene expression. For these studies, we used murine L929 fibroblasts transfected with the MMTV-driven glucocorticoid-responsive CAT reporter vector pGMCS (37, 38).

[1] The abbreviations used are: CAT, chloramphenicol acetyltransferase; MMTV, mouse mammary tumor virus.

In parallel transfections, cells were transfected with the hormone-independent constitutive CAT reporter vector pBLCAT2 (39) in order to distinguish between specific effects of intracellular pyridoxal phosphate concentration on the glucocorticoid receptor and potential effects of the vitamin on other transcription factors involved in induction of reporter gene expression. Results of these studies are presented in Fig. 1. As described above, transcriptional activation by the endogenous human glucocorticoid receptor in HeLa cells was modulated by alterations in intracellular pyridoxal phosphate concentration, with a 45% decrease in the level of receptor-induced gene expression under conditions of elevated vitamin concentration, and an approximate 2-fold increase in the opposite state of vitamin deficiency. Constitutive expression from the glucocorticoid nonresponsive plasmid pBLCAT2 was unaffected by alterations in pyridoxal phosphate concentration.

The data shown in the right portion of the Figure demonstrate that transcriptional activation by the endogenous murine glucocorticoid receptor in L cells was similarly modulated, with a 66% decrease in the level of receptor-induced CAT activity in the presence of pyridoxine and a 2-fold increase after exposure to the synthesis inhibitor 4-deoxypyridoxine. As shown in the lower portion of the Figure, these effects were observed under conditions in which glucocorticoid-independent constitutive gene expression remained unaffected. Thus, modulation of glucocorticoid receptor-mediated gene expression by pyridoxal phosphate concentration was neither restricted to the human form of the glucocorticoid receptor nor limited to HeLa cells.

These observations suggested that transcriptional modulation by vitamin B_6 may be a general property of glucocorticoid receptor-mediated gene expression, and we next wished to examine this possibility. Many properties of transfected glucocorticoid receptor, including nuclear translocation and subcellular localization, specific DNA binding, transcriptional activation, and ligand-induced down-regulation are identical

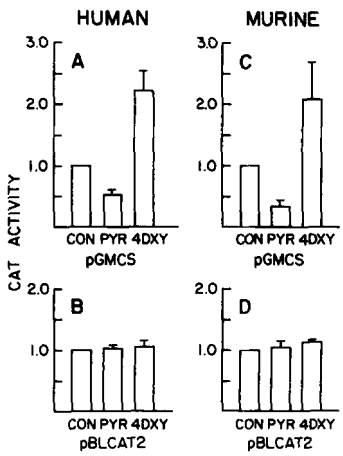

FIG. 1. **Vitamin B_6 similarly influences the transcriptional activation capacity of both the human and murine glucocorticoid receptors.** *Left*, HeLa cells were transfected with either pGMCS (*A*) or pBLCAT2 (*B*), then cultured in unaltered medium (*CON*), medium supplemented with 1 mM pyridoxine (*PYR*), or medium supplemented with 5 mM 4-deoxypyridoxine (*4DXY*) prior to an 8-h exposure to 100 nM dexamethasone or vehicle. Cells transfected with pBLCAT2 were exposed to vehicle only. *Right*, murine L929 fibroblasts (L cells) were transfected with either pGMCS (*C*) or pBLCAT2 (*D*) and treated as above, with 2 mM pyridoxine or 1 mM 4-deoxypyridoxine. Determination and quantitation of CAT activity was as described under "Materials and Methods." The data shown are representative of at least three individual transfection experiments.

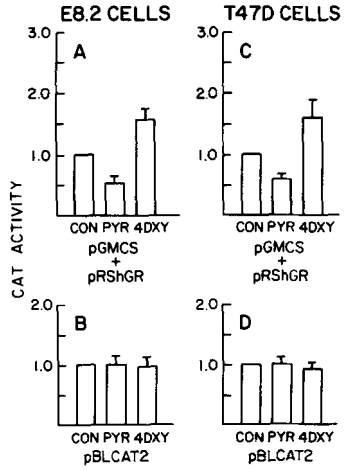

FIG. 2. **Vitamin B_6 influences the transcriptional activation capacity of the glucocorticoid receptor expressed in heterologous cell types.** *Left*, E8.2 cells were transfected with either pGMCS and pRShGR (*A*) or pBLCAT2 (*B*). After transfection, cells were cultured in unaltered medium (*CON*), medium supplemented with 2 mM pyridoxine (*PYR*), or medium supplemented with 3 mM 4-deoxypyridoxine (*4DXY*) for 48 h prior to an 8-h exposure to 100 μM dexamethasone or vehicle. *Right*, T47D cells were transfected with either pGMCS and pRShGR (*A*) or pBLCAT2 (*B*), then treated as described above, with 3 mM pyridoxine or 2 mM 4-deoxypyridoxine. Determination and quantitation of CAT activity was as described under "Materials and Methods." The data shown are representative of at least three individual transfection experiments.

