Effects of Pyridoxal 5'-Phosphate on Uterine Estrogen Receptor

I. INHIBITION OF NUCLEAR BINDING IN CELL-FREE SYSTEM AND INTACT UTERUS*

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The present study was undertaken to establish whether pyridoxal 5'-phosphate interacts with various states of the uterine estrogen receptor (i.e. unbound receptor, receptor-estrogen complex (R-E)). Binding of this reagent (20 mM) to unfilled cytoplasmic receptors at 0°C or 28°C had no effect on subsequent formation of receptor-[3H]estradiol (R-[3H]E2) complexes. Similarly, at 28°C, no effects on the stability of R-E2 were observed. Pyridoxal-5'-P did not alter the nuclear receptor capacity for heat-activated R-E2 complexes. Pyridoxal-5'-P and pyridoxal, but not pyridoxine, inhibited nuclear binding of cytoplasmic estrogen receptor complexes heat-activated in the presence of these reagents. The inhibition of nuclear binding was more pronounced when pyridoxal-5'-P was added before rather than after R-E2 heat activation. This inhibitory effect was reversed by reacting pyridoxal-5'-P-treated cytosol with dithiothreitol (0.1 m) or 1-lysine (0.1 m); however, these reagents did not reverse the inhibitory effect when the pyridoxal-5'-P-treated cytosol was first reacted with NaBH4. Pyridoxal-5'-P was also efficient in solubilizing nuclear-bound R-E2 complexes. This extraction effect was less pronounced with nuclei in which R-E2 was formed by incubating intact uterine tissue with estradiol than with nuclei in which R-E2 was formed by adding heat-activated cytosol to R-E2. The inhibitory effect of pyridoxal-5'-P and pyridoxal, but not pyridoxine, on nuclear binding of R-E2 was observed with intact uterine tissue. This effect could be reversed by extensive washing of the tissue with pyridoxal-5'-P-free buffer containing estradiol.

The data presented here suggest that pyridoxal-5'-P interferes with both activation and nuclear binding of uterine R-E2 by forming a Schiff base with nucleophilic residues of the inactive and heat-activated receptor. Furthermore, pyridoxal-5'-P reacts with nuclear bound R-E2 and thereby extracts these complexes.

The cytoplasm of uterine cells contains specific estrogen-binding proteins, referred to as ‘receptors.’ Upon estradiol entry into the cell, a hormone-receptor complex is formed and rapidly translocated to the nucleus. It is generally accepted that nuclear binding of R-E2 complexes is an essential prerequisite for initiation of a series of biochemical reactions which eventually lead to estrogen-induced modulation of the physiological state of uterine cells. At a molecular level, the reactions leading to nuclear translocation of the cytoplasmic R-E2 complex are poorly understood. In vitro studies with a cell-free system suggest that the cytoplasmic hormone-receptor complex formed at 0°C must undergo physicochemical alterations in order to translocate and bind to nuclei (1, 2). ‘Transformation’ and ‘activation’ are the two attributes most frequently used to describe the reactions which modify the hormone-receptor complexes in such a fashion that they can bind to nuclei. Such transformation activation of R-E2 complexes can be induced by a variety of experimental manipulations, such as exposure to heat or high ionic strength, dilution, and dialysis (3-5). Recent studies by Notides and his collaborators (6-8) suggest that this receptor activation consists of an estrogen-dependent 4 S monomer to 5 S dimer transformation. Such activation occurs in cell-free systems and in intact cells even at 0°C, although at greatly reduced rates (9).

Previous studies with receptors of progesterone or glucocorticoid-responsive tissues have shown that a variety of chemical agents may interfere with the interaction of cytoplasmic steroid-receptor complexes with isolated nuclei, ATP-Sepharose, DNA-cellulose, or phosphocellulose (10-12). One of these reagents, pyridoxal 5'-phosphate, was of particular interest to us not only because of its possible value as a chemical probe for the analysis of uterine estrogen receptor in the activated and inactive state, but also because of the biological relevance of this B6 vitamer which acts as a coenzyme in numerous enzymatic reactions. Recently, it has been shown that pyridoxal 5'-phosphate could be a modulator of glucocorticoid action in liver cells (12). In this report, we present data on the effects of pyridoxal 5'-phosphate, pyridoxal, and pyridoxine on the formation, activation, nuclear translocation, and nuclear retention of uterine R-E2 in a cell-free system and in intact uterine tissue.

MATERIALS AND METHODS

Animals—Immature 21- to 23-day-old female Sprague-Dawley CD rats (Charles River) were used in these studies.

