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Effect of Dexamethasone and Epinephrine on Metallothionein Level in the Perfused Rat Liver

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The effect of dexamethasone (10^{-5}M) and epinephrine (10^{-6}M) on the biosynthesis of metallothionein (MT) in the perfused rat liver was investigated. MT synthesis was determined by measuring the incorporation of ^{14}C-L-aspartic acid into liver MT fraction after the perfusion for five hours of isolated liver by artificial blood containing ^{14}C-L-U-aspartic acid (0.2uci) with dexamethosone or epinephrine.

MT was isolated by Sephadex G-75 column chromatography and DEAE Sephadex column chromatography. Incorporation of radioactive ^{14}C into the MT fraction of perfused liver cytosol (9.0grams of liver) from dexamethasone treated, epinephrine treated and control groups were, respectively, 0.72, 0.34 and 0.33% of total radioactivity infused. Total protein content in the MT fraction of liver perfused with dexamethasone and epinephrine were 0.80, 0.64mg/g liver compared to 0.52mg/g liver in the control. MT, a protein having a high content of cystein and metals is synthesized in the perfused rat liver and its induction is stimulated by dexamethasone, while epinephrine increased the accumulation of Zn in the MT fraction of the perfused rat liver.

The present experiment confirms that MT synthesis and degradation are somewhat regulated by glucocorticoid hormone and epinephrine.

Metallothionein (MT), a soluble low molecular weight protein(−10,000 MW) has been described by Kägi and Vallee (1961). The protein contains high levels of cystein and various kind of metals(Zn, Cd, Hg, Cu), and it was identified in several tissues of various kinds of animals (Bremner and Young, 1976; Chen *et al.*, 1975; Margoshes and Vallee, 1957: Nordberg *et al.*, 1971). Although the biological function of MT has not been defined, several investigators (Nordberg, 1972, Yoshikawa, 1970) proposed its role in heavy metal detoxification while others (Bremner and Davies, 1975; Chen *et al.*, 1974; Richards and Cousine, 1975) insisted on its involvement in the normal Zn metabolism.

MT has been shown to be induced not only by metals but also by a variety of stresses employed or by restriction of food intake (Bremner and Davies, 1975; Oh *et al.*, 1978; Shaikh and Smith, 1976). It has been also demonstrated that MT levels in liver and kidney of rats are markedly influenced by

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the age of the animals and the metabolism of Zn, including the protein moiety of MT, was also dependent on the age (Oh *et al.*, 1979)

Glucocorticoids have been shown to influence the Zn uptake by HeLa cell cultures (Cox, 1963). Recently, Karin and Herschman, (1979) have reported that dexamethasone stimulates MT synthesis in HeLa cell cultures. Secretion of glucocorticoid hormone, together with epinephrine, is known to be changed under stressful conditions and both hormones are increased in circulating blood during gluconeogenesis (Cox, 1968; Hales, 1967).

The present study describes the influence of dexamethasone and epinephrine on the MT synthesis in perfused rat liver and a possible role of these hormones in the regulation of MT synthesis is discussed.

METERIALS AND METHODS

Nine female rats weighing 170-210g were fed with a normal diet (Commercial broiler diet) supplemented with 500ppm Zn as $ZnSO_4$ for two weeks before sacrifice.

Sepadex G-75 and DEAE Sephadex (A-50) were purchased from Pharmacia Fine Chemicals Co., Sweden and ^{14}C-L-Aspartic acid was purchased from Amersham, England. Dexamethasone was supplied by Dong-Hwa Pharmaceutical Co., Seoul, Korea and epinephrine was supplied by Dae Han Pharmaceutical Co. Seoul, Korea. Bovine serum albumin used in liver perfusate was purchased from Sigma Chem. Co. U.S.A.

Three rats were used for each group; control, dexamethasone treated and epinephrine treated.

Perfusion of isolated rat liver

The liver from each rat was removed after cannulating the bile duct, hepatic artery and vein under ether anesthesia. The isolated liver was perfused by a modified method of Miller (1951). The perfusate is composed of 80ml of Ringer solution, 25ml of packed bovine erythrocytes, 2.4g of bovine serum albumin, 2ml of heparin and 20 umoles of all amino acids (L-from) except L-as-partic acid. 0.2ml of ^{14}C-L-aspartic acid (1.0 uci/ml, specific activity of 100 uci/umole) was added to the perfusate at the beginning of perfusion. For the dexamethasone treated group, 0.02ml of dexamethasone (5mg/ml) was infused into the circulating blood and 5 ul of epinephrine (0.1%solution) was used for the epinephrine treated group. The control group did not receive any of these hormones. Livers were perfused for 5 hours under physiolegical conditions (pH 7.4 37'C under O_2)