to those of the endogenous receptor (10, 52–58), and it is generally held that effects detected with transfected receptors are reflective of events which occur with the endogenous ones. Thus, we examined the effect of altered vitamin status on transcriptional activation by the glucocorticoid receptor transfected into two different cell lines, E8.2 and T47D cells. E8.2 cells are derived from the murine L929 fibroblast line and are devoid of glucocorticoid receptors (45). The T47D cell line is derived from a human breast cancer, and contains insufficient levels of glucocorticoid receptors to induce gene expression in response to dexamethasone treatment.[2] However, both of these cell types express functional receptors following transfection with a glucocorticoid receptor expression vector (45).[2] After co-transfection with both the glucocorticoid-responsive CAT reporter plasmid pGMCS and the human glucocorticoid receptor expression vector pRShGR, the cells were cultured in the presence of pyridoxine or 4-deoxypyridoxine, to elevate or reduce, respectively, intracellular pyridoxal phosphate concentration, prior to stimulation with dexamethasone. The results from these studies, shown in Fig. 2, demonstrated that transcriptional activation mediated by the glucocorticoid receptor expressed in these different cell types was reduced under conditions of elevated intracellular vitamin concentration, with a 50% decrease observed in E8.2 cells and a 40% decrease measured in T47D cells. In contrast, under the opposite state of vitamin deficiency, the level of glucocorticoid receptor-induced gene expression was enhanced by approximately 60% in each cell type. As shown in the lower panels, glucocorticoid-independent constitutive expression remained unaffected under these conditions of altered vitamin concentrations. Thus, the modulatory effect of pyridoxal phosphate on transcriptional activation by the glucocorticoid receptor was neither mediated through a factor restricted to a specific cell type, nor limited to the endogenously expressed receptor.

[2] V. E. Allgood, unpublished data.

The results in Figs. 1 and 2 clearly demonstrate that the biological function of the glucocorticoid receptor, transcriptional activation, was affected by vitamin B_6 concentration. Since there is direct evidence that vitamin B_6 has similar effects on several physical and structural properties of the glucocorticoid and other hormone receptors (reviewed in Refs. 59 and 60), and since a high degree of sequence homology exists among the receptors (61), we next sought to determine if exposure to agents which alter intracellular pyridoxal phosphate concentration might affect transcriptional activation by other steroid hormone receptors in a manner analogous to that which we have seen with the glucocorticoid receptor. We first examined the effect of pyridoxine and 4-deoxypyridoxine supplementation on induction of reporter gene expression by the androgen and progesterone receptors. Extensive characterization of DNA binding and transcriptional activation has demonstrated that the glucocorticoid, androgen, and progesterone receptors can act through the same hormone response element to mediate transcription of target genes (62, 62). In fact, these three receptors can each bind to and activate transcription from the hormone response element present in the MMTV promoter, first identified as being responsive to the glucocorticoid receptor (64). Therefore, we have utilized the same MMTV-driven glucocorticoid-responsive plasmid pGMCS for analysis of transcriptional activation by these three different receptors. Use of this common reporter plasmid, however, necessitated the use of cells which expressed each receptor individually in order to unequivocally establish effects of pyridoxal phosphate on induction of transcription specifically by the androgen or progesterone receptors. Thus, we used E8.2 cells, which contain endogenous androgen receptors but neither glucocorticoid nor progesterone receptors (45), to allow investigation of vitamin effects on the androgen receptor. In companion studies, we used T47D cells to examine transcriptional activation by the progesterone receptor; these cells express significant levels of progesterone receptors (46) but contain insufficient levels of either glucocorticoid or androgen receptors to activate transcription from a transfected MMTV-driven CAT reporter construct.[2] Both cell types were transfected with the MMTV-driven CAT reporter vector pGMCS, and exposed to the same pyridoxine or 4-deoxypyridoxine conditions demonstrated in Fig. 2 to modulate glucocorticoid receptor-mediated transcriptional activation but not hormone-independent constitutive expression. After subsequent treatment with the appropriate hormone, induction of CAT gene expression was determined. Results of these experiments are presented in Fig. 3. It is apparent that under the same vitamin conditions which resulted in modulation of transcriptional activation by the glucocorticoid receptor, both androgen receptor-mediated and progesterone receptor-mediated gene expression were analogously affected. The level of androgen receptor-mediated gene expression was suppressed by 28% following pyridoxine treatment, and enhanced by 57% after exposure to the synthesis inhibitor 4-deoxypyridoxine. Similarly, progesterone receptor-mediated gene expression was reduced by 41% following exposure to pyridoxine, and increased by 90% after growth in media supplemented with 4-deoxypyridoxine. The magnitude of these effects were quantitatively similar to those observed with both endogenous and transfected glucocorticoid receptors (Figs. 1 and 2). Thus, the effects of pyridoxal phosphate on steroid receptor-mediated transcriptional activation were not restricted to the glucocorticoid receptor, but extended to other members of the steroid hormone receptor family, including at least the androgen and progesterone receptors.