Isotopes and Chemicals—[17β-3H]-Estradiol (98 to 109 Ci/mol) was obtained from Amersham/Searle; diethyldithiobetol was obtained from Steraloids. All reagents were reagent grade obtained from commercial sources: Dextran grade C (clinical grade) and sucrose; crystalline RNase-free (Schwarz/Mann); hydroxylapatite (Bio-gel HTP), (Bio-Rad Laboratories); ethylenediaminetetraacetic acid (EDTA) (Eastman); C10 cryptate, A grade (dithiothreitol), (Calbiochem); pyridoxal 5'-phosphate, pyridoxal, pyridoxine, 1-lysine, and activated charcoal (Norit A), (Sigma Chemical Co.); scintillation fluids: Liquidint and Betaflow (National Diagnostics).

Buffers and Solutions—Buffer BS (0.2 m boric acid, 0.25 m sucrose, 3 mM MgCl2, pH 8.0 at 0°C). Buffer TED (10 mM Tris, 1.5 mM EDTA, 0.5 mM diethiothreitol, pH 7.5 at 0°C). Buffer KRBG (calcium-free Krebs-Ringer bicarbonate buffer: 6.923 g/liter of NaCl, 0.353 g/liter of KCl, 0.162 g/liter of KH2PO4, 1 g/liter of glucose, 2.1 g/liter of NaHCO3, pH 7.4 at 25°C).

Pyridoxal 5'-phosphate, pyridoxal, and pyridoxine were dissolved...
in Buffer KRBG or Buffer BSM and the pH adjusted at 0°C to 7.4 and 8.0, respectively; these solutions as well as the experimental incubations were always kept from exposure to light.

Preparation of Cytosol and Nuclear Fractions—All operations were done at 0-4°C, unless otherwise stated. Excised uteri were rinsed with Buffer BSM, homogenized in this buffer (five uteri per ml) and prepared of nuclear and cytoplasmic fractions by centrifugation, were performed exactly as described elsewhere (13).

Formation of Nonactivated and Temperature-activated R-E2—Cytosol was incubated with [3H]E2 for 1 h at 0°C in the presence or absence of a 200-fold excess of unlabeled diethylstilbestrol, [3H]E2 was prepared in Buffer BSM and 1 volume added to 9 volumes of cytosol to yield a final [3H]E2 concentration of 1 x 10^-11 M; protein concentrations ranged from 2 to 5 mg/ml. Temperature activation was achieved by incubating the R-E2 preparation at 28°C for 30 min.

Nuclear Binding of Activated R-E2—Unless otherwise stated, 1 volume of temperature-activated R-E2 was added to 1 volume of uterine nuclei suspended in Buffer BSM. The incubation was performed at 0°C for 2 h with frequent blending on a Vortex mixer. Nuclei were then sedimented by centrifugation for 20 min at 800 x g and washed three times by resuspending the pellet in 3 ml of the same buffer followed by centrifugation for 10 min at 800 x g.

R-E2 Formation in Intact Uterine Tissue—Uteri were excised and placed in Buffer KRBG at 22°C. The tissue was rinsed in that buffer and transferred to Buffer KRBG (one uterus per ml) containing [3H]E2 (1 x 10^-8 M) in the presence or absence of diethylstilbestrol (2 x 10^-7 M). The incubation was performed under air at 37°C for 1 h. The tissue was then rinsed extensively in ice-cold Buffer BSM and the cytoplasmic and nuclear fractions prepared as described above, except that the 800 x g supernatant of the tissue homogenate was used as a cytosol source without further centrifugation at 105,000 x g.

Assay of Nuclear-bound and Cytosolic R-E2—The washed nuclear pellets were suspended in 1 ml of 95% ethanol and [3H]E2 extracted overnight at 22°C. The entire suspensions were transferred to scintillation vials, the test tubes were rinsed with 1 ml of ethanol which was combined with the extracts, and radioactivity was counted (2 ml of ethanol to 10 ml of Betaplate).

Cytosolic R-E2 was determined by the dextran-coated charcoal technique (14) or by the hydroxyapatite technique (15). Throughout this study, specific estrogen binding was assessed by subtracting [3H]E2 bound in the presence of an excess of unlabeled diethylstilbestrol (nonspecific binding) from [3H]E2 bound in parallel incubations in the absence of unlabeled diethylstilbestrol (total binding). The data shown in this paper represent specifically bound [3H]E2.

