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IN VIVO EFFECTS OF CADMIUM ON RAT LIVER GLUCOCORTICOID RECEPTOR FUNCTIONAL PROPERTIES

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Abstract—1. Cadmium (Cd^{2+}) administered *in vivo* induced a 40% reduction of rat liver glucocorticoid receptor (GR) capacity and inhibition of glucocorticoid–receptor complexes binding to mouse mammary tumor virus (MMTV) DNA fragment containing GR consensus sequence.

2. The effect of Cd^{2+} on the GR binding activity can be reversed with DTT, suggesting Cd^{2+} interaction with thiol groups.

3. Cd^{2+}-related GR modification seems to be mediated by Cd^{2+} binding to cytoplasmic components included in the regulation of the receptor function, although the direct binding of the metal to the receptor thiols could not be ruled out.

INTRODUCTION

Heavy metals, such as cadmium, exert many toxic effects on living systems (for review see Chowdhury and Chandra, 1987), but the molecular mechanisms underlying their toxicity are not well understood. Cd^{2+} shows the affinity for thiol groups of proteins (Stacey, 1986), the best known example being the coordination of the metal by metallothionein, a protein playing an important role in Cd^{2+} metabolism and detoxification (Kagi and Hunziker, 1988; Waalkes and Goering, 1990). Several lines of evidence show that at least some of Cd^{2+} toxic effects are mediated by direct inhibition of enzymes containing essential thiols (Ahammadsahib *et al.*, 1989; Zhang *et al.*, 1990; Wahba *et al.*, 1990).

GRs from different sources contain 20 cystein residues (Hollenberg *et al.*, 1985; Miesfeld *et al.*, 1986; Danielsen *et al.*, 1986), 5 of which are localized within steroid-binding and 9 within DNA-binding domain of the molecule. Numerous *in vitro* studies employing thiol-reactive reagents have shown that sulfhydryl groups of the receptor are required for three important receptor functions: steroid binding (Grippo *et al.*, 1985; Simons *et al.*, 1987; Smith *et al.*, 1988), transformation (Tienrungroj *et al.*, 1987; Blicq *et al.*, 1988) and binding to DNA (Bodwell *et al.*, 1984a; Tienrungroj *et al.*, 1987). All of these functions could be inhibited by oxidizing agents which promote the formation of intramolecular disulfide bonds (Bresnick *et al.*, 1988; Silva and Cidlowski, 1989; Hutchison *et al.*, 1991). The evidence has been provided that sulfhydryl groups required for steroid binding are different from those necessary for DNA-binding of the receptor (Bodwell *et al.*, 1984b; Meshinchi *et al.*, 1990a). Miller and Simons (1988) have shown that two closely spaced thiols in the steroid-binding domain of the receptor are responsible for its steroid binding activity.

In regard to Cd^{2+} affinity for thiol groups, it is reasonable to predict the formation of the metal–dithiol adduct on the GR in the presence of Cd^{2+} and the resulting conformational change of the receptor which could influence its biological activity. Simons *et al.* (1990) have recently published the results of the *in vitro* studies showing that Cd^{2+} affects multiple functions of the GR from hepatoma tissue culture (HTC) cells, probably by binding to vicinal thiols in the steroid binding domain of the receptor. Since it is known that some thiol-containing cytoplasmic components, included in the maintenance of the redox equilibrium, play an important role in the regulation of the GR function (Grippo *et al.*, 1983, 1985), it is also possible to assume that Cd^{2+} might influence the activity of GR by an indirect mechanism.

The aim of the present study was to check for a possibility that the GR modification is a component of Cd^{2+} toxicity. This work summarizes the effects of *in vivo* Cd^{2+} administration on rat liver GR binding capacity and affinity, transformation and DNA-binding. The investigations along these lines could prove to be useful for better understanding of the mechanisms underlying Cd^{2+} toxic effects and of the structure and function of the GR.