Because the glucocorticoid, progesterone, and androgen receptors activate transcription as a result of interaction with the same DNA sequence (62, 63), these studies did not eliminate the possibility that the effects of vitamin B_6 might be restricted to specific MMTV sequences present in the reporter plasmid. Therefore, we next sought to determine if the effects of pyridoxal phosphate on transcriptional activation were restricted to or mediated specifically through the hormonally regulated MMTV promoter. To this end, we examined transcriptional activation mediated by a different steroid receptor, the estrogen receptor, which acts through a distinct DNA sequence (65, 66). This sequence, the estrogen-response element, is associated with estrogen-regulated genes and mediates effects of the estrogen receptor specifically (42). In particular, we have used a well-characterized fragment from the estrogen-inducible vitellogenin gene promoter (42) to direct expression of the reporter CAT gene.

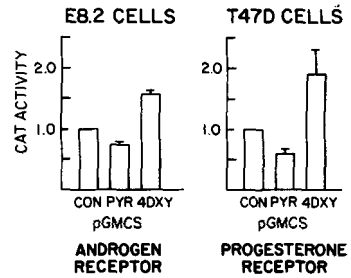

FIG. 3. **Vitamin B_6 influences transcriptional activation of the androgen and progesterone receptors.** *Left*, 16 h after transfection with pGMCS, E8.2 cells were cultured for 48 h in control medium (*CON*), medium supplemented with 2 mM pyridoxine (*PYR*), or medium supplemented with 3 mM 4-deoxypyridoxine (*4DXY*). Cells were then treated with 10 nM R1881 or vehicle for 12 h prior to CAT activity determination. *Right*, 16 h after transfection with pGMCS, T47D cells were cultured for 48 h in control medium, medium supplemented with 3 mM pyridoxine, or medium supplemented with 2 mM 4-deoxypyridoxine prior to a 24-h treatment with 1 nM progesterone or vehicle and CAT activity determination. The data shown are representative of at least three individual experiments.

HeLa cells are devoid of endogenous estrogen receptors, but express functional receptors and exhibit estrogen-responsive transcriptional activation after transfection with an estrogen receptor expression vector (40, 67–69). Since we have previously characterized in these cells the effects on intracellular pyridoxal phosphate concentration that arise from supplementation of the culture medium with synthesis precursors or inhibitors (36), we chose to use these cells as the experimental model for examination of vitamin effects on estrogen receptor-mediated transcription. For these studies, cells were transfected with both an expression vector that directs production of functional human estrogen receptor, pHEO, and the estrogen-responsive CAT reporter plasmid pERENF1CAT. After transfection, cells were exposed to the concentrations of pyridoxine or 4-deoxypyridoxine which we have shown alter intracellular pyridoxal phosphate concentration and modulate glucocorticoid receptor-mediated induction of transcription (Ref. 36 and Fig. 1). Results from these experiments, presented in Fig. 4, demonstrated that estrogen-induced CAT activity was suppressed by 30% under pyridoxine conditions that elevate intracellular pyridoxal phosphate concentration, while mild vitamin deficiency resulted in an 85% enhancement in the level of estrogen-induced CAT activity. Thus, the effects of vitamin concentration on estrogen receptor-mediated transcriptional activation were analogous to those which we had previously observed with the glucocorticoid receptor endogenously present in these same cells, as demonstrated in Fig. 1. Another significant point to be made from this series of experiments is that modulation of receptor-

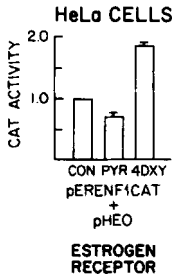

FIG. 4. **Estrogen receptor-mediated gene expression is modulated by alterations in intracellular pyridoxal phosphate concentration.** HeLa cells were transfected with the estrogen receptor expression vector pHEO and the estrogen-regulated CAT reporter pERENF1CAT. Sixteen hours after transfection, cells were exposed to control medium (*CON*), medium supplemented with 1 mM pyridoxine (*PYR*), or medium supplemented with 5 mM 4-deoxypyridoxine (*4DXY*) prior to a 24-h treatment with 5 nM 17β-estradiol or vehicle and CAT activity determination. The data shown reflect results from at least three individual transfection experiments.

induced gene expression was not restricted to the MMTV promoter. This finding supports the hypothesis that alterations in intracellular vitamin B_6 concentration can affect transcription mediated through other hormonally regulated promoters.