DNA was determined by the method of Burton (16) using calf thymus DNA as a standard. Protein was determined by the procedure of Lowry et al. (17), using bovine serum albumin as a standard.

RESULTS

When R-E2 complexes formed at 0°C are incubated at that temperature with uterine nuclei for 2 h, nuclear binding is negligible. In contrast, heating (28°C) of R-E2 for 30 min modifies the properties of these complexes, such that during subsequent incubation at 0°C, 20 to 40% of R-E2 bind to nuclei (18, 19). In this report these two receptor states are defined as inactive (R-E2) and active (R+E2), respectively.

The Presence of Pyridoxal 5'-Phosphate, Pyridoxal, but not Pyridoxine, during Heat Activation Inhibits R-E2 Binding to Nuclei—Fig. 1 shows that when R-E2 is heated, approximately 40 to 50% of the hormone-receptor complex binds to nuclei at 0°C (cf. ordinate). In contrast, when pyridoxal-5'-P or pyridoxine are added to R-E2 and the sample is heated-activated, subsequent binding to nuclei at 0°C is reduced markedly. This effect is concentration-dependent, with maximum inhibition being obtained at 20 mM concentrations. Pyridoxal-5'-P is more effective than pyridoxine, with 50% inhibition being achieved at 5 and 10 mM concentrations, respectively. The presence of pyridoxine during heat activation has no effect on nuclear binding of R-E2. This inhibition was not due to pyridoxal-5'-P, or pyridoxine-induced instability of R-E2 prior to or during the heat activation step, since upon prolonged incubations at 0°C and 25°C the presence or absence of these reagents did not alter the concentration of estradiol-receptor complexes (data not shown).

The data in Fig. 1 also demonstrate that the presence of these reagents during the nuclear translocation assay does not cause a loss of receptor, since the untranslocated R-E2 was recovered stoichiometrically in the supernatant of the nuclear pellets by adsorption to hydroxyapatite.

We also tested the hypothesis that the presence of pyridoxal 5'-phosphate during the translocation assay may have modified specific and nonspecific nuclear acceptor sites, thereby preventing R-E2 retention. Uterine nuclei were incubated with or without pyridoxal-5'-P; one pyridoxal-5'-P-treated aliquot was then reacted with NaBH4, to reduce putative Schiff bases formed with nuclear proteins and to eliminate unreacted pyridoxal-5'-P. These three nuclear preparations were then extensively washed and incubated with temperature-activated R-E2. The slight decrease in acceptor capacity (Fig. 2) caused by pyridoxal-5'-P does not account for the 80 to 90% inhibition of nuclear binding observed with R-E2 heat-activated in the presence of 20 mM pyridoxal-5'-P (Fig. 1).

Effect of Pyridoxal 5'-Phosphate on Nuclear R-E2 Retention—Since pyridoxal-5'-P was present during the translocation assay, the inhibition of nuclear binding could have been the result of pyridoxal-5'-P-induced dissociation of E2 from translocated R-E2 or extraction of R-E2 into the medium, rather than inhibition of R-E2 activation, or translocation, or both. Therefore, we examined the effects of this reagent on the retention of R-E2.

Nuclear R-E2 was formed either by incubating nuclei with heat-activated R-E2, or by incubating intact uteri with E2 at 37°C to achieve nuclear translocation. Nuclei were extracted with BSM buffer containing pyridoxal-5'-P and R-E2 in the nuclei and extracts were measured. Fig. 3 shows that pyridoxal-5'-P extracts more E2 from nuclei labeled in a cell-free system than from nuclei labeled in intact tissue. This effect is concentration-dependent and is not due to R-E2 dissociation into R and E2, since the extracts contained the solubilized R-E2 complexes. The presence of 5 and 20 mM pyridoxal-5'-P during heat activation of R-E2 causes 50% and 90% inhibition of nuclear binding, respectively (Fig. 1). Since
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Pyridoxal-5'-Phosphate Addition Prior to or after Temperature Activation Inhibits Nuclear Translocation of R-E2 to a Different Extent—Pyridoxal-5'-P could be acting directly on R-E2 by preventing the temperature activation of the complex. This reagent could also interact with amino acid residues which become accessible upon heat activation. Finally, pyridoxal-5'-P may react with both the activated and nonactivated R-E2 complexes, but at different sites. Two experiments were performed in which pyridoxal-5'-P (20 mM) was added before or after heat activation of R-E2. Subsequent nuclear binding was reduced to 20% and 27% of control when pyridoxal-5'-P was added before heat activation. When pyridoxal-5'-P was added to heat-activated R-E2, the inhibition of nuclear binding was less marked, with 36% and 47% of control being translocated.