Isolation and Quantitation of MT

For the isolation of MT, each perfused liver was washed with 300ml of isotonic saline solution in the perfusion system. Three gram portions of 3 individual livers in each group were pooled and homogenized in a Potter Elvejhem glass homogenizer with 2 volumes of 0.005M Tris HCI buffer(pH 8.6). The homogenate was then centrifuged at 10,000g for 10min. The supernatant was further centrifuged at 100,000g for 90 min to obtain the soluble fraction. Ten mililiters of the soluble fraction were chromatographed on a Sephadex G-75 column (2.2×90cm). One ml aliquot from each elution fraction was counted for ^{14}C radioactivity in a Packard liquid scintillation counter(Model 3320). Zn content in each fraction was determined by the

Table 1. **Effect of Dexamethasone and Epinephrine on MT Synthesis in Perfused Rat Livers***

Treatment	Zn content (ug)		Radioactivity		% ^{14}C incorporated into total MT	Total MT (mg)
	MT I	MT II	MT I	MT II		
			cpm	cpm		
Control	12.1	14.0	560	615	0.33+	4.68
+Dexamethasone	18.0	19.1	905	1623	0.72	7.20
+Epinephrine	22.3	25.4	420	784	0.34	5.76

* All values represent 9.0g of pooled sample from each3.0g of 3 livers perfused for 5 hours.
+ Values are % ^{14}C in MT for 9.0g of liver of total ^{14}C (0.2 uCi) infused.

Dithizon Method II (Rand *et al.*, 1976).

The MT fraction obtained from Sephadex G-75 column chromatography was further purified by DEAE sephadex (A-50) column (2.0×14.0cm) chromatography. To quantitate the protein content in the final fractions (MTs), each peak corresponding to MT was pooled and subjected to freeze drying. The dried sample was then weighed by chemical balance and the total protein was calculated. Radioactivities in each sample were also counted and expressed as cpm/g liver.

RESULTS

MT was synthesized in the perfused rat liver, regardless of the presence of dexamethasone or epinephrine, as evidenced by the incorporation of ^{14}C -L-aspartic acid into the MT fraction of the control group. However, the infusion of dexamethasone increased the ^{14}C -L-aspartic acid incorporation into the MT fraction with increased Zn accumulation. The infusion of epinephrine to the liver caused a marked increase in Zn accumulation in MT with a small increase in ^{14}C incorporation into it (Table 1). Zn concentrations and ^{14}C activities in the high molecular weight protein peaks (Fig. 1.) were similar between groups. Zn concentration in MT fractions of both hormone treated groups were much higher as compared to the control. Accumulation of Zn in the MT fraction in livers of the epinephrine treated group was most pronounced.

As shown in Fig. 1, the increased amount of ^{14}C-radioactivity was observed especially in the MT fraction of livers treated with dexamethasone, indicating the stimulation of MT synthesis by dexamethasone was greater than that by epinephrine.

The response to dexamethasone was 214% of the control and to the epinephrine was only 102% of the control.

Two types of MT (MTI, MT II) appeared when the MT fraction from Sephadex G-75 column chromatography was further chromatographed on a DEAE-Sephadex column. The results are shown on Fig 2 and were confirmed in many previous studies (Nordberg *et al.*, 1972, Bremner and Davies, 1975).

Both MT types (MT I, MT II), obtained from DEAE Sephadex chromatography, showed little absorption at 280nm (Fig. 2) which is chracteristic for the typical MT's. There were slight differences in protein and Zn content between the two MT types, but MT II had a higher Zn concentration than MT I (Table 1).

DISCUSSION

MT synthesis is in a dynamic state in the rat liver and its induction is influenced by

