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SHORT COMMUNICATIONS

Influence of metal ions on renal cytosolic lead-binding proteins and nuclear uptake of lead in the kidney

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Previous studies [1, 2] on the subcellular distribution of lead within the rat kidney after a single low dose have shown that a high proportion of lead is initially present in the cytosol, but that there is a gradual increase in the nuclear fraction with time. After chronic or acute exposure, however, a high proportion of the cellular lead within the nuclei of proximal tubule cells exists in the form of inclusion bodies which consist of a dense core of protein and lead with a protein fibrillar structure [3, 4]. During the initial stages of lead exposure, cytoplasmic inclusion bodies have also been observed [4]. Recent reports [5, 6] from this laboratory have demonstrated the presence of two high-affinity lead-binding proteins (PbBP*) in rat kidney cytosol with approximate molecular masses of 11,500 (11.5 K) and 63,000 (63 K) daltons. *In vitro* studies [6, 7] have also suggested that these proteins are involved in regulating the bioavailability of lead within the cell and in the transport of this metal ion into kidney nuclei.

Other investigations [8, 9] concerning the effects of *in vivo* exposure to various combinations of metals have clearly demonstrated that cadmium markedly influences the accumulation and biological effects of lead in the kidney. In particular, these studies showed a complete inhibition of lead-induced intranuclear inclusion body formation by cadmium. In the present studies, the effects of various metals on the binding of lead to PbBP, and the subsequent transport of lead into the nucleus were investigated to provide insight into the mechanism(s) by which metals, such as cadmium, may influence the bioavailability of lead.

Materials and methods

Preparation of renal cytosol fraction. Male Sprague–Dawley rats, 65- to 80-days-old, were used in the experiments. Kidneys were homogenized in 5 vol. of TGM buffer [10% (v/v) glycerol, and 0.5 mM monothioglycerol in 10 mM Tris–acetate buffer, pH 7.4] at 4°, and the cytosol fraction was prepared by centrifuging at 105,000 *g* for 45 min. The cytosol was used immediately either for competition studies or for isolation of partially purified lead proteins. The 63 K dalton protein fraction was isolated using a Sephacryl S-200 column (65 × 2.6 cm) at a flow rate of 30 ml/hr, and the 11.5 K dalton protein fraction using a Sephadex G-75 column (40 × 1.6 cm) at a flow rate of 45 ml/hr as described previously [5, 6].

Competition studies. Plastic-ware was used throughout unless stated otherwise. Duplicate aliquots (100 μl) of cytosol or isolated 63 K and 11.5 K protein fractions were incubated with lead acetate (^{203}Pb) solution (100 μl, 250 nM final concentration) alone or in the presence of various concentrations of radioinert competitor metal cations for 18 hr at 4° under N_2. Equilibration of ^{203}Pb binding was observed under these incubation conditions [6]. After incubation bound and free ligand were separated by using Sephadex G-25 minicolumns [6], and the results were calculated as percent displacement of total bound ^{203}Pb (in the absence of competitor).

Sedimentation analysis. Aliquots of cytosol (2.8 ml) or 63 K fraction (400 μl) were incubated with ^{203}Pb solution (200 μl) alone (250 nM final concentration) or in the presence of 100-, 250- or 500-fold excess of various competitor metal cations for 18 hr at 4°. Free and loosely bound ligand were removed by elution through Sephadex G-25 mini-columns (Pharmacia PD-10 columns) for cytosol or by dextran coated charcoal adsorption for the 63 K fraction [6]; the aliquots (200 μl) were analyzed on 10–30% sucrose gradients as described previously [6].

Cell-free nuclear uptake studies. All procedures were performed at 4° unless stated otherwise. The kidney cortex was homogenized in 5 vol. of 0.25 M STKM buffer (25 mM KCl, 5 mM $MgCl_2$, 0.25 M sucrose in 50 mM Tris–HCl buffer, pH 7.6, at 4°). Cytosol was prepared as before and incubated with ^{203}Pb solution (250 nM final concentration) with and without a 250-fold excess of various metal cations (Pb^{2+}, Cd^{2+}, Zn^{2+}, Ca^{2+} and Fe^{2+}) for 18 hr at 4°. After incubation the bound and free ligand were separated using Sephadex G-25 minicolumns. The presaturated cytosol was then incubated with purified nuclei from kidney cortex (purity was checked by electron microscopy) for 60 min at 29° and the nuclear uptake of ^{203}Pb was measured as described before [6]. Maximum nuclear uptake of ^{203}Pb was observed after 60 min.

