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ACCUMULATION OF NEWLY SYNTHESIZED SERUM PROTEINS BY CADMIUM IN CULTURED RAT LIVER PARENCHYMAL CELLS

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Abstract—Effects of cadmium on secretion of the two major serum proteins, albumin and transferrin were studied in primary cultured rat liver parenchymal cells. Exposure to 7 μM cadmium for 8 hr did not inhibit synthesis of both albumin and transferrin whereas secretions of the two proteins were effectively depressed. In the cells exposed to cadmium serum type albumin and proalbumin were accumulated. Cadmium may affect intracellular processing of secretory proteins and also retard the discharge of these proteins resulting in inhibition of protein secretion from the liver parenchymal cells.

Cadmium (Cd) is a toxic element, which preferentially accumulates in the liver and kidney and impairs the functions of these organs [1]. Previously we found in *in vivo* studies that Cd depresses serum levels of albumin, total protein and serum cholinesterase (EC 3.1.1.8), an enzyme secreted from the liver at a relatively small dose [2–4]. As many serum proteins are synthesized in the liver and secreted into the bloodstream, these findings suggest that Cd inhibits the secretory function of the liver at the early stage of Cd intoxication. The mechanism for this inhibitory action of Cd seems important to understand hepatotoxicity of Cd at molecular level and needs to be clarified. Recently, using primary cultured rat liver parenchymal cells, we showed that secretion of total protein is inhibited by Cd at lower concentrations where cellular protein synthesis is not disturbed [5].

In the present study we investigated the effects of Cd on secretion of two major serum proteins, albumin and transferrin, in cultured rat liver parenchymal cells to elucidate in more detail how Cd inhibits protein secretion from the liver.

MATERIALS AND METHODS

Preparation of monolayer cultures of primary rat liver parenchymal cells

Rat liver parenchymal cells were isolated from male Wistar rats (body weight about 150 g) according to the method of Seglen [6]. The preparation and incubation of monolayer cultured parenchymal cells were carried out as described previously [5] except for extra additions of dexamethasone (10^{-6} M) and Hepes (0.025 M) to the culture medium (modified William's medium E supplemented with insulin (10^{-6} M), antibiotics, and 5% fetal calf serum [7]). All the following experiments were performed after a 21–22 hr culture.

Radiolabeling procedures

The cells were exposed to 0–20 μM Cd as $CdCl_2$ in the culture medium for 7–8 hr before radiolabeling.

(1) *Labeling with* [^{14}C]*leucine.* To examine synthesis and secretion of total protein the cells were washed twice with the medium without Cd, serum and leucine, and 0.85 ml of the same medium containing [^{14}C]leucine (270 mCi/m mole, ICN Radiochemicals, CA) at the concentration of 0.2 μCi/ml was added to the dishes (35-mm culture dishes, Falcon®, Becton Dickinson, CA). After incubation for 2 hr the cells were separated from the medium, washed twice with Hanks' balanced salt solution and lysed in 0.2 ml of lysis buffer (1% sodium deoxycholate and 1% Triton X-100). Trichloroacetic acid-insoluble materials in the cell lysate or the medium were collected on Whatmann GF/C® filters (Whatmann International Ltd., U.K.) and radioactivity was counted in a scintillation counter.

(2) *Labeling with* [^{35}S]*methionine.* For pulse-chase experiments the cells were washed twice with the medium without Cd, serum and methionine. A 0.5 ml solution of the same medium containing 40 μCi of [^{35}S]methionine (1100 Ci/mmol, New England Nuclear Co., MA) was added to each dish. After incubation for 10 min the medium was aspirated. The cells were washed twice with a phosphate buffered saline solution (PBS) and the medium supplemented with 300 mg/l methionine (0.85 ml) was added. Serum and Cd were not added during the actual pulse-chase experiments. At the end of the chase period, the cells and the medium were harvested as described above and used for immunoprecipitation.

Immunoprecipitation

Rabbit antisera raised against rat albumin and rat transferrin were purchased from Cappel Laboratories Inc. (PA). Either the cell lysate (50–150 μl) or the medium (100–200 μl) was allowed to react with 100 μl of anti-rat albumin antiserum (1:100) for 4 hr at 4° and the albumin–antibody complex was precipitated by addition of 150 μl of 10% (v/v) protein A-Sepharose 4B (Pharmacia Fine Chemicals, Sweden) as described by Johnston and Thrope [8].

For rat transferrin analysis anti-rat albumin antiserum was replaced with anti-rat transferrin antiserum (1:20). The immunoprecipitates were dissolved in sample buffer (1% sodium dodecyl sulfate–1% β-mercaptoethanol–0.06 M Tris–HCl (pH 6.8)–10% glycerol), incubated at 70° for 20 min and used for determination of radioactivity or gel isoelectric focusing.