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MATERIALS AND METHODS

Chemicals

[1,2,4(*n*)-^{3}H]Triamcinolone acetonide (TA, sp. act. 958 GBq/mmol), adenosine 5′-[γ-^{32}P]triphosphate, triethylammonium salt (ATP, sp. act. >185 TBq/mmol), T4 polynucleotide kinase (*E. coli B*), *Hind* III and *Bam* HI were obtained from Amersham International (Amersham, U.K.). Unlabeled TA, dextran 60, $CdCl_2$ and DNA-cellulose (double stranded calf thymus DNA) were purchased from Sigma Chemical Co. (St Louis, Mo., U.S.A.) and Chelex 100 chelating resin (200–400 mesh) from Bio-Rad (Richmond, Calif., U.S.A.).

Treatment of animals

Male Wistar rats (200–250 g) were reared under standard laboratory conditions with 12:12 hr light–dark cycle, at 22°C. Cadmium chloride dissolved in 0.9% saline was administered intraperitoneally at 2 mg Cd/kg. Control rats received saline. Animals were sacrificed by decapitation 2, 24 or 48 hr after the injection and livers were perfused *in situ* with cold saline.

Preparation of the cytosol

Liver homogenates were prepared in 2 vol (w/v) of cold buffer containing 1 mM EDTA and 10 mM Hepes, pH 7.35 and centrifuged at 6000 *g* for 10 min and at 150,000 *g* for 1 hr. The clear supernatants were stored in liquid nitrogen until use.

Equilibrium binding studies

Saturation analysis was performed by incubation (18 hr, 0°C) of triplicate cytosol aliquots (0.1 ml) with dried [^{3}H]TA at final concentrations ranging from 2 to 90 nM. Nonspecific binding was determined from the parallel incubations in 100-fold molar excess of radioinert TA. Unbound steroid was removed by dextran-charcoal competitive binding technique (Beato and Feigelson, 1972). The number and affinity of specific glucocorticoid binding sites were calculated by "Equilibrium Binding Data Analysis" computer program (Elsevier-BIOSOFT, Cambridge, U.K., 1985).

Dissociation kinetics

After labeling of the receptor with 100 nM [^{3}H]TA in the presence and absence of 10 μM TA at 0°C for 18 hr, the unbound steroid was removed by pelleted dextran–charcoal and 10 μM radioinert TA was added to preclude the reassociation of dissociated [^{3}H]TA with unactivated receptors. The cytosol was transferred to 37°C and the aliquots were withdrawn at zero time and at different time intervals for assaying the binding activity. Curve fitting and estimation of dissociation rate constants (k_{-1}) was done by "Kinetic" computer program (Elsevier-BIOSOFT, Cambridge, U.K., 1985).

Chelex-treatment of cytosol

Chelex-treatment of the cytosol was done at 0°C, essentially as described by Meshinchi *et al.* (1990b). Chelex 100 resin, extensively washed and equilibrated with 10 mM Hepes–1 mM EDTA, pH 7.35, was loaded in 10 ml syringe and centrifuged inside a 30 ml Corex tube at 10,000 *g* in order to remove the interstitial liquid. Liver cytosol was placed on top of the resin in 1:4 ratio (cytosol vol:resin vol), centrifuged immediately and collected in a clean tube.

DNA-cellulose binding assay

[^{3}H]TA–receptor complexes were formed by incubation (18 hr, 0°C) of 100 μl cytosol aliquots with 100 nM steroid. After transformation of the receptors to the DNA binding state by incubation for 45 min at 25°C, cytosols were mixed with 200 μl of 12.5% (v/v) DNA–cellulose suspension and incubation was continued for 45 min at 0°C, with occasional mixing. DNA–cellulose pellets were washed three times with 1 ml of homogenization buffer, resuspended in scintillation cocktail and measured for radioactivity.