Results and discussion

The binding of lead to rat kidney cytosolic PbBP was influenced markedly by the presence of other divalent metal ions, and these effects were much more pronounced in the isolated 63 K and 11.5 K protein fractions (Figs. 1–3). The order of displacement (and thus the order of affinity of binding) in all three fractions was $Cd^{2+} > Zn^{2+} > Pb^{2+}$. A 50% reduction in ^{203}Pb binding to cytosol, 63 K and 11.5 K protein was achieved by a 270-, 13.5- and 5-fold excess of Cd^{2+}; by a 270-, 125- and 12-fold excess of Zn^{2+}; and by a 300-, 190- and 100-fold excess of Pb^{2+} respectively. Ca^{2+} showed no displacement of ^{203}Pb even at a 1000-fold excess concentration. Addition of Fe^{2+} to the incubation mixture caused a marked increase in ^{203}Pb binding by the isolated 63 K and 11.5 K fractions, showing increases of 2.3- and 2.0-fold, respectively, at a 500-fold excess of Fe^{2+}. However, cytosolic displacement studies showed a 22% decrease in total ^{203}Pb binding in the presence of 500-fold excess of Fe^{2+}. When this cytosolic fraction was analyzed on a sucrose gradient, the profile showed that a 500-fold excess of Fe^{2+} did indeed increase binding of ^{203}Pb to the 63 K (4.6 S peak) and 11.5 K (2 S peak) fraction and that the decrease in total cytosolic ^{203}Pb binding was due to displacement from the high molecular weight protein fraction (approximately 7 S peak (Fig. 4). The possible significance of the cooperative effort of Fe^{2+} on Pb^{2+} binding is discussed below.

Previous *in vitro* nuclear translocation studies have shown that there is a time- and temperature-dependent uptake of ^{203}Pb into renal nuclei and that the 11.5 K, and possibly the 63 K, proteins may be involved in this translocation process [6]. The present *in vitro* nuclear uptake studies performed by incubating purified rat kidney nuclei with cytosol that had been preincubated with ^{203}Pb alone or

* Abbreviations: PbBP, lead-binding proteins; and TGM, Tris-glycerol monothioglycerol.

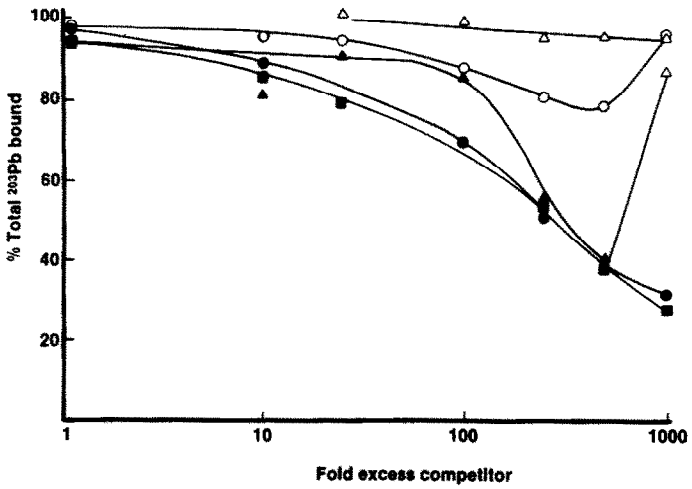

Fig. 1. Competitive displacement of ^{203}Pb binding from cytosolic proteins by various metals. Aliquots of kidney cytosol were incubated with ^{203}Pb (250 nM) alone or together with various concentrations of Pb^{2+} (▲), Cd^{2+} (●), Zn^{2+} (■), Fe^{2+} (○), or Ca^{2+} (△) for 18 hr at 4°. After incubation, bound and free ligand were separated on Sephadex G-25 minicolumns.

