The Inductive Effect of Cadmium on Protein Synthesis of Rat Intestine

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INTRODUCTION

There are many reports about the localization of cadmium in the liver and kidney (NORDBERG et al 1972a; SHAIKH et al 1972a). The cumulative property of cadmium is explained by the distribution of the cadmium binding protein, thionein, in these organs (SHAIKH et al 1972b; WISNIEWSKA-KNYPL et al 1971). Thionein has a high affinity for cadmium because of the abundance of SH groups. It is also known that cadmium induces thionein in the liver and kidney and cadmium toxicity is masked by a trapping effect of the protein (SHAIKH et al 1970; WISNIEWSKA-KNYPL et al 1970).

In this report, the inductive effect of cadmium is shown in intestinal protein containing thionein.

METHOD

The experiment was carried out on male albino rats of the Wistar strain. They were fed with commercial rat laboratory chow (Oriental Kobo Co.). Cadmium chloride was given in the driking water for 105 days in concentration of 0, 50 or 100 ppm. After starvation overnight, animals were anesthesized with ether and intravenously injected with ^{3}H-cystine (4μC$_i$/100g body weight, RCC, Amersham). At 3 hours after the injection, intestine was taken out 30cm length from the pyloric end. It was homogenized with 3 volumes of 135mM NaCl-30mM Tris HCl buffer pH 7.4, using a teflon homogenizer. A soluble fraction, obtained as the supernatant of 105,000G centrifugation for 1 hour, was further fractionated by EtOH-CHCl$_3$ (PULIDO et al 1966). After the precipitant by EtOH-CHCl$_3$ was centrifuged off, the protein in this supernatant was recovered by TCA (EtOH sup. protein). Cadmium was determined with an atomic absorption spectrometer (Hitachi 208 type) and ^{3}H was counted on a liquid scintillation spectrometer (Horiba LS-700 type). All data were analyzed statistically by t-test or Welch's test.

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RESULT

The intestine weight, expressed in grams, 2.34±0.38 (M±SD) of the Cd-50 ppm group and 2.36±0.31 of the Cd-100 ppm group were significantly increased, compared with 1.71±0.20 of the Cd-0 ppm group. As shown in TABLE 1, the relative weight of intestine (mg/g body weight) was also increased in experimental groups, especially in the Cd-100 ppm group.

When these intestines were homogenized with 3 volumes buffer per weight, the concentration of protein in the soluble fraction did not decrease but increased. There may be several reasons that explain the increase of intestinal weight. In this case, edema does not appear to be the explanation.

TABLE 1

Cadmium in drinking water (ppm)	Rat (head)	Weight of intestine (mg/g body weight)	Soluble protein (mg/ml)	Cadmium in soluble fraction (10^{-1} µg/mg protein)
0	7	4.94±1.23	10.4±0.9	0.1±0.1
50	6	5.79±0.80	11.8±2.2	1.2±0.3**
100	7	7.17±1.37*	12.9±2.0*	2.2±0.7**

30cm of the upper part of the intestine was sampled. These data represent M±SD and were analyzed statistically by t-test. Asterisks * and ** represent $P<0.05$ and $P<0.01$ respectively.

TABLE 2

Cadmium in drinking water (ppm)	Total protein of EtOH sup. (mg)	Incorporation of ^{3}H-cystine (cpm/mg EtOH sup.protein)	^{3}H-cystine in serum (cpm/µl serum)
0	0.85±0.35	498± 91	16.5±2.2
50	1.24±0.64	703±172*	18.5±1.7
100	1.68±0.65*	1048±308**	16.4±1.8

EtOH sup. was obtained by the fractionation of soluble fraction with EtOH- $CHCl_3$.
For details see TABLE 1.

In this experiment, protein synthesis of soluble fraction was investigated. In particular, the EtOH-supernatant fraction, containing thionein, was examined with ^{3}H-cystine. As the result of cadmium intake, specific activity (cpm/mg protein) was increased (TABLE 2). In the Cd-100 ppm group, both specific activity and total protein were increased two-fold compared with the Cd-0 ppm group.

DISCUSSION

Eighteen years ago, metallothionein was first isolated from equine renal cortex by MARGOSHES et al (1957). Thereafter it has been reported in liver, duodenum, spleen, pancreas, blood and so on. The protein, thionein, is characteristic in amino acid composition (NORDBERG et al 1972b). SH groups constitute about 30% so that thionein has high affinity for heavy metals, especially for cadmium. It has been confirmed that when cadmium is injected, thionein is induced in the liver and kidney. This phenomenon is interpreted as the body defence mechamism against toxic cadmium ions.

In an investigation of the mode of copper absorption from the gastrointestinal tract of chicks, STARCHER (1969) reported the presence of thionein in the duodenum. EVANS et al (1970) reported the protein in bovine duodenum, too. Recently TANAKA et al (1973) fed rats with water containing 50-200 ppm cadmium for 30 days. In the gel filtration of intestinal mucosa, a cadmium peak appeared in the position of thionein. But in their study there are no data about protein induction itself.

In this experiment, the inductive effect of cadmium was confirmed by the study of ^{3}H-cystine incorporation. In the EtOH supernatant fraction of the Cd-100 ppm group, specific activity was significantly increased two-fold, compared with the Cd-0 ppm group. The recovery of total protein was also doubled.

There are many articles on the toxic effect of cadmium on the gastrointestinal tract, for example, effects on the absorption of calcium, phosphorus, copper,glucose and alanine. After feeding rats with cadmium-containing food, a significant decrease in calcium absorption followed by a slight restoration has been observed by the authors (SUGAWARA et al 1974).

Because of its high affinity for cadmium, the induced thionein possibly has a masking effect in the intestine, too.

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