Reversibility of Pyridoxal 5'-Phosphate Inhibition of Nuclear Binding—It has previously been shown that dithiothreitol and gel filtration reverse the inhibitory effects of pyridoxal-5'-P on the binding of the heat-activated glucocorticoid cytosol receptor to DNA-cellulose. Furthermore, it has been shown that the inhibitory effects of pyridoxal-5'-P are not reversed by 3-h dialysis. Similarly, the effects of pyridoxal-5'-P could not be reversed by adding Tris (0.1 M) to the cytosol R-E2 preparation (data not shown). One molar Tris restored...
nuclear binding to 29% of control value. However, Tris-treated cytosol of control incubations gave only 40% of the nuclear binding obtained with cytosol and nuclei exposed only to BSM buffer. Thus, Tris as such may alter nuclear binding by ionic strength effects and therefore the capacity to reverse pyridoxal-5'-P inhibition cannot be evaluated. When R-E2, heat-activated in the presence of 20 mM pyridoxal-5'-P, was incubated for 30 min with dithiothreitol (100 mM) or L-lysine (100 mM) subsequent nuclear binding was restored to 40% and 64% of control, respectively.

Pyridoxal 5'-Phosphate and Pyridoxal, but not Pyridoxine, Inhibit Nuclear Binding of R-E2 in Intact Uteri. The Effect is Reversible—In the experiments described so far the inhibitory effect of pyridoxal-5'-P was observed in a cell-free system. It was important to verify whether inhibition of nuclear translocation occurs also in intact tissue. Uteri were incubated at 37°C for 30 min in calcium-free buffer KRBG containing either pyridoxal-5'-P, pyridoxal, or pyridoxine; R-E2 formation and nuclear translocation was then achieved by adding E2 to the medium and continuing the incubation for 60 min at 37°C. The nuclear and cytoplasmic fractions were prepared and specifically bound E2 was measured as described. The data in Table 1 show that pyridoxal-5'-P and pyridoxal, but not pyridoxine inhibit nuclear binding. The cytoplasmic fraction of those uteri in which inhibition of nuclear R-E2 binding was observed, did not contain correspondingly higher amounts of R-E2, nor did reincubation of this cytosol with [3H]E2 reveal empty receptor sites, since no additional binding was detected.

Although in these experiments uteri were rinsed extensively before homogenization, a certain amount of pyridoxal-5'-P was still present in the homogenate; therefore pyridoxal-5'-P could have prevented E2 binding to empty cytoplasmic estrogen receptor sites. To test this hypothesis, uterine cytosol was incubated at 0°C for 1 h or 28°C for 10 min with or without pyridoxal-5'-P (20 mM). Subsequent incubations with [3H]E2 (1 X 10^-10) for 3 h at 0°C followed by measurement of R-E2 by the dextran-coated charcoal technique showed that pyridoxal-5'-P does not interfere with formation of R-E2 (data not shown). Therefore, failure to detect receptors not translocated to the nucleus cannot be attributed to an effect of pyridoxal-5'-P on unoccupied estrogen binding sites.

To assess whether the pyridoxal-5'-P effect is reversible, one group of uteri was washed four times for 15 min at 37°C in KRBG buffer containing [3H]E2 but without pyridoxal 5'-phosphate. [3H]E2 was added to the rinsing medium to maintain the level of R-E2 formed in the presence of pyridoxal-5'-P and to allow E2 binding to unoccupied receptor sites. The data in Table 1 show that nuclear and cytoplasmic R-E2 was restored to control values. This treatment reversed the inhibitory effect of pyridoxal-5'-P, since cytoplasmic and nuclear R-E2 were restored to control values.