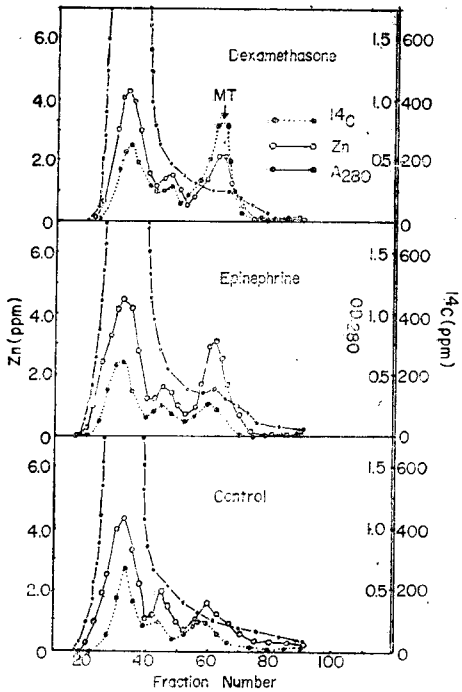

Fig. 1. Effect of dexamethasone and epinephrine on the metallothionein synthesis in rat liver. Liver cytosol from each group were applied on Sephadex G-75 column (2.2×90cm) and eluted with 0.05M Tris HCl buffer (pH 8.6) at a flow rate of 20ml/hour.

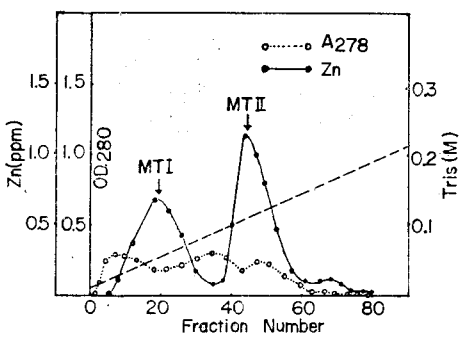

Fig. 2. DEAE Sephadex (A-50) column chromatography of metallothionein fraction obtained from Sephadex G-75 column chromatography. Samples are diluted 10 time with 0.005 M Tris HCl buffer (pH8.6) then applied on a column (2.0×14.0cm). The column was eluted with a linear gradient of 0.005M to 0.30MTris HCl buffer (pH 8.6)at a flow rate of 40ml/hour.

many nutritional and physiological factors. The increased ^{14}C-L-aspartic acid incorporation into MT of livers treated with dexamethasone or epinephrine in the present study implies the possible involvement of these hormones in the regulation of MT synthesis. A previous study (Oh *et al.*, 1979) on MT induction by a variety of stresses could be explained as the consequence of the release of hormones under stressful conditions because stress usually increases the concentration of glucocorticoid hormone and epinephrine in circulating blood (Hodges *et al.*, 1962).

Zn accumulation in the MT fraction has been considered an estimation of MT content by many investigators (Feldman and Cousins, 1976; Oh *et al.*, 1978). Because MT contains no aromatic aminoacids in its composition, it has been a difficult problem to quantitate the amount by conventional analysis methods. Therefore, the measurement of Zn concentration in MT fraction would be an useful mean to estimate the MT content. The increased amount of Zn in MT of liver treated with hormones in the present study may be an event resulting from an increased protein synthesis or a decreased degradation of MT. The increased amount of ^{14}C incorporation into the MT fraction in rat liver perfused for 5 hours would represent an increase in synthetic rate of MT rather than a decreased degradation rate, because the half life of rat liver MT is much longer than 5 hours. The half life of rat liver MT has been reported to be about 1.2 day (Oh *et al.*, 1978). The use of ^{14}C-L-aspartic acid in the labelling of MT in the present study was employed because there is a considerable amount of aspartic acid in MT (7% of total residues). Leucine content in MT represents only 0.3 or 1.0% of total amino acid residues (Bremner and Davies, 1975).

Therefore, ^{14}C-leucine may not be recommended for the labelling of MT. ^{14}C-cystein or ^{35}S-methionine are widely used in labelling of MT. Inclusion of epinephrine in the perfusate caused a marked increase in Zn content with small increase in ^{14}C incorporation into MT in the present study. From this result, we can assume that epinephrine has somewhat different mode of action from glucocorticoid hormone in the regulation of MT metabolism.

It should not be excluded from the consideration that epinephrine may have an inhibitory effect on MT degradation or that L-aspartate is more actively used for gluconeogenesis during the perfusion. The purity of isolated MT through DEAE Sephadex chromatography was questionable, so the homogeneity of MT I and MT II was tested by polyacrylamide disc gel electrophoresis. Both MT types showed a major single band with minor impurities, and MT II was more homogeneous than MT I (unpublished data). Although the role of MT in heavy metal metabolism is emphasized, its synthesis and degradation are influenced by hormones which are related to glucose metabolism. Lee *et al.* (1978) reported that dexamethasone pretreatment alleviated endotoxin-indued hepatocellular injury. The protective effect of dexamethasone against cell membrane damage is implicated. Therefore, the possible involvement of MT in the stabilization of membrane integrity by protecting the cells from various stresses is suggested.

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