Influence of Adrenalectomy and Dexamethasone on Rat Liver Metallothionein and Superoxide Dismutase Activity

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There is increasing evidence that the hepatic metabolism of both copper and zinc is regulated by hormones [1]. For example, glucocorticoids influence the synthesis of the metal binding proteins caeruloplasmin [2] and metallothionein [3–6]. The influence of glucocorticoids on synthesis of hepatic copper–zinc superoxide dismutase (SOD), however, has not been previously studied. The technique that is frequently used to examine these relationships is chromatographic separation of tissue extracts. Gel filtration chromatography resolves rat liver cytosol into three major zinc-containing peaks [5–7]. The highest molecular weight fraction contains the bulk of the zinc metalloproteins. The intermediate peak has been assumed to represent a mixture of proteins including carbonic anhydrase and SOD [8]. Etzel *et al.* observed that the amount of zinc bound by this intermediate peak was sensitive to treatment by glucocorticoids [5]. In particular, dexamethasone treatment markedly decreased the amount of zinc bound in this peak and substantially increased zinc associated with metallothionein, the third chromatographic peak. It has also been found that expression of the metallothionein gene is enhanced in liver cells by this glucocorticoid [5, 6]. This induction may be responsible in part for the redistribution of hepatic zinc observed in response to dexamethasone.

In order to define further how these glucocorticoid-dependent changes in copper and zinc distribution influence cellular function and to ascertain if SOD is involved, we have determined the SOD activity of the chromatographic fractions. SOD has a molecular weight of approximately 31,000 daltons and contains two zinc and two copper ions per molecule. The working hypothesis for these experiments was that the dexamethasone-induced change in zinc redistribution might influence SOD zinc and thus, alter cytoplasmic SOD activity.

Experimental

Male rats of the CD strain (250 ± 50 g) were maintained on a commercial diet and tap water. Bilateral adrenalectomy (ADX) was executed under sodium pentobarbetol anesthesia by the paravertebral dorsal approach [5]. ADX rats received 0.9% NaCl in place of drinking water and were used for experiments seven days after surgery. Dexamethasone was administered ip (2 mg/kg) twelve hours before sacrifice. Livers were homogenized (1:1, w/v) in ice-cold, 0.25 *M* sucrose (10 m*M* Tris-acetate; pH 7.4). A 166,000 g supernatant fraction was prepared and subjected to gel filtration chromatography (Sephadex G-75; 2.5 × 60 cm column). These fractionation procedures are the same as described previously [5], except NaN_3 was not included in any of the buffers. Copper and zinc content of fractions was measured by atomic absorption spectrophotometry. Protein concentration was measured by the dye binding procedure [9]. SOD activity was determined by measuring the inhibition of 6-hydroxy dopamine autoxidation [10]. Each treatment was replicated at least twice.

Results and Discussion

Chromatography resolved the liver cytosol into two or three zinc containing peaks depending on the hormone treatment. Apparent average molecular weights for these peaks were >65,000 (I), 34,000 (II) and 11,000 (III) based on comparison with standards [11]. Peak III has been well characterized and contains metallothionein (MT). In rats not treated with dexamethasone, peak III is typically absent or very small. As shown in Fig. 1, dexamethasone treatment markedly increased MT-bound zinc. This response involves *de novo* synthesis of this cytoplasmic metal

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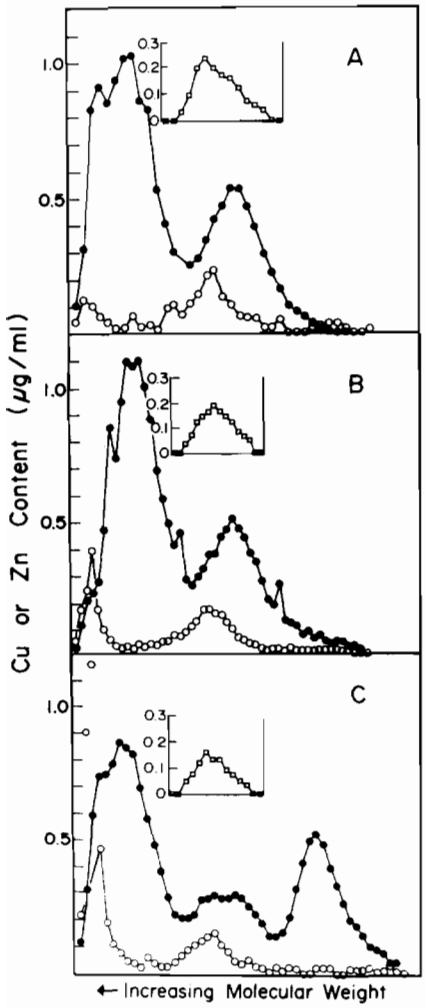

Fig. 1. Gel filtration chromatography of liver cytosol prepared from control (A), adrenalectomized (B), or adrenalectomized-dexamethasone treated (C) rats. Cu (○) and Zn (●) content was measured by atomic absorption spectrophotometry. SOD activity was determined by the inhibition of 6-hydroxy dopamine autoxidation. SOD concentrations (□, see insert) were calculated by comparison of the SOD activity of the sample with the SOD activity of a standard solution of bovine liver SOD (Diagnostic Data Inc.) of known concentration. The three peaks (see C) represent fractionation ranges of >65,000, 34,000 (average) or 11,000.

binding protein [5]. It is of interest that dexamethasone does not enhance the binding of copper to MT. This is in agreement with the suggestion of Cousins and Weiner that glucocorticoids influence hepatic copper metabolism mainly via secretion of copper from hepatocytes as caeruloplasmin [2]. The only SOD activity detected was in peak II and this was on the higher molecular weight range of that peak (see inserts, Fig. 1). The amount of SOD activity in the individual chromatographic fractions was closely correlated to copper content. Assuming that all of the SOD activity in peak II was due to Cu, Zn SOD, we were able to calculate the copper and zinc content in peak II that can be attributed to this protein alone. In each treatment group the (Total Cu):(SOD Cu) ratio was approximately 1.0, (based upon an SOD content of about 300 mg/ml calculated from activity). However, the (Total Zn):(SOD Zn) ratio was between 2.8 and 3.9 indicating that peak II contains a significant amount of non-SOD protein. We were also able to determine that, based on its activity, Cu, Zn SOD represents 6.5–7.0% of the total protein in liver cytosol for treated rats and for controls. Typical cytosol preparations contained 45–50 mg total protein prior to chromatography. The total amount of SOD per experiment (determined by its activity) in mg divided by the total concentration of cytosol preparation used was 3.10–3.24 mg/ml. It is clear from Fig. 1 that neither ADX nor dexamethasone treatment of ADX rats had a significant effect on the level of Cu, Zn SOD in peak II. We conclude, therefore, the decrease in zinc content of peak II must be associated with changes in the concentrations of zinc metalloproteins other than Cu, Zn SOD, but in that same molecular weight range.

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