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THE EFFECT OF ADRENALECTOMY ON ZINC THIONEIN LEVELS IN RAT LIVER

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SUMMARY The concentrations of zinc thionein and cytosolic zinc in rat liver were examined in male rats five days after bilateral adrenalectomy. Zinc in metallothionein increased 10 fold, as compared with control animals. Cytosolic zinc increased 79% as compared with controls. 65% of this increase could be accounted for bound to metallothionein. Sham operated animals after five days showed a 4 fold increase in hepatic zinc thionein and a 23% increase in cytosolic zinc, 71% of this increase being bound to metallothionein. Adrenalectomized rats, maintained on daily injections of corticosterone (4mg/100g b.w.), exhibited the same levels of zinc thionein and cytosolic zinc as adrenalectomized rats receiving no treatment. Adrenalectomized rats, maintained on daily injections of aldosterone (5μg/100g b.w.), exhibited the same levels of zinc thionein as the sham operated rats, but the cytosolic zinc remained elevated at the level found in adrenalectomized rats receiving no treatment. These results indicate that there is adrenal involvement in the control of hepatic zinc and zinc thionein levels in the rat.

Metallothionein is a 6500 dalton molecular weight protein, found in eucaryotic tissues, which binds 6-7 g-atoms of metal (Cd, Zn, Cu) per mole protein. It has been implicated as functioning in cadmium detoxification and in zinc and copper homeostasis. Several reviews are available which deal with the properties and potential functions of metallothionein (1-3).

Recently, reports have appeared which indicate that the levels of hepatic zinc and zinc thionein are susceptible to changes caused by various environmental stresses. Oh, et al. (4) reported the induction of zinc thionein in the livers of rats subjected to the stresses of cold environment, hot environment, heat burn, strenuous exercise, and CCl_4 intoxication. Concomitant with increases in hepatic zinc thionein, a general decline in plasma zinc levels was seen. Stresses involving inflammation and infection have been reported to affect zinc distribu-

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tion within the rat(5), and this has been shown to be correlated with changes in hepatic zinc thionein levels(6).

Cousins' group(7-10) has studied the effects of glucocorticoids on zinc, cadmium, and copper uptake by isolated rat liver parenchymal cells in primary monolayer culture. Dexamethasone in the presence of insulin or glucagon stimulated a 48% (7) and a 106% (8) increase in zinc uptake, a 93% increase in cadmium uptake (9), and a 35% increase in copper uptake (10) by liver cells. Aldosterone had no effect on zinc uptake (8). Stimulation by dexamethasone of metallothionein synthesis in HeLa cell cultures has also been reported (11). Daily prednisolone administration for three weeks, however, was reported to decrease weight gains and liver zinc concentrations in male weanling rats (12).

In view of these reported effects of glucocorticoids on the uptake and levels of zinc in rat liver and the subsequent changes in zinc thionein levels, a study was designed to determine the effects of adrenalectomy with and without steroid replacement therapy on the levels of zinc and zinc thionein in rat liver cytosol.

MATERIALS AND METHODS

Male, Sprague-Dawley rats, 350-450g, were obtained from Sasco, Inc., Omaha, Nebraska. The rats were housed in wire bottomed cages in a temperature and light controlled room and were maintained on Purina rat chow and tap water ad libitum. Following adrenalectomy, rats were maintained on 5% glucose in saline instead of tap water.

Aldosterone, corticosterone, and Tris·HCl were obtained from Sigma Chemical Co. Liver cytosol was obtained by homogenization of frozen livers in two volumes of 0.02 M Tris·HCl, pH 8.6, 0.25 M sucrose and centrifugation at 4° at 40,000 rpm in a Type 50.2 Ti rotor in a Beckman L5-65 centrifuge to obtain the high speed supernatant or cytosol. Zinc was determined by flame atomic absorption spectrometry, using a Perkin Elmer Model 303. Zinc thionein was quantitated using atomic absorption analysis of the eluate obtained from Sephadex G-75 column chromatography of liver cytosol (13-17). The zinc content of liver cytosol was determined by direct atomic absorption analysis of 100 fold diluted samples.