DISCUSSION

The first suggestion that glucocorticoid receptor function was influenced by vitamin B_6 was derived from early studies in which it was shown that modulation of vitamin B_6 concentration in cultured cells or tissue explants affects the levels of glucocorticoid-induced tyrosine aminotransferase (70) and alkaline phosphatase enzymatic activities (71), as well as casein mRNA (72). Moreover, gestational pyridoxine administration was demonstrated to reduce the incidence of glucocorticoid-induced cleft palate formation in rats (73). It is clear from the studies described in Figs. 1 and 2 that the transcriptional activation capacity of the glucocorticoid receptor is influenced by vitamin B_6 concentration. This modulatory effect is observed in diverse cell types and with glucocorticoid receptor of different species. It is quite likely, then, that vitamin B_6 influences glucocorticoid receptor-mediated transcription of the tyrosine aminotransferase, alkaline phosphatase, and casein genes. A clear mechanism is not so apparent in the cleft palate study. However, glucocorticoid receptor-mediated gene expression is undoubtedly a fundamental component in the process of hormone-induced cleft palate formation, and our studies indicate that vitamin B_6 would also act to affect this process at the level of modulation of hormone-induced gene expression.

From the studies shown in Figs. 3 and 4, it is apparent that alterations in vitamin concentration analogously affect the transcriptional activation capacities of other steroid hormone receptors, suggesting that the vitamin may act at the transcriptional level to modulate steroid hormone action in general, and not just glucocorticoid hormone action. This hypothesis is supported by the observations of vitamin B_6-induced alterations in the level of estrogen-induced uterine growth and peroxidase activity (34), as well as the enhancement in androgen-mediated prostate growth observed in vitamin deficiency (33).

It is important to note that these different hormonally regulated genes (tyrosine aminotransferase, alkaline phosphatase, and casein), with different promoters, are similarly affected by modulation of vitamin concentration: in all cases where it has been examined, hormone response is reduced under conditions of elevated vitamin concentration and enhanced under conditions of vitamin deficiency. This consistent modulatory response is also observed in the studies on the hormone-regulated physiological processes of uterine and prostate growth (33, 34). The data presented in Fig. 4 demonstrate that vitamin concentration modulates gene expression mediated through different hormonally regulated promoters, and we conclude that vitamin B_6 acts at the level of receptor-mediated transcriptional activation to modulate the biological actions of different steroid hormone receptors.

The data presented in this report are consistent with the idea that the transcriptional modulatory effects of the vitamin are mediated through the receptor molecules themselves. There is a preponderance of evidence suggesting a direct interaction of pyridoxal phosphate with the glucocorticoid receptor, including interaction of the receptor with an antibody directed against pyridoxal phosphate (60), and prevention of receptor cleavage with exogenously applied trypsin by prior exposure to pyridoxal phosphate (2). In its classical role as a cofactor in many enzymatic reactions, pyridoxal phosphate interacts with specific amino acids, predominantly lysine residues (74). Thus, it is possible that pyridoxal phosphate also interacts with the glucocorticoid receptor through a similar association with specific amino acids, potentially lysines. If this is, in fact, the mechanism through which the transcriptional modulatory effects of the vitamin are achieved, then it follows that pyridoxal phosphate would also interact with the estrogen, androgen, and progesterone receptors. In fact, with the significant degree of sequence homology which exists between these proteins, there may be a consensus sequence or region common to these four receptors with which pyridoxal phosphate interacts to mediate its effects on transcriptional activation. Mutational analysis will determine if there exists a binding site for pyridoxal phosphate on the glucocorticoid receptor, as evidence in the literature suggests. Further studies will then be necessary to evaluate the possibility of a common site among other members of the highly conserved steroid hormone receptor superfamily which allows modulation of gene expression by alterations in intracellular vitamin B_6 concentration.

In conclusion, the studies described here demonstrate that pyridoxal phosphate concentration affects the level of glucocorticoid receptor-induced gene expression without dependence on receptor species or cell type. In addition, transcriptional activation by other steroid hormone receptors is analogously modulated, and is observed with different hormonally regulated promoters. These observations together are important when considering the concept of regulation of hormone action: a regulator, or regulatory mechanism, would be required to affect multiple hormone receptors similarly, functioning in a variety of cell types to modulate the expression of a diverse group of target genes. It is clear that vitamin B_6 acts through a specific mechanism, modulation of transcriptional activation, to regulate the physiological actions of multiple members of the steroid hormone receptor superfamily.

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