Cd^{2+} Toxicity in the Kidney: Inhibition of Zn-finger Transcription Factor Sp1

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A characteristic of human Cd^{2+} toxicity is the inability of the kidney to resorb glucose and other nutrients from the glomerular filtrate. Mouse kidney cortical (KC) cells incubated with Cd^{2+} lose their Na^{+}-dependent glucose transport (SGLT) activity in a concentration and time dependent manner. The depression of SGLT activity is paralleled by the down-regulation of SGLT1 and 2 mRNA. Since Cd^{2+} does not alter the biodegradation rate of the SGLT 1 message, it appears to inhibits the rate of mRNA synthesis. The sglt 1 gene and its upstream region have been cloned, revealing transcription factor binding sites in the promoter for HNF-1 and the Zn-finger protein, Sp1, as in the human and ovine promoters. Investigation of the functionality of the upstream region coupled to a luciferase reporter gene in KC cells showed that Cd^{2+} decreases the expression of luciferase. It also down-regulates thymidine kinase and SV-40 promoter-driven luciferase constructs that depend upon Sp1 binding for transcriptional activity. In control KC cell extract, Sp1 binds to a consensus Sp1 DNA binding site and the two Sp1 binding sites of sglt1 according to electrophoretic mobility shift assays. In contrast, Sp1 from Cd^{2+}-exposed cells has a significantly lower binding affinity for cognate DNA despite its similar cellular concentration. One mechanism may involve direct displacement of Zn^{2+} by Cd^{2+} in Sp-1. At stoichiometric concentrations of Sp1 and its DNA binding site, there is a Cd^{2+}-dependent reduction in Sp1-DNA binding in the same concentration range. Thus, a target of Cd^{2+} may be Sp1, itself. Nevertheless, treatment of Cd-treated KC cell extract with serine-threonine Protein Phosphatase I, at least partially restores Sp1 binding to its cognate sequences, pointing strongly to phosphorylation of Sp1 as a mechanism of inhibition of SGLT 1 mRNA expression. We hypothesize that the Zn_2-cysteine rich domain of protein kinase C (PKC) undergoes Cd^{2+}-Zn^{2+} exchange, leading to the activation of PKC. In turn, this stimulates a signal transduction pathway that results in the phosphorylation of Sp1. Consistent with this hypothesis, phorbol ester, a specific activator of PKC, inhibits glucose uptake by KC cells. The onset of SGLT1 and 2 inhibition occurs as the Cd-protective protein, metallothionein, is being synthesized by KC cells. The lack of protection afforded by MT will also be considered.
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