Rats were bilaterally adrenalectomized under ether anesthesia. Anesthetized rats were secured in the prone position on a surgical board. Their backs were shaved and cleaned with 70% ethanol. Bilateral incisions were made just below the last rib and lateral to the deep back muscles. The adrenals were located just above the exposed kidneys

and removed by pinching off with a curved forceps. After removal of the adrenals the posterior abdominal wall muscle layer was stitched and the skin wound was sealed with stainless steel autoclips. The surface on and around the incision was coated with bacitracin ointment. The entire process required 10-15 minutes. Rats were housed individually and maintained on Purina rat chow and 5% glucose in saline ad libitum before killing by decapitation on day 5. No animals who survived the surgery died during the five days post adrenalectomy. Sham operated animals received similar surgical and postsurgical treatment except the adrenals were not removed. Stock animals, maintained on Purina rat chow and tap water ad libitum, served as controls.

Two groups of adrenalectomized animals received daily steroid therapy, either corticosterone (4mg/100g b.w.) or aldosterone (5μg/100 g b. w.) intraperitoneally on days 1,2,3, and 4 postadrenalectomy. Corticosterone was administered as a suspension in olive oil, containing 5% ethanol, and aldosterone was administered in saline. All surgical procedures, injections, and killings were done between 1000 and 1200 hours to avoid effects due to circadian changes in endogenous hormones. Serum corticosterone levels were determined using a fluorometric assay (18). Serum aldosterone levels were not determined.

Differences between means were statistically evaluated using Fisher's "t" test of significance (19).

RESULTS

The levels of zinc in liver cytosol and in liver zinc thionein increased significantly following sham operation and even greater increases were seen following adrenalectomy (Table 1). When compared with control animals, zinc in hepatic metallothionein increased 4 fold in sham operated animals and 10 fold in adrenalectomized animals, while zinc in liver cytosol increased 23% and 79% respectively, in these same groups. On an absolute basis, as compared to controls, 71.1% of the increase in cytosolic zinc is accounted for bound to zinc thionein in the sham operated group and 64.7% in the adrenalectomized group.

Daily corticosterone administration did not significantly decrease the hepatic zinc thionein ($P < 0.4$) or cytosolic zinc ($P < 0.2$) levels, as compared with adrenalectomized animals receiving no treatment. In contrast daily aldosterone treatment significantly decreased hepatic zinc thionein levels ($P < 0.001$), as compared with adrenalectomized animals, reaching the levels of sham operated animals ($P < 0.9$). Surprisingly, cytosolic zinc levels in aldosterone treated adrenalecto-

Table 1: Zinc concentrations in Hepatic Cytosol and Metallothionein

Group	N	Zinc in Metallothionein[1] (µg/g liver)	Zinc in Cytosol[1] (µg/g liver)	Percent of Cytosolic Zinc Increase in Metallothionein
Control	4	0.65 ± 0.09	12.02 ± 0.69	-
Sham Operated	4	2.64 ± 0.42	14.82 ± 0.50	71.1
Adrenalectomized	6	6.79 ± 0.71	21.51 ± 0.59	64.7
Adrenalectomized + Corticosterone	8	5.77 ± 0.70	19.62 ± 1.38	67.4
Adrenalectomized + Aldosterone	7	2.69 ± 0.29	22.26 ± 0.25	19.9

[1] Values are presented as the mean ± standard error.

Table 2: "t" Test of Significance of Differences Between Means, P<

Group	Control	Sham	Adx	Adx + Corticosterone	Adx + Aldosterone
Control	-	0.01[1]	0.001	0.001	0.001
Sham	0.02[2]	-	0.01	0.02	0.9
Adx	0.001	0.001	-	0.4	0.001
Adx + Corticosterone	0.01	0.05	0.2	-	0.01
Adx + Aldosterone	0.001	0.001	0.4	0.2	-

[1] Upper right hand numbers are comparisons between groups for the zinc in metallothionein.

