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Effects of Lead on the Kidney: Roles of High-Affinity Lead-Binding Proteins

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Lead-induced nephropathy produces both tubular and interstitial manifestations of cell injury, but the pathophysiology of these leisons is not completely understood. Delineation of the molecular factors underlying renal handling of lead is one of central importance in understanding the mechanisms of renal cell injury from this agent. Recent studies from this laboratory have identified several distinct high-affinity lead-binding proteins (PbBP) from rat kidney and brain that appear to play critical roles in the intracellular bioavailability of lead to several essential cellular processes in these target tissues at low dose levels. The PbBP from rat kidney has been shown to be a specific cleavage product of α_2-microglobulin, which is a member of the retinol-binding protein superfamily. Recent preliminary Western blot and immunohistochemical studies have shown that a polyclonal antibody to the renal PbBP does not recognize the brain PbBP, which appears to be a chemically similar, but distinct molecule. These studies have also shown that the renal PbBP is selectively localized in only certain nephrons and only specific segments of the renal proximal tubule. The striking nephron and cell-type specificity of the localization reaction could result from physiological differences in nephron functional activity or selective molecular uptake mechanisms/metabolism differences that act to define target cell populations in the kidney. In addition, other preliminary studies have shown that short-term, high-dose lead exposure produces increased excretion of this protein into the urine with concomitant decreases in renal concentrations.

Introduction

The kidney is a major target organ system for lead (*1,2*) with documented effects on a number of essential physiological and biochemical functions. In addition, there also appears to be variation between individuals, and in rats (*3*), differences between sexes with regard to susceptibility to lead nephropathy. There is hence a pressing need to delineate the factors that predispose individuals to the nephrotoxic effects of lead and the relationships of those factors to the mechanisms of lead action.

Studies from this laboratory (*4–10*) have identified several high-affinity lead-binding proteins (PbBP) from rat kidney and brain that appear to act as receptors for lead and mediate its activity within these target tissues (*11*). The present report is an update of these studies with regard to purification of these molecules, their immunohistochemical localization within the kidney, and responsiveness to short-term, high-dose lead exposure. Such data are clearly essential to understanding the role(s) of these molecules in determining target cell/tissue susceptibility to lead and the potential use of these molecules as kidney-specific biological indicators of lead nephropathy early in the toxic process.

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Materials and Methods

Purification of Renal PbBP. Purification of the renal PbBP was carried out as previously described (*10,12*), using a combination of Sephadex G-75 and DEAE anion-exchange column chromatography followed by reverse-phase, high-performance liquid chromatography using both nondenaturing columns.

Preparation of Polyclonal Antibodies. Preparation of rabbit anti-rat polyclonal antibodies was conducted by injecting rabbits with purified renal PbBP in Freunds adjuvant according to established methods (G. E. DuVal, manuscript submitted).

Immunohistochemical Localization of PbBP within the Kidney. Immunohistochemical localization of PbBP was accomplished by exposing paraffin-embedded sections of control rat kidney to the polyclonal antibody followed by reaction with goat anti-rabbit antibody conjugated with peroxidase. The sections were then incubated with diaminobenzidine (DAB) for varying time periods to develop color (*13*).

Dot Blot Assays of Kidney and Urine. To assess the effects of prolonged, high-dose oral exposure of rats to lead in drinking water, a dot-blot assay using the

methods of Domin et al. (*14*) was employed. Kidney and urine samples of untreated control rats and rats exposed to lead acetate in drinking water at concentrations of 0, 1, or 3% for 1 to 7 weeks were blotted onto nitrocellulose paper, reacted with the rabbit polyclonal antibody described above, followed by reaction with an ^{125}I-labeled goat anti-rabbit antibody. Densitometric scans were used to quantify the reaction, and the assay was calibrated using purified renal PbBP standards.