Gel isoelectric focusing

Slab gel isoelectric focusing was performed by some modifications of the method described by Ames and Nikaido [9]. Samples prepared as described above were diluted with equal volumes of sample dilution buffer (2% Ampholines, 8% Nonidet P-40 and 9.5 M urea), absorbed into pieces of filter paper and laid on the surface of the gels (4% acrylamide gels containing 2% ampholines [pH 5–7:pH 3.5–10 = 4:1] and 8 M urea). Electrophoresis was performed initiating at 200 V followed by gradual increase to 1800 V and maintaining this voltage for 2 hr. Protein bands were fixed with 5% perchloric acid–5% sulfosalicylic acid and treated with Amplify® (Amersham, U.K.) before drying. Radioactive bands were detected by fluorography on X-ray films (X-O mat, Kodak). Chromatographically purified rat albumin (Cappel Laboratories, Inc.) was subjected to isoelectric focusing at the same time and stained with Coomasie Brilliant Blue.

RESULTS AND DISCUSSION

Total protein synthesis and secretion

Synthesis of total protein was not inhibited by exposure to Cd at the concentrations less than 20 μM (Fig. 1a). On the other hand protein secretion into the medium was depressed at concentrations of 5 μM and more (Fig. 1b). Since the inhibition occurred in the medium without Cd, it seems that the Cd accumulated in the cells affects the secretory process.

Synthesis and secretion of albumin and transferrin

It is known that intracellular proteins and secretory proteins are synthesized by different processes; the former is synthesized on free ribosomes, whereas the latter is synthesized on membrane-bound polysomes and secreted without passage through the cytosolic spaces [10]. Thus, to confirm that disturbance of protein synthesis is not responsible for Cd-induced inhibition of protein secretion, we further studied the effect of Cd on synthesis and secretion of albumin and transferrin, two major, well characterized secretory proteins from the liver. Figure 2 shows dose effect of Cd on synthesis and secretion of albumin. Synthesis of albumin was estimated by incorporation of [^{35}S]methionine into intracellular albumin during pulse-labeling for 10 min because newly synthesized albumin is not secreted within this time [11]. Exposure to Cd at concentrations of 0–7 μM did not disturb albumin synthesis (Fig. 2a). However, secretion of albumin was inhibited at Cd concentrations of 5 μM and 7 μM, with concomitant accumulation of newly synthesized albumin in the cells (Fig. 2b).

Time course of albumin secretion is shown in Fig. 3. In the control cells approximately 90% of newly synthesized albumin was secreted into the medium during chase period of 90 min. On the other hand, after the same period more than 50% of albumin was retained in the cells pre-exposed to 7 μM Cd for 8 hr.

Similar results were observed with transferrin (Table 1). Although the secretion rate of transferrin was slower than that of albumin, exposure to Cd depressed transferrin secretion without affecting the synthesis. After 90 min of the chase period 34% of newly synthesized transferrin was retained in the control cells while 55% was retained in the cells exposed to Cd.

These results support that Cd-induced inhibition of protein secretion from the liver is not caused by depression of protein synthesis. Cadmium may affect

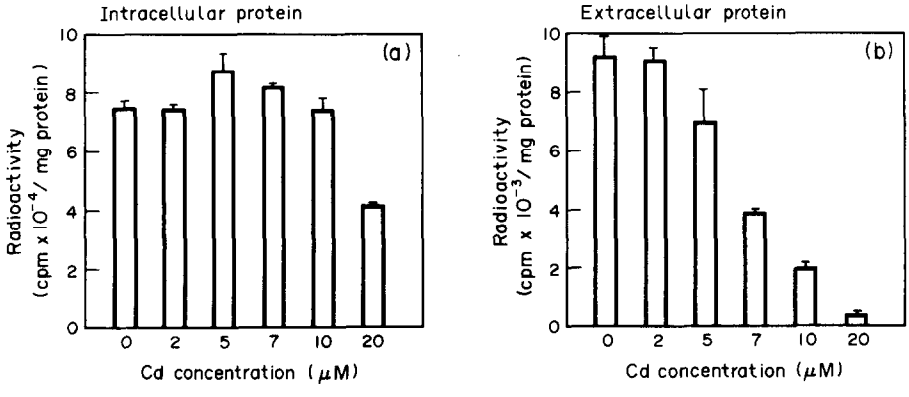

Fig. 1. Dose effects of Cd on total protein synthesis and secretion. After exposure to 0–20 μM Cd for 8 hr, rat liver parenchymal cells were labeled with [^{14}C]leucine (0.2 μCi/ml) for 2 hr in the absence of Cd. Total protein synthesis and secretion were measuring acid-insoluble radioactivity incorporated into the cells and the medium, respectively. The data represent mean ± SD of three monolayers.