Gel retardation analysis

Cytosols were pretreated with DNA–cellulose in order to remove a bulk on nonspecific DNA-binding proteins and charged with 100 nM TA at 0°C. GR content in each cytosol was determined in parallel incubations with 100 nM [^{3}H]TA ± 10 μM TA, by dextran–charcoal technique. The 50-bp *Hind* III–*Bam* HI fragments (GRE1) containing the sequence (−190/−160) of the promoter distal glucocorticoid binding site of mouse mammary tumor virus (MMTV) were isolated from the pTK.CAT 3 plasmid (Cato *et al.*, 1986) and 5′-labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase (Maniatis *et al.*, 1982). Labeled fragments (20,000 dpm) were incubated (30 min at 25°C) with the aliquots of heat-activated cytosols containing about 0.2 pmol of the receptor. The incubation was performed in a final volume of 40 μl containing 10 mM Hepes, pH 7.35, 1 mM EDTA, 150 mM NaCl and 0.4 μg of calf thymus DNA sheared by passage through a 30 G needle. The retarded fragments were resolved on 5% nondenaturing acrylamide gels prepared and run in Tris–borate buffer (Maniatis *et al.*, 1982) and visualized by autoradiography.

Miscellaneous

Protein content in the cytosol samples was determined by the method of Lowry *et al.* (1951). For radioactivity measurements the samples were directly introduced into 5–10 ml of OptiPhase "HiSafe" II scintillation cocktail and counted in 1219 Rackbeta liquid scintillation counter (LKB) at an efficiency of 45–55%, with automatic cpm/dpm calculation.

RESULTS

Equilibrium binding parameters of [^{3}H]TA interaction with the GR were determined in liver cytosols from rats sacrificed 2, 24 and 48 hr after single Cd^{2+} injection and compared to corresponding parameters of the control animals. Figure 1 shows the representative Scatchard plots for each cytosol. Statistically significant 40% decrease in [^{3}H]TA binding to the GR was registered in a group of animals treated with Cd^{2+} for 24 hr as compared to the controls ($P < 0.01$). Maximal number of available specific binding sites (B_{max}), estimated from three independent experiments done in triplicate, was 0.49 ± 0.03 and 0.29 ± 0.02 pmol/mg protein (mean ± SE) for control and rats treated with Cd^{2+} for 24 hr, respectively. GR binding capacity 2 hr after Cd^{2+} administration was unchanged in comparison to the control, while after 48 hr it was still under the control level, B_{max} being 0.45 ± 0.03 and 0.40 ± 0.03 pmol/mg protein, for 2 and 48 hr Cd^{2+} treatments, respectively.

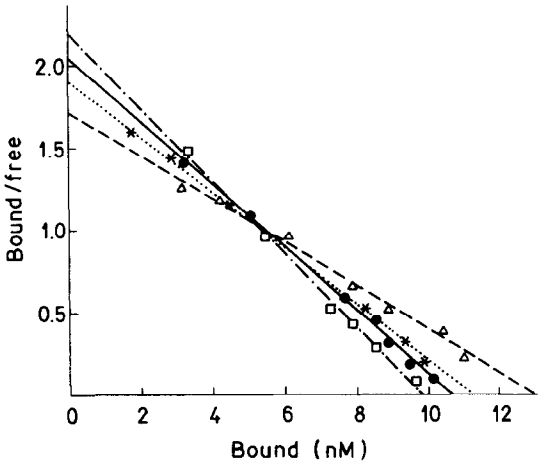

Fig. 1. Scatchard plots of [^{3}H]TA saturable binding. Saturation analysis was performed by incubating (18 hr, 0°C) aliquots of liver cytosol from control (●—●) and rats treated with Cd^{2+} for 2 (△---△), 24 (□-·-□) or 48 hr (★···★) before sacrifice with increasing concentrations of [^{3}H]TA ranging from 2–90 nM in the absence and presence of 100-fold molar excess of unlabeled steroid. Specific binding was measured by dextran–charcoal adsorption technique. The representative plots are presented.

No significant changes in the GR affinity for [^{3}H]TA among the experimental groups were found. The apparent equilibrium dissociation constants (K_d) were determined to be: 1.15 ± 0.08, 1.13 ± 0.18, 1.10 ± 0.21 and 0.89 ± 0.02 nM (mean ± SE) for controls and rats treated with Cd^{2+} for 2, 24 and 48 hr, respectively.