[2] Lower left hand numbers are comparisons between groups for the zinc in cytosol.

mized animals did not decrease in parallel with the zinc thionein levels, remaining instead at the same levels as the adrenalectomized animals who received no steroid treatment ($P < 0.4$). The complete set of "t" tests of significance of differences between means for all the groups is presented in Table 2.

Plasma corticosterone levels were 7.4, 8.8, and 7.6 μg/100 ml respectively, in controls, sham operated, and adrenalectomized animals receiving daily corticosterone treatment. In adrenalectomized animals receiving no treatment and in adrenalectomized animals receiving daily aldosterone treatment plasma corticosterone levels decreased to 0.5-1.5 μg/100 ml. Daily consumption of 5% glucose in saline in the drinking water should have eliminated any sodium or glucose effects due to lack of glucocorticoids and mineralocorticoids in the various groups.

DISCUSSION

Adrenalectomy dramatically increases the levels of cytosolic zinc and zinc thionein in rat liver, when examined five days after surgery. Sham operation also increases these levels, but not as greatly as does adrenalectomy. The stress caused by surgery is sufficient to cause a mobilization of zinc to the liver, as was seen in previous studies from this laboratory (14,15), in which levels of cytosolic zinc of 15.4-21.6 μg/g liver were attained in livers surgically removed under ether anesthesia for isolated liver perfusion. These levels are reached in adrenalectomized rat livers in this study, probably during and after surgery, but the levels of cytosolic zinc do not return to control levels after five days, as do the sham operated animals. Hormonal control of the entry of zinc into the liver, and its subsequent incorporation into zinc thionein, during the stress of surgery, may involve epinephrine, produced by the adrenal medulla. This question is currently being pursued in this laboratory.

The question of hormonal control of the exit of zinc from the liver seems to be more complicated. Adrenalectomized rats, receiving corticosterone or aldosterone therapy, maintain their elevated levels of cytosolic zinc in the liver five days after surgery. Corticosterone does not affect the levels of hepatic zinc thionein, but aldosterone lowers these levels to that found in sham operated animals. All the surgically treated animals received 5% glucose in saline as drinking water; consequently, effects due to sodium or glucose imbalances should have been minimized.

Our results with corticosterone therapy cannot be directly compared with reports on the stimulation of zinc uptake in isolated hepatocytes by dexamethasone (7,8), as these cells may have started at a normal level of zinc, while our studies were with livers which had already taken up extrahepatic zinc. On the other hand one of these studies (7) used cortisone and found that this natural form of the steroid does not stimulate zinc uptake. Recently, dexamethasone has been shown to stimulate metallothionein production in HeLa cells, while physiological levels of hydrocortisone did not (11). The explanation for this differential effect may relate to the greater potency of dexamethasone, which is not metabolized, and the lesser potency of hydrocortisone, which is metabolized. Perhaps the strong influence of both ether anesthesia and surgical stress on ACTH secretion, followed by marked increases in corticosterone synthesis and release (20), may account for the transitory elevation of hepatic metallothionein and zinc in the sham operated animals in our study. If this is true, then the elevated levels of zinc thionein seen in sham operated animals may be caused by a totally different mechanism than that in adrenalectomized animals.

It appears that aldosterone may have an inhibitory influence on metallothionein levels just as progesterone does (11). Whether this is a direct influence on metallothionein production or an indirect effect

mediated either by zinc availability or the binding capability of metallothionein remains to be investigated.

The basic questions remain concerning the mechanisms of control of the uptake of zinc by the liver, and what subsequent signals, or removal of signals, are necessary to allow the levels of hepatic cytosolic zinc to return to normal. The involvement of zinc thionein in binding a great deal of this transitory zinc is quite clear, but the hormonal control of zinc uptake and release by the liver must be investigated in more detail.

While this manuscript was in preparation, another report (21) appeared in which adrenalectomized rats receiving a single dose of dexamethasone at least 6 days after surgery showed a 50% increase in zinc thionein 14 hours after injection, as compared to controls.

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