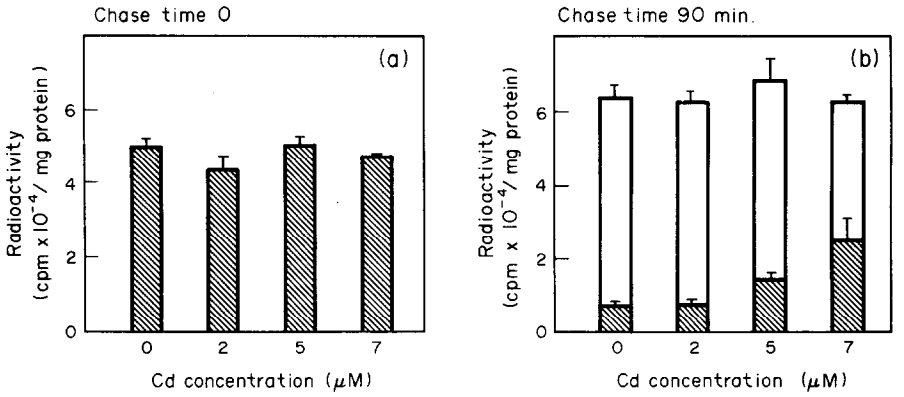

Fig. 2. Dose effects of Cd on synthesis and secretion of albumin. Rat liver parenchymal cells were preincubated with or without Cd for 8 hr and labeled with 40 μCi of [^{35}S]methionine for 10 min. (a) For the determination of albumin synthesis, the radioactivity in the immunoprecipitate prepared from the cell lysate was determined without chase. (b) For the determination of secretion of albumin, the parenchymal cells were chased for 90 min in the absence of Cd after the [^{35}S]methionine labeling. Secreted albumin and intracellular albumin were estimated from radioactivity in immunoprecipitate prepared from medium and cell lysate, respectively. Open and shaded portions of each bar represent the amounts of secreted albumin and intracellular albumin, respectively. The data represent mean ± SD of three monolayers.

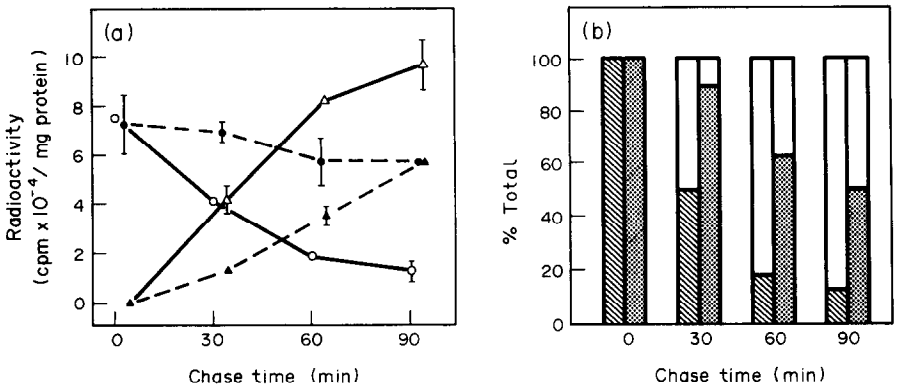

Fig. 3. Effect of Cd on secretion of albumin. Rat liver parenchymal cells were exposed to Cd and radiolabeled as described in the legend to Fig. 2 and chased for indicated periods. Radioactivity of intracellular and medium albumin were measured (a) and intracellular albumin was expressed as percentage of total synthesized albumin (b). The data represent mean ± SD of three monolayers. Open circle and shaded bar, intracellular albumin of control cells; closed circle and dotted bar, intracellular albumin of Cd treated cells; open triangle, medium albumin of control cells; closed triangle, medium albumin of Cd treated cells.

Table 1. Effect of Cd on transferrin secretion in cultured rat liver parenchymal cells

Chase time (min)		Radioactivity (cpm/mg cellular protein)		
		Intracellular transferrin (A)	Medium transferrin (B)	$\frac{A}{A+B}$ (%)
0	Control	10,800 ± 600	—	(100)
	Cd	10,100 ± 1600	—	(100)
30	Control	8130 ± 2420	2310 ± 180	78
	Cd	8150 ± 3650	1830 ± 1000	82
60	Control	5110 ± 2100	7210 ± 2260	42
	Cd	7240 ± 1420	4900 ± 3400	60
90	Control	5180 ± 1620	10,000 ± 1030	34
	Cd	8010 ± 2990	6670 ± 1420	55

Rat liver parenchymal cells were preincubated with or without Cd, pulse-labeled with [^{35}S]methionine and chased as described in the legend to Fig. 3. Intracellular and extracellular transferrin were immunoprecipitated and counted for radioactivity. Data were expressed as mean ± SD for three monolayers.