**Table 1**

| Specificity and reversibility of the pyridoxal 5'-phosphate effect on nuclear binding of R-E2 in intact uteri |
|--------------------------------------------------|
| **Experiments 1 and 2:** uteri (one uterus per ml) were preincubated under air for 30 min at 37°C in Buffer KRBG, pH 7.4, containing the indicated compounds at a final concentration of 20 mM. To each group, [3H]E2 in KRBG buffer was added (final concentration 1 X 10^-9 M) and the incubation extended for 60 min at 37°C. The uteri were rinsed extensively in ice-cold Buffer BSM and homogenized in the same buffer (five uteri per ml). Nuclear and cytoplasmic fractions were then assayed for specifically bound [3H]E2. To determine the presence of empty receptor sites, aliquots of cytosol were incubated at 0°C for 18 h with [3H]E2 and R-E2, measured by the dextran-coated charcoal technique. Experiment 3: uteri were preincubated as above, without (control) or with 20 mM pyridoxal-5'-P, followed by 1 h labeling with [3H]E2 (1 X 10^-9 M). At this point, uteri were either homogenized and R-E2 in the nuclear and cytoplasmic fractions was measured, or washed four times at 37°C for 15 min in KRBG buffer containing [3H]E2 (1 X 10^-9 M) but without pyridoxal-5'-P. Tissue was then rinsed, homogenized, and cytoplasmic and nuclear R-E2 determined. Furthermore, cytosol was reincubated for 18 h at 0°C with [3H]E2 and R-E2, measured as described above. |
| fmol nuclear R-E2/uterine equivalent | fmol cytoplasmic R-E2/uterine equivalent | Direct assay | Assayed after 18-h incubation at 0°C with [3H]E2 |
|--------------------------------------|------------------------------------------|--------------|------------------------------------------|
| **Experiment 1**                     |                                          |              |                                          |
| Control                              | 840                                      | 250          | 230                                      |
| Pyridoxal-5'-P                       | 420                                      | 278          | 245                                      |
| Pyridoxine                           | 415                                      | 55           | 105                                      |
| Pyridoxine                           | 415                                      | 55           | 105                                      |
| **Experiment 2**                     |                                          |              |                                          |
| Control                              | 640                                      | 200          | 185                                      |
| Pyridoxal-5'-P                       | 420                                      | 150          | 140                                      |
| Pyridoxine                           | 300                                      | 100          | 90                                       |
| Pyridoxine                           | 668                                      | 260          | (Not measured)                           |
| **Experiment 3**                     |                                          |              |                                          |
| Control                              | 732                                      | 168          | 180                                      |
| Control (washed 4x)                  | 794                                      | 150          | 145                                      |
| Pyridoxal-5'-P                       | 398                                      | 196          | 178                                      |
| Pyridoxal-5'-P                       | 824                                      | 125          | 138                                      |

**DISCUSSION**

This study demonstrates that pyridoxal-5'-P inhibits the binding of R-E2 to nuclei in a cell-free system and in the intact uterus. We have attributed this effect to the binding of pyridoxal-5'-P to the estrogen receptor (Fig. 5), although we cannot exclude that pyridoxal-5'-P inhibited nuclear binding by reacting with other cytoplasmic proteins (e.g., enzymes) required for R-E2 activation and nuclear binding. We consider the latter to be unlikely, since so far such entities have not been identified and the majority of data on estrogen action suggests that receptor transformation into an activated state capable of nuclear translocation is solely dependent upon the binding of the hormone to the receptor, with the energy thereby released being sufficient to drive the transformation of R-E2 into R+E2. Furthermore, with the chick oviduct progesterone receptor, Nishigori and Toft (21) have presented data supporting the concept that pyridoxal-5'-P reacts with the receptor.

We have shown that the reduction of nuclear binding is not due to pyridoxal-5'-P inhibition of R-E2 formation, nor to pyridoxal-5'-P-induced dissociation of cytoplasmic or nuclear R-E2 complexes into their binding moieties. Furthermore, the pyridoxal-5'-P effect could not be attributed to decreased nuclear acceptor capacity (Fig. 2). The scheme in Fig. 5 is based on the finding that pyridoxal-5'-P binds to the various states of the receptor, i.e., R-E2, R+ E2, and intranuclear R-E2 either free, or bound to specific and nonspecific nuclear acceptors (R-E2 ACC).

The various reactions with pyridoxal-5'-P are reversible as suggested by the experiments with the cell-free system, in which L-lysine and dithiothreitol reversed the action of pyridoxal-5'-P, thus restoring nuclear-binding capacity (Fig. 4). Reversibility was lost when the pyridoxal-5'-P-R-E2 preparation was reduced with NaBH4, prior to addition of the above nucleophile reagents. Pyridoxal-5'-P was also a reversible inhibitor of nuclear binding of R-E2 in the intact tissue (Table 1). These findings are consistent with the fact that pyridoxal-5'-P forms a Schiff base with nucleophilic residues of proteins and that this reaction is reversible. In our system, L-lysine and dithiothreitol displaced pyridoxal-5'-P by transimination. The
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The multiple points of action of pyridoxal 5'-phosphate (PLP) leading to reduced nuclear binding of receptor-estrogen complex. R. = inactive unbound receptor; R.E2 = untransformed, inactive R.E2; R.E2 = transformed, activated R.E2; Acc = specific and nonspecific acceptor sites for translocated R.E2.