Figure 2 represents the time–course of [^{3}H]TA–receptor complexes dissociation in liver cytosols from control and Cd^{2+}-treated rats. Dissociation kinetics, followed at 37°C, conformed to first-order reaction and dissociation rate constants (k_{-1}) of $1.06 \pm 0.18 \times 10^{-2}$, $0.89 \pm 0.14 \times 10^{-2}$, $2.02 \pm 0.31 \times 10^{-2}$ and $0.75 \pm 0.12 \times 10^{-2}$ min^{-1} were determined as mean ± SE ($n = 3$) for control and rats treated with Cd^{2+} for 2, 24 and 48 hr, respectively. These results indicate that the stability of glucocorticoid–receptor complexes in rat liver cytosol was not affected by Cd^{2+} treatment and confirm that the affinity of the receptor for the steroid in the livers of Cd^{2+}-treated rats was unchanged in comparison with the controls.

In order to establish whether the reduction of the GR binding capacity observed in liver cytosol of rats treated with Cd^{2+} for 24 hr is mediated by Cd^{2+} binding to thiol groups, the reversal of this Cd^{2+} effect by dithiothreitol (DTT) was examined. It was found (Fig. 3) that the incubation for 3 hr at 0°C of liver cytosol from rats injected with $CdCl_2$ 24 hr before sacrifice with DTT concentrations between 1 and 5 mM partially reversed the Cd^{2+} inhibitory effect on [^{3}H]TA binding. At 10 mM DTT, Cd^{2+} effect was almost completely reversed. The experiments aimed to reveal the time–course of the reversal with DTT (data not shown) showed that 10 mM DTT acts almost instantaneously.

To approach the question whether Cd^{2+} influences the GR capacity by binding to the receptor itself, or by an indirect action through other cytoplasmic components, mixing experiments with cytosols from control and Cd^{2+}-injected animals were performed (Fig. 4). Before the mixing of the two cytosols, free metal was removed from Cd^{2+}-treated rat liver cytosol by means of metal chelating Chelex 100 resin. This procedure diminished the steroid binding in the cytosol for only about 8% (bar 3 vs bar 1). The results show that the reduction of the GR binding capacity in the liver cytosol of control rats achieved by the addition of Chelex-treated cytosol from Cd^{2+}-treated rats (bar 4 vs bar 3) was almost of the same magnitude as that seen after Cd^{2+} administration (bar 2 vs bar 1), suggesting that the inhibition could be accounted for by Cd^{2+} binding to the GR and to other cytoplasmic components, or solely by its binding to molecules other than the receptor. Further on, Chelex-treated cytosol from control and Cd^{2+}-treated rats was boiled, or presaturated with radioinert TA and then added to the cytosol of control rats. Since boiling or TA presaturation of the Chelex-treated control cytosol completely eliminated its [^{3}H]TA binding capacity, its addition to the control cytosol gave an expected overall inhibition of about 50% as compared to the control (bar 5 and bar 7 vs bar 3). An additional inhibition of about 15% (bar 6 vs bar

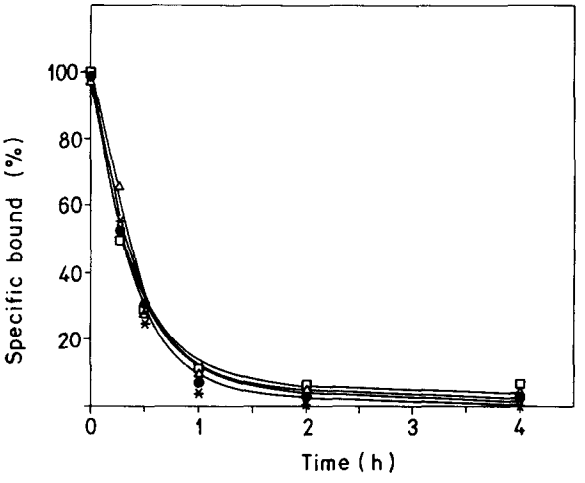

Fig. 2. Dissociation kinetics of [^{3}H]TA–receptor complexes. Liver cytosols from control (●—●) and rats injected with $CdCl_2$ 2 (△—△), 24 (□—□) and 48 hr (★—★) before the experiment, were preincubated at 0°C to equilibrium with 100 nM [^{3}H]TA in the presence and absence of unlabeled TA, cleared from the excess steroid and at zero time transferred to 37°C and supplemented with 10 μM TA. At indicated time intervals triplicate 0.1 ml aliquots were withdrawn and assayed for specific binding. The regression curves fitted to the binding data from three independent experiments by exponential regression program are shown. The values are presented as percent of specific binding of time zero in individual cytosols.

