—SHORT COMMUNICATIONS

EFFECTS OF CADMIUM ON RAT HEPATIC POLYAMINES

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It is well known that ingested cadmium accumulates mainly in the liver and kidney and creates functional disturbances (1, 2), however, the mechanisms of this toxic action remain obscure. Previously, we demonstrated that cadmium induces an increase in rat hepatic polyamines (3). Recently, however, Kacew et al. reported that cadmium exposure enhanced DNA biosynthesis while depressing polyamine formation in rat liver (4). Recent biochemical studies have suggested that increases in polyamine concentrations usually precede or are simultaneous with increases in nucleic acid and protein levels in tissues (5). The purpose of this study was to determine the effects of cadmium on rat hepatic polyamines in cases of acute toxicity, so induced in rats.

Male Wistar rats weighing about 200 g were used. After s.c. administration of 0.01 mM, 0.025 mM or 0.05 mM/kg CdCl₂ (aqueous solution, 0.1 ml injected/100 g), the animals were decapitated, always between the hours of 2:00 and 3:00 p.m.. On the other hand, to study the earlier changes of polyamine concentrations, a second group of rats was given i.p. 0.005 mM/kg CdCl₂ and sacrificed after 1–48 hr. A portion of the liver was immediately excised and frozen on dry-ice. The polyamine extraction procedure was carried out using Endo & Ogura's method (6). The extracted mixture was applied on a CM-Cellulose column (0.6×11 cm) previously equilibrated with phosphate buffer pH 6.2. After washing with the same buffer, polyamines were separated in a stepwise elution of borate buffers, as follows: (1) 0.02 M, pH 8.0, (2) 0.02 M, pH 8.0 containing 0.02 M NaCl, (3) 0.02 M, pH 8.0 containing 0.05 M NaCl (putrescine was eluted), (4) 0.02 M, pH 9.0, (5) 0.02 M, pH 9.0 containing 0.06 M NaCl (spermidine was separated), and (6) 0.02 M, pH 9.0 containing 0.15 M NaCl (spermine was eluted). The amount of putrescine was measured fluorimetrically according to the method of Endo (7). Spermidine and spermine were determined spectrophotometrically by the TNBS method (8). Results were expressed as nmole/g wet weight of the tissues.

The figure shows the hepatic polyamine changes induced by cadmium administration (0.05 mM/kg, s.c.). After 6 hr, putrescine content reached its first maximum (110.9 ± 27.52 nmole/g) and, after 24 hr, again increased. The putrescine contents at 6 hr induced by cadmium (0.01 mM or 0.025 mM/kg, s.c.) were 56.8 ± 17.07 and 74.5 ± 10.18 nmole/g. These results suggest that hepatic putrescine content changes dose-dependently following increases in the dose of cadmium. On the other hand, spermidine increased slowly and reached a

maximum at 48 hr and then decreased, whereas spermine showed a reverse response (Fig. 1). This indicates that cadmium induced spermidine accumulation in liver. Table 1 shows the earlier changes induced by 0.005 mM/kg CdCl_2 (0.56 mg Cd/kg, i.p.). Putrescine reached a maximum at 6 hr, whereas spermidine changes were very slight. This suggests that hepatic putrescine formation responds sensitively to cadmium exposure.

Kacew et al. reported that cadmium depressed hepatic polyamine synthesis and did not affect putrescine contents (4), whereas we found that cadmium induced increases of both putrescine and spermidine. These differences seemed to be caused by differences in the dose of cadmium and the experimental time. We previously demonstrated that large dose injections of cadmium, which induced severe histological changes, decreased polyamine contents in the rat liver and testis (3). Kacew et al gave CdCl_2 (1 mg/kg, i.p., twice daily) repeatedly and they observed severe hepatic lesions (4, 9).

FIG. 1. Time course of polyamine changes in rat liver (mean $\pm$ S.D.), after CdCl_2 (0.05 mM/kg, s.c.).

TABLE I. Hepatic Content of Polyamines After Cadmium Chloride (0.56 mg Cd/kg, i.p.)

Time After Injection (hr)	No. of animals	Putrescine (nmole/g)	Spermidine (nmole/g)	Spermine (nmole/g)
CONTROL	8	26.7 $\pm$ 9.1 ^a	970.5 $\pm$ 72.0 ^a	557.3 $\pm$ 66.7 ^a
1	7	34.4 $\pm$ 9.7	846.7 $\pm$ 76.8	426.5 $\pm$ 113.5
3	7	47.5 $\pm$ 15.3*	871.0 $\pm$ 112.0	432.2 $\pm$ 53.8*
6	7	66.9 $\pm$ 15.1**	968.2 $\pm$ 118.7	532.0 $\pm$ 69.7
12	7	30.5 $\pm$ 8.8	939.3 $\pm$ 118.0	638.5 $\pm$ 21.0
24	5	47.7 $\pm$ 21.1	1128.6 $\pm$ 242.5	567.0 $\pm$ 38.3
48	5	42.6 $\pm$ 4.0	1006.8 $\pm$ 47.7	529.2 $\pm$ 50.3

^aMean $\pm$ S.D. Statistically significant differences between the control (mean) and experimental values. $p < 0.02^*$, $< 0.01^{**}$.

Heby et al indicated that spermidine accumulation is an event primarily associated with an activation of cell metabolism (10) and other workers have suggested that the peak rate of metallothionein synthesis in the liver occurs 6–12 hr after cadmium exposure (11). In the present study, increases of hepatic putrescine and spermidine occurred between 6–48 hr following a single injection of cadmium. This result is consistent with the possibility that cadmium exposure enhances protein synthesis in the liver. The cause of increases in polyamines and their relationship, if any, to the possible induction of metallothionein synthesis in liver is now under investigation.

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A SIMPLE METHOD FOR MEASURING DEPTH AND DURATION OF ANESTHESIA OF LOCAL ANESTHETICS USING THE MENTAL NERVE BLOCK IN GUINEA PIGS

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The anesthetic potency of local anesthetics has been evaluated by various indexes, such as duration, onset, minimum effective concentration, spread or depth of anesthesia, etc. (1–3). Of these indexes, “depth of anesthesia” is often used as a parameter that expresses the