Results

Purification studies of the renal PbBP coupled with N-terminal analyses demonstrated that the renal PbBP exists as two forms in the kidney, which are differentiated only by the cleavage of the first 9-N terminal residues from higher molecular weight form. Insertion of the N-terminal sequences into a computerized protein sequence data base showed a 100% sequence homology with the rat protein α_2-microglobulin. This protein, which is a member of the retinol-binding protein superfamily (*15*), is produced most extensively in the livers of male rats and to a much lesser extent in female rats of breeding age. The protein has been shown to be under positive androgen control (*15*). Western blot analyses using our polyclonal antibody demonstrated that the cleaved form is the major entity present in the kidney and that it is this cleaved form that is complexed with nuclear chromatin as judged by KCl extracts of control rat kidney nuclei (unpublished observations). These data confirm and extend previous *in vitro* (*8–9*) nuclear translocation studies with isolated nuclei. Immunohistochemical studies (Fig. 1) showed that the α_2-microglobulin was primarily localized in lysosomes the descending second segment (S_2) of only selected proximal tubules. Some faint staining also appeared to be present in the cytoplasm of these cells and some third (S_3) segments (Fig. 1). The importance of these data is that they confirm and extend previous reports by others (*15,16*) that indicated that the second segment is the chief localization site for α_2-microglobulin in the kidney. The knowledge that two forms of the protein are present in the kidney and that only certain nephrons appear to contain reactive material has not been previously reported.

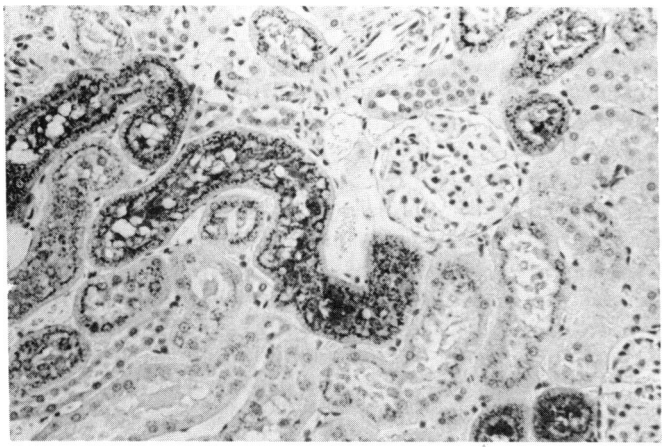

FIGURE 1. Immunohistochemical localization of α_2-microglobulin (PbBP) in S_2 segments of specific nephrons of rat kidney. Note that the peroxidase staining is observed in both the lysosomes and cytoplasm of the cells.

Further, preliminary studies using the dot-blot assay for α_2-microglobulin in kidney and urine (Fig. 2) showed an apparent dose-related increase in the excretion of this protein into the urine and concomitant decrease in renal concentrations of rats exposed to lead in drinking water at 1 or 3% for 7 weeks. The onset of the increase occurred after 2 weeks of exposure but appeared to vary in magnitude between weeks, perhaps as a function of toxicity, water intake, or urine volume/osmolality. Further studies are needed to evaluate these modulating factors. The significance of the data rests with the apparent rapidity with which this response occurs and that these increases in PbBP excretion probably occur prior to the onset of chronic renal damage. The association of the response with the renal-specific cleaved form of α_2-microglobulin is also important because it confers a renal specificity to this protein as a biological indicator of renal tubular dysfunction. The sensitivity of the radioimmunological methods used for this demonstration are also of great potential value in providing an early end point for chemical-induced cell injury.

Low-dose and chronic lead exposure studies are in progress to determine the threshold of this response under these conditions. In addition, other studies are in progress to purify hypothesized analogous PbBP from nonhuman primate and human tissues. Given the ubiquitous nature of the retinol-binding protein family in mammals and similarities in renal responses to lead, it is considered likely that chemically similar proteins will be identified and purified, which will permit the development of radioimmunoassays such as those demonstrated in the present report for human populations.