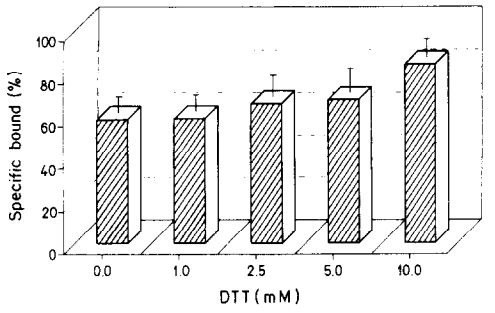

Fig. 3. Reversal of Cd^{2+} inhibition of [^{3}H]TA binding to the GR by DTT. Liver cytosols of rats treated with Cd^{2+} for 24 hr before the sacrifice were preincubated with the indicated concentrations of DTT at 0°C. After 3 hr, binding activity of the receptor was assayed by incubating the cytosols (2 hr, 0°C) with 100 nM [^{3}H]TA ± 10 μM TA and removing the free steroid by dextran–charcoal. The amount of specific [^{3}H]TA binding after DTT preincubation was expressed as a percentage of binding in the cytosol of control rats.

5) or 30% (bar 8 vs bar 7) was recorded after mixing the control cytosol with Chelex-treated boiled, or Chelex-treated TA presaturated cytosol from Cd^{2+}-treated rats, respectively. The obtained additional inhibition could be explained by Cd^{2+} binding to thermostabile and thermolabile cytoplasmic factors influencing the binding activity of the receptor, although direct binding of the metal to the receptor could not be ruled out.

The finding that Cd^{2+} administration affected the binding activity of rat liver GR prompted the examination of the receptor transformation and binding to DNA. Figure 5 shows that there were no significant differences in the binding of the transformed hepatic [^{3}H]TA–receptor complexes from different experimental groups to calf thymus DNA immobilized to cellulose.

However, when the transformed glucocorticoid–receptor complexes were incubated with a 50 bp DNA fragment of MMTV promoter region containing specific GR binding site (GRE1), considerable differences in the DNA binding between the complexes from control and Cd^{2+}-treated rats were noticed. Gel retardation analyses (Fig. 6) revealed several retarded bands. The appearance of only one band (indicated by the arrow) was strictly dependent on preincubation of the cytosol under the conditions that allow transformation of the GR to DNA-binding form. Therefore, this band was taken to represent GR–DNA complex. Glucocorticoid–receptor complexes derived from rats injected with Cd^{2+} 2 and 48 hr before the sacrifice showed the most prominent decrease in the ability of binding to the specific DNA response element, while binding of the complexes deriving from cytosol of animals treated with Cd^{2+} for 24 hr was inhibited to a lower extent in comparison with the controls.

DISCUSSION

There are no reports on *in vivo* effects of Cd^{2+} and very few are related to *in vitro* effects of Cd^{2+} on the steroid receptors (Colvard and Wilson, 1984; Freedman *et al.*, 1988; Simons *et al.*, 1990). The