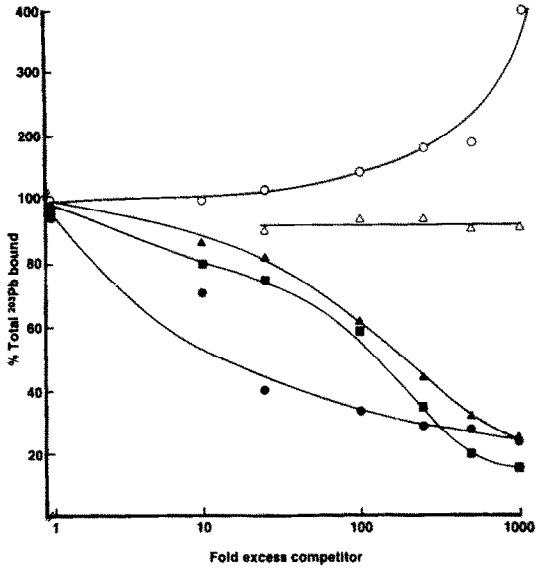

Fig. 2. Competitive displacement of ^{203}Pb binding from the isolated 63 K protein by various metals. For details see legend for Fig. 1.

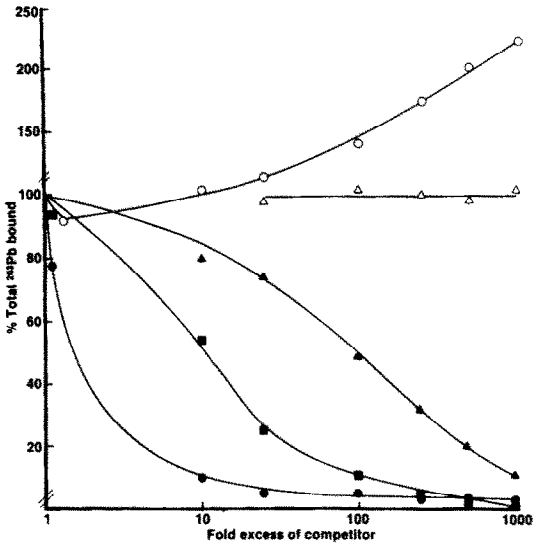

Fig. 3. Competitive displacement of ^{203}Pb binding from the isolated 11.5 K protein by various metals. For details see legend for Fig. 1.

in the presence of a 250-fold excess of metal ion competitor showed displacement results similar to those observed in cytosol or isolated fractions (Table 1). Excess Cd^{2+} reduced nuclear ^{203}Pb uptake by up to 70% followed by Pb^{2+} which decreased uptake by 50%. Zn^{2+}, however, increased nuclear uptake of ^{203}Pb despite the fact that this element reduced cytosolic binding, and the reasons for this are unclear. Ca^{2+} had no effect on cytosolic binding or on nuclear uptake of ^{203}Pb, whereas a 500-fold excess of Fe^{2+}, which increased ^{203}Pb binding to both the 63 K and the 11.5 K proteins, also increased nuclear uptake. Thus, there is a good correlation between cytosolic binding and patterns of intranuclear uptake.

The marked displacement of lead from cytosolic proteins and the inhibition in nuclear uptake of lead by Cd^{2+} are consistent with the 60% reduction in total kidney lead concentration observed in rats treated *in vivo* with lead plus cadmium compared to those treated with lead alone [8]. These results may also explain the reported [9] inhibition of lead-induced intranuclear inclusion bodies *in vivo* by concomitant exposure to Cd^{2+}. Furthermore, the increase in intranuclear uptake and cytosolic binding of lead by Fe^{2+} may be of importance in formation of inclusion bodies, particularly if Fe^{2+} is present in high concentration in close proximity to those proteins. Indeed, ferritin molecules may have been observed [4] in the immediate vicinity

Table 1. Effects of various metal ions on cytosolic lead binding and uptake by renal nuclei

Competitor	Fold excess of competitor	Cytosol bound ^{203}Pb (% of control)*	Nuclear uptake of ^{203}Pb (% of control)*
Pb^{2+}	250	70.2 ± 2.3†	50.2 ± 18.6
Cd^{2+}	250	52.2 ± 1.1	31.7 ± 15.6
Zn^{2+}	250	81.9 ± 9.5	144.5 ± 11.9
Fe^{2+}	500	91.8 ± 0.6	122.9 ± 12.3
Ca^{2+}	250	99.7 ± 3.3	94.5 ± 6.2

* ^{203}Pb (250 nM) alone represents 100%.
† Values represent mean ± S.E.M., N = 3 separate determinations.