Discussion

Results of the above studies are consistent with the hypothesis (*11*) that the renal PbBP now identified as a cleaved form of α_2-microglobulin acts as a target tissue-specific receptor for lead and mediates the action of this ubiquitous toxicant in mammals. A diagrammatic representation of the current hypothesis is shown in Figure 3. Under this hypothesis, lead entering into renal proximal tubule cells is complexed with α_2-microglobulin, which is highly resistant to complete degradation (*15*). The elevated presence of the cleaved form of α_2-microglobulin in the kidney nuclei suggests that it is this form that is capable of surviving proteolysis and entering the nucleus with subsequent binding to regulatory-promoting regions of DNA. These events lead to the observed (*17,18*) novel alterations in renal gene expression associated with lead

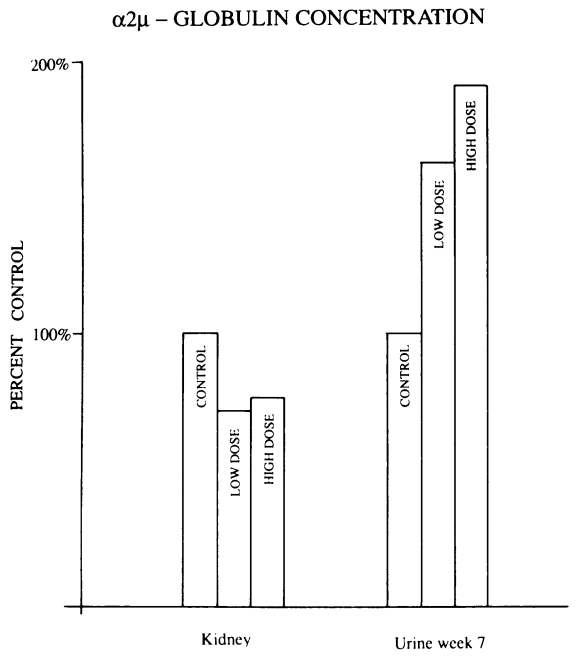

FIGURE 2. Results of dot-blot assay for α_2-microglobulin (PbBP) in kidney and urine of rats exposed to 0, 1, or 3% lead in drinking water for 7 weeks showing dose-related increase in urine and concomitant decrease in renal content.

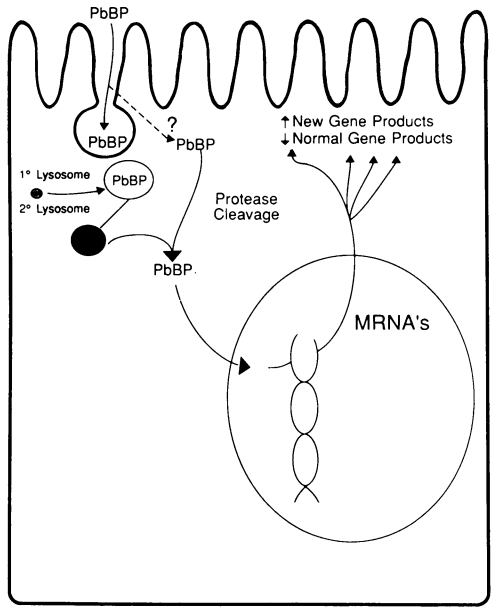

FIGURE 3. Diagram of current hypothesis for α_2-microglobulin (PbBP) as a lead receptor in renal proximal tubule cells showing apical uptake, cleavage of 9 N-terminal residues, uptake into the cell nucleus, and attendant changes in renal gene expression.

uptake into the nucleus. It is further hypothesized that some of these novel gene products may be oncogenes whose expression may account for the carcinogenic effects of lead in rodents and persons with high expression levels of the analogous human proteins.

The apparent increase in excretion of α_2-microglobulin in rats following short-term, high-dose oral exposure to lead in drinking water indicates that the rat kidney is highly responsive to elevated lead exposure prior to the onset of a general nephropathy. A similar response in humans with increased urinary excretion of an analogous protein could hence also permit early detection of lead-induced nephropathy at an early stage of development. Further studies are currently in progress to evaluate this possibility in nonhuman primates and humans exposed to lead over prolonged time periods.

Finally, we hypothesize that given the propensity for α_2-microglobulin to aggregate in the presence of lead into higher molecular weight species (*12*), this protein and its hypothesized analogs in other species also play a major role in the formation of the pathognomic lead intranuclear inclusion bodies (*19,20*) within kidney tubule cell nuclei. Further studies are needed to fully evaluate this idea.

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