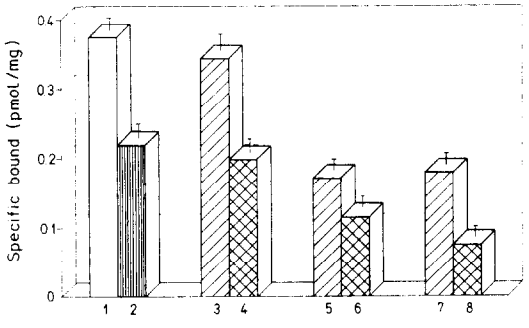

Fig. 4. The effect of thermolabile and thermostabile Cd^{2+}-binding components from Cd^{2+}-treated rat liver cytosol on [^{3}H]TA binding to untreated rat liver GR. Liver cytosol from control rats was mixed with an equal volume of Chelex-treated cytosol from control rats (▨) or Chelex-treated cytosol from rats injected with $CdCl_2$ 24 hr before sacrifice (■). Before the mixing Chelex-treated cytosols were either boiled (bar 5 and 6), presaturated with unlabeled TA (bar 7 and 8), or used without any additional treatment (bar 3 and 4). Specific binding was measured after 2 hr incubation at 0°C with 100 nM [^{3}H]TA ± 10 μM TA and expressed as pmol of bound steroid per mg of protein in the control cytosol. Bars 1 (□) and 2 (▥) show specific binding of [^{3}H]TA in control and Cd^{2+}-treated rat liver cytosol, respectively. The values represent the mean ± SE from three independent experiments done in triplicates.

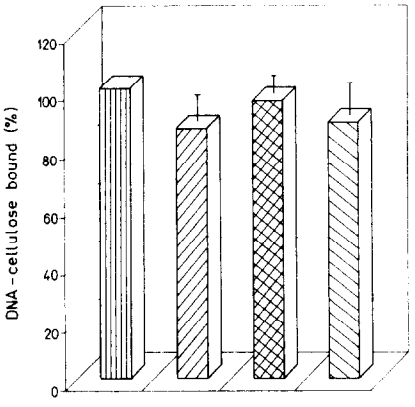

Fig. 5. Binding of [^{3}H]TA–receptor complexes to DNA–cellulose. Liver cytosols from control (▥) and rats injected with $CdCl_2$ 2 (▨), 24 (■) or 48 hr (▧) before the sacrifice were labeled 18 hr at 0°C with 100 nM [^{3}H]TA, cleared from the unbound steroid by dextran–charcoal and preincubated 45 min at 25°C. Triplicate 0.1 ml aliquots were incubated for 45 min at 0°C with DNA–cellulose and the radioactivity of the pellets was measured after three washes. The number of DNA-bound complexes was expressed as percentage of total number of [^{3}H]TA–receptor complexes determined in the respective cytosol. The values are mean ± SE from three independent experiments.