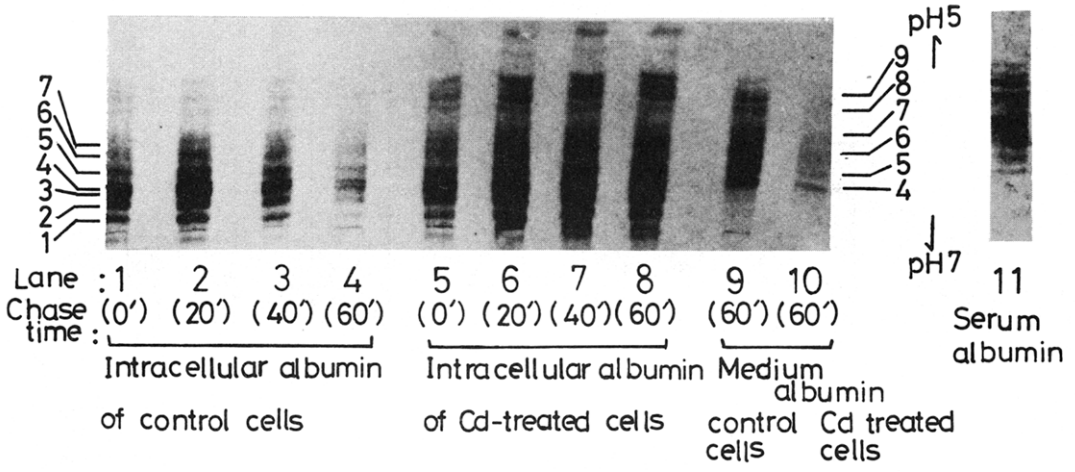

Fig. 4. Effect of Cd on gel isoelectric focusing profile of intracellular and medium albumin. The cells preincubated with or without Cd for 7 hr were radiolabeled as described in the legend to Fig. 2 and chased for indicated periods. Intracellular and medium albumin were immunoprecipitated and subjected to slab gel isoelectric focusing followed by fluorography. Authentic serum albumin was also co-electrofocused and stained (lane 11).

other secretory processes, e.g. intracellular transport of secretory proteins from the sites of synthesis to secretory vesicles and/or final discharge into bloodstream.

Effects of Cd *on conversion of proalbumin to serum type albumin*

Albumin exists for a time in the liver cell as an intermediate precursor termed proalbumin [12]. Conversion of proalbumin to serum albumin by removal of a N-terminal hexapeptide occurs at the Golgi apparatus [13]. Therefore we examined the effect of Cd on intracellular processing of proalbumin to serum albumin to find out the target sites for the inhibitory action of Cd.

Proalbumin and serum albumin are not immunologically distinguishable but the two albumins can be separated by ion-exchange chromatography or isoelectric focusing [14, 15]. Thus, we analyzed intracellular and medium albumins labeled with [^{35}S]methionine by gel isoelectric focusing followed by fluorography (Fig. 4). Intracellular albumin at the chase time 0 was resolved into four major bands and three minor bands (Fig. 4 lanes 1 and 5), while medium albumin at the chase time 60 min was resolved into four major bands and two or more minor bands (Fig. 4 lanes 9 and 10). These bands were numbered in the order of basicity as shown in Fig. 4. Since the bands 4–9 detected in the medium were almost identical with those chromatographically purified serum albumin (Fig. 4 lane 11), these bands were assigned to the bands of serum albumin. Proalbumin is somewhat more basic than serum albumin [16]. Thus the bands 1–3, isoelectric points of which were higher than that of serum albumin (bands 4–7), were assigned proalbumin. On assumption of these assignments we can explain our results as follows. In the control cells ratio of proalbumin to serum type albumin was decreased and both proteins were diminished with time. On the other hand, in the cells exposed to Cd both proalbumin and serum type albumin were retained in the cells and serum type albumin was only slightly detected in the medium.

These results suggest that Cd depresses both the intracellular processing of proalbumin to serum type albumin and the discharge of serum type albumin outside the cells. Either one or both depression may be responsible for the inhibition of protein secretion from the liver.

Since Cd inhibits not only albumin secretion but also secretion of transferrin and probably other secretory proteins, which undergo different intracellular processing, there should be a common mechanism in such depressive action of Cd. Further studies are necessary on intracellular localization of unsecreted proteins and their accumulated forms to reveal an exact mechanism for Cd-induced inhibition of protein secretion from the liver.

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