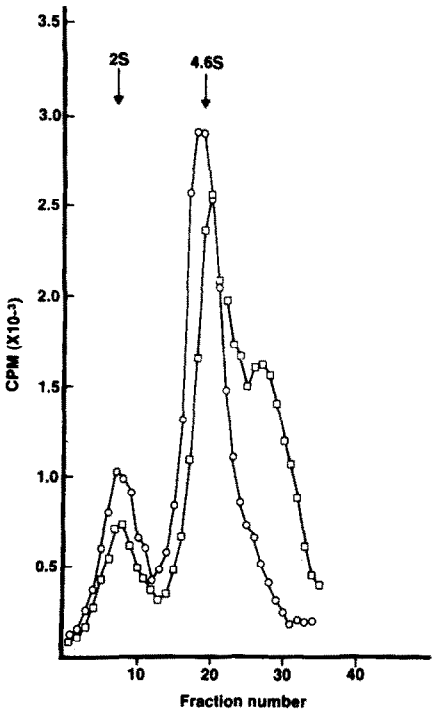

Fig. 4. Effect of Fe^{2+} on ^{203}Pb binding by cytosolic proteins. Aliquots of kidney cytosol were incubated with ^{203}Pb alone (□) or together with 500-fold excess of Fe^{2+} (○) for 18 hr at 4°. After incubation, bound and free ligand were separated on Sephadex G-25 minicolumns and the sample was analyzed on a 10–30% sucrose gradient. The gradients were centrifuged at 310,000 g for 20 hr.

of cytoplasmic inclusion bodies during the early period of lead exposure and the Fe^{2+} suggested as important to the formation process. These results suggest that cytosolic lead-binding proteins are involved in regulating the subcellular distribution and bioavailability of lead within the kidney and that the concomitant exposure to other metal ions may alter this distribution by inhibiting or enhancing the binding of lead to these proteins. In addition, these results also suggest that the lead-binding proteins may be involved in the formation of inclusion bodies in renal cells of lead-exposed rats and that the presence of other metals, such as Cd^{2+} and Fe^{2+}, could play an important role in mediating this process. Further studies are currently under way to determine the role of these proteins in the formation of renal inclusion bodies, and also if they are involved in mediating the reported [10] lead-induced increase in protein and DNA synthesis in renal cells following the intranuclear movement of this metal.

In summary, the specificity of Pb^{2+} binding by kidney cytosol lead-binding proteins (PbBP) was examined together with the effects of various metal ion competitors on PbBP-mediated nuclear uptake of Pb^{2+}. The order of displacement of ^{203}Pb binding (and thus the order of affinity of binding) in cytosol or to the isolated 63,000- and 11,500 dalton proteins was $Cd^{2+} > Zn^{2+} > Pb^{2+}$. Ca^{2+} had no effect on Pb^{2+} binding, whereas the presence of a 500-fold excess of Fe^{2+} had a cooperative effect, increasing Pb^{2+} binding by the 63,000- and the 11,500-dalton proteins by 2.3- and 2.0-fold respectively. The effects of these metals on renal nuclear uptake of Pb^{2+} showed similar trends. These results, which are consistent with previously reported *in vivo* data, suggest that concomitant exposure to other metals may alter the nuclear uptake of Pb^{2+} within the kidney by altering Pb^{2+} binding to these cytosolic proteins.

*Laboratory of Pharmacology and
†Biometry and Risk Assessment Program
National Institute of Environmental Health Sciences
National Institutes of Health Research, Triangle Park, NC 27709, U.S.A.

PRAKASH MISTRY*
CALLIOPI MASTRI†
BRUCE A. FOWLER*‡

‡ Send all correspondence to: Dr. Bruce A. Fowler, Laboratory of Pharmacology, NIEHS/NIH, P.O. Box 12233, Research Triangle Park, NC 27709.

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