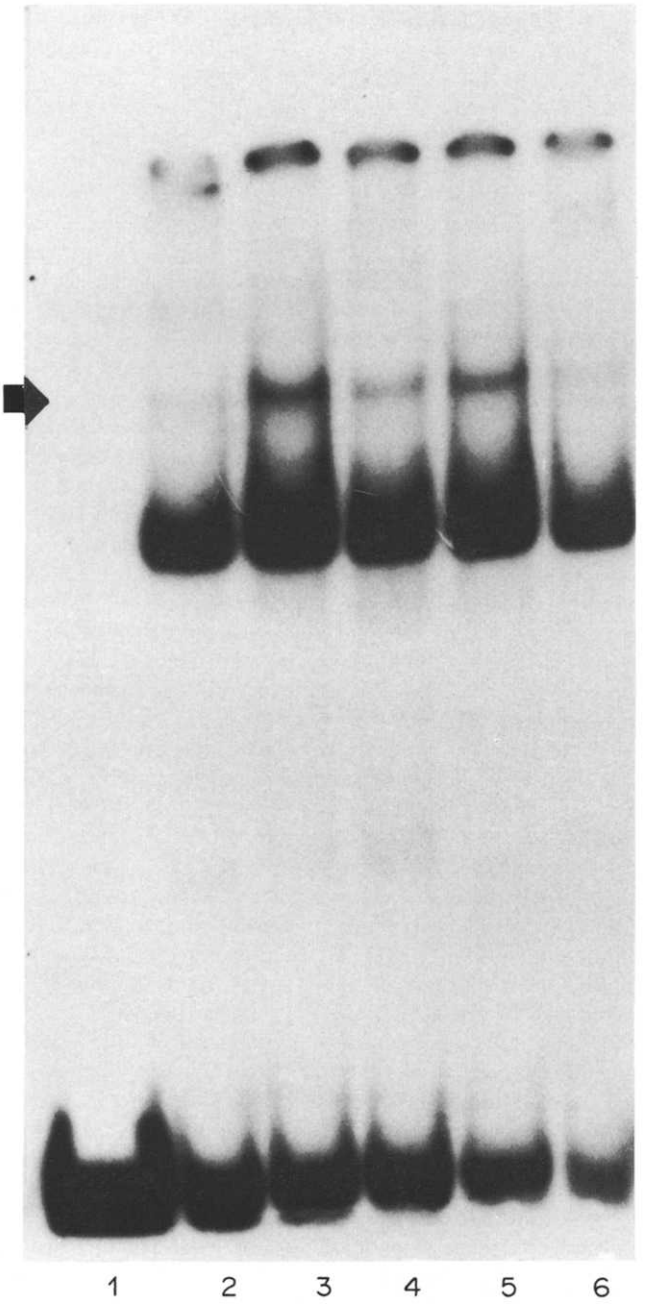

Fig. 6. Gel retardation analysis of the GR binding to a 50 bp MMTV DNA fragment harboring glucocorticoid response element (GRE). DNA–cellulose filtered liver cytosol from control (lane 3) and rats treated with Cd^{2+} for 2 (lane 4), 24 (lane 5) or 48 hr (lane 6) before the sacrifice were incubated with 100 nM TA to saturation and heat-activated for 45 min at 25°C. A parallel sample of the control cytosol (lane 2) was kept at 0°C. End-labeled MMTV DNA fragments (GRE1) harboring specific GR-binding site (20,000 dpm) were added to cytosol aliquots containing about 2 pmol of the GR and the incubation proceeded for 30 min at 25°C in 10 mM Hepes, pH 7.35 containing 1 mM EDTA, 150 mM NaCl and 10 ng/μl calf thymus DNA (final vol 40 μl). Retarded DNA fragments were resolved on 5% nondenaturing polyacrylamide gel and visualized by autoradiography. For lane 1 the binding buffer was used instead of cytosol. The band indicated by the arrow corresponds to the GR–GRE1 complex.

experimental results presented in this work show that *in vivo* administration of Cd^{2+} led to the decrease in binding capacity (Fig. 1) and ability of rat liver GR to bind to specific DNA binding sites (Fig. 6). The alterations in the receptor affinity for the steroid (Fig. 1) and in stability of the transformed glucocorticoid–receptor complexes (Fig. 2) were not detected upon Cd^{2+} injection.

Glucocorticoid binding in rat liver cytosol was reduced for about 40% 24 hr after a single intraperitoneal Cd^{2+} injection. The decline of Cd^{2+} effect, observed after 24 hr, may be connected to the sequestering of the metal by metallothionein, concentration of which is considerably higher 48 hr than 24 hr after Cd^{2+} injection (data not shown). Cd^{2+}-induced reduction of the GR capacity could be reversed by DTT (Fig. 3), providing strong evidence that the effect is based on Cd^{2+} reaction with thiol groups.