reversibility of the Schiff base reaction explains why complete inhibition of nuclear binding was not achieved. As shown in Fig. 5, reversibility implies that a certain fraction of R.E2 will escape pyridoxal-5'-P inhibition and bind to nuclei. The specificity data in Fig. 1 also point to a reaction involving Schiff base formation, since pyridoxine is inactive as an inhibitor and the latter lacks the aldehyde group. At equal concentrations, pyridoxal-5'-P was a better inhibitor than pyridoxal, suggesting that positively charged groups of R.E2 attract pyridoxal-5'-P, thus increasing the effective concentration of this reagent at the sites of Schiff base formation. Similar observations were reported in studies on 6-phosphogluconate dehydrogenase (22) and glutamic dehydrogenase (23).

The chemical nature of the transformation reaction is not yet understood, but it appears that in a temperature-accelerated reaction the R.E2 complex undergoes dimerization and changes its conformation with concomitant appearance of positively charged residues on the surface of the receptor. These changes mediate R.E2 binding to polyanions, nuclei, etc. The data in Fig. 1 demonstrate that pyridoxal-5'-P addition to R.E2 inhibits subsequent heat activation. In a separate study (24), we have shown that this effect is in part due to inhibition of transformation, as judged by kinetic and sucrose gradient analysis. In this respect, the estrogen receptor differs from the glucocorticoid receptor, where only the activated state of the hormone-receptor complex reacted with pyridoxal-5'-P (12).

Pyridoxal-5'-P also binds to R.E2 and thereby decreases its nuclear-binding capacity. This suggests that pyridoxal-5'-P has either formed a Schiff base with those residues on the activated R.E2 complex which are essential for nuclear binding, or has reacted with residues which are distal to this site, and has thereby altered the spatial arrangement of the amino acid residues which interact with nuclear acceptor sites. Both reactions would result in decreased affinity for nuclear components, and, as indicated in the scheme in Fig. 5, pyridoxal-5'-P.R.E2 is either not translocated, or translocated without retention.

Finally, pyridoxal-5'-P binds to translocated R.E2 and thereby decreases its affinity for specific and nonspecific nuclear binding sites; this causes a release of R.E2 into the medium as judged by hydroxylapatite adsorption of the extracted R.E2 (Fig. 3). On sucrose density gradients this pyridoxal-5'-P-extracted R.E2 has the same sedimentation coefficient as heat-activated R.E2. As already explained under "Results," this extraction effect is too small to account for the decreased nuclear binding of R.E2 heat-activated in the presence of pyridoxal-5'-P.

The data discussed so far were obtained with a cell-free system. With intact uterine tissue pyridoxal-5'-P had a similar effect (Table I). These data provide the first evidence for a possible role of this substance as a modulator of estrogen receptor activity in the intact tissue. At equal pyridoxal-5'-P concentration, the inhibition of nuclear binding was smaller than that observed with the cell-free system, and pyridoxal appeared to be somewhat more effective than pyridoxal-5'-P. Differences in the mechanism of uptake of these two compounds and competition for binding by other proteins are just some of the factors which may have contributed to the quantitative differences observed between the cell-free system and intact tissue. With the intact uterus the pyridoxal-5'-P inhibition of nuclear binding was not associated with a corresponding increase of nontranslocated cytoplasmic R.E2, nor could receptor sites be detected in the subcellular fractions of tissue homogenate. Since we have shown that the inhibitory effect of pyridoxal-5'-P could be reversed by extensively rinsing the intact tissue at 37°C in the presence of E2 (Experiment 3, Table I), our failure to detect empty receptor sites in the cytosol fraction of the homogenate cannot be attributed to the absence of such receptors, but more likely to some yet unidentified experimental artifact.

In conclusion, this study demonstrates that pyridoxal-5'-P inhibits nuclear binding of R.E2 by interference with transformation of R.E2 into R.E2 by binding to activated R.E2, and by extracting R.E2 already translocated to the nucleus. Each of these reactions is the result of reversible Schiff base formation. Although pyridoxal-5'-P inhibits nuclear binding of R.E2 also in the intact uterus, no evidence is available to suggest a similar role in vivo. So far, this compound should be considered only as a useful probe for the characterization of receptor in its various states (21).

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