It is generally accepted that the binding activity of the GR could be altered in a rapid, reversible manner, modulating the capacity of a cell to respond to hormone. The regulation of GR binding activity is rather complex and involves synthesis and degradation of the receptor, migration of the hormone–receptor complexes to nuclei, phosphorylation of the receptor, oxido-reduction of the receptor thiols and influence of low and high molecular weight cytoplasmic factors. To explain the mechanism of Cd^{2+} action on GR binding properties, further investigation is necessary. It is important to undoubtedly establish whether Cd^{2+} binds directly to the receptor thiols, or to other cytoplasmic molecules among which the thermostabile and thermolabile modulators of the receptor function could be the most important candidates. In the case of direct binding of the metal to the receptor, the alteration of the receptor binding activity could be explained by a conformational change of the receptor affecting its steroid binding site. Recently, Simons *et al.* (1990) have shown that Cd^{2+} added to cytosol of HTC cells reacts with thiol groups, blocking steroid binding to the GR, destroying existing glucocorticoid–receptor complexes and decreasing the DNA binding of activated complexes. The observation that Cd^{2+} is able to block [^{3}H]dexamethasone 21-mesylate labeling of the receptor, but not of other thiol-containing proteins in the HTC cell cytosol led the authors to the proposal that Cd^{2+} effects were mediated by its binding to the vicinal dithiol group in the steroid binding domain of the receptor. However, the mixing experiments presented in this paper (Fig. 4) suggest that Cd^{2+}-induced decrease of the GR binding capacity might result from its binding to thiol groups of cytoplasmic components other than the receptor, although the direct binding to the receptor thiols can not be excluded. It has been shown (Grippo *et al.*, 1983, 1985) that rat liver cytosol contains an endogenous heat-stable reducing system, comprising of thioredoxin—a small, thermostabile, thiol-containing protein acting as a donor of reducing equivalents in protein reduction reactions, and NADPH. This system is involved in the regulation of GR function activating the receptors to the steroid binding form. On the basis of our results, it is reasonable to propose that Cd^{2+} administered *in vivo* could occupy thiol groups of thioredoxin, since the addition of metal-depleted boiled liver cytosol from Cd^{2+}-treated rats to the control one gave rise to an additional inhibition of the GR binding activity over that seen by the addition of Chelex-treated and boiled control cytosol (Fig. 4, bar 6 vs bar 5). Our results also imply the possibility that Cd^{2+} might bind to the thiol groups of the thermolabile molecules in the liver cytosol (Fig. 4, bar 8 vs bar 7). Since Cd^{2+} is a potent inhibitor of many thiol containing enzymes, it is likely that the reduction of the GR binding capacity could be a consequence of the metal binding to thioredoxin reductase and/or other enzymes involved in the receptor synthesis, degradation, phosphorylation and transport to the nuclei.

Heavy metals, like many other physical and chemical agents, elicit heat shock response (Bourmias-Vardiabasis *et al.*, 1990). New data from Sanchez (personal communication) show that arsenite poisoning and thermal stress induce translocation of unliganded GR to the nuclei, suggesting that GR nuclear binding may be inducible by any condition which can elicit cellular stress response. It would be interesting to examine migration of GR to the nuclei upon Cd^{2+} administration, since an increased nuclear translocation of the receptor could be the reason for the observed decreased glucocorticoid binding in the cytoplasm of liver cells. It is premature to draw any conclusion from the time–course of the changes in GR binding capacity and DNA-binding ability after *in vivo* administration of Cd^{2+}, but these data could prove to be useful for further elucidation of the mechanism of Cd^{2+} action.

As far as Cd^{2+} effects on steroid–receptor complexes transformation and DNA binding are concerned, the available data in the literature are rather obscure. Colvard and Wilson (1984) have shown that Cd^{2+} added *in vitro* had no influence on the androgen receptor–nuclear interactions and Freedman *et al.* (1990) have demonstrated that truncated GR, encompassing the DNA-binding domain of the molecule, could reversibly ligate two Cd^{2+} instead of two Zn^{2+} ions without any effect on specific DNA binding and proper folding of the receptor. However, Simons *et al.* (1990) stated that Cd^{2+} may decrease DNA binding of HTC cell glucocorticoid–receptor complexes. Our results show that DNA-binding ability of rat liver GR was diminished after Cd^{2+} injection, but the effect was obvious only when relatively short (50 bp) DNA fragment containing the GR consensus sequence was used (Fig. 6), probably because of the high level of nonspecific binding of the glucocorticoid–receptor complexes to calf thymus DNA that could mask Cd^{2+} effect (Fig. 5). It is not possible to conclude whether Cd^{2+} affects the process of the

receptor transformation, or the interaction of the transformed glucocorticoid–receptor complexes with DNA. The observed Cd^{2+}-induced decrease of the GR binding to specific DNA binding sites might result from Cd^{2+} binding to low and high molecular weight factors included in the regulation of the receptor function at the level of transformation and DNA binding, or by its binding to the receptor itself.

In conclusion, *in vivo* Cd^{2+} administration led to modification of some rat liver GR essential functions: capacity to bind the hormone and ability to bind to specific DNA sequences. These effects of Cd^{2+} are mediated by its binding to thiol groups of the receptor and/or to thiol groups of thermostabile and thermolabile cytoplasmic molecules included in the regulation of the receptor function.

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