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Degradation and re-synthesis of injected liver cadmium-thioneins in rat kidney

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Metallothionein has been considered to be a protective protein for the toxicity of heavy metals [1]. Contrary to the assumed biological role, Nordberg *et al.* [2], Cherian *et al.* [3], and Webb and Etienne [4] demonstrated the selective and more apparent toxicity of the injected metallothionein to renal tubular lining cells compared to cadmium ion. Recently we have compared the lesions induced by the injection of metallothioneins with differing Cd/Zn ratios and the time-dependent changes of the distribution patterns of cadmium, zinc, and copper among protein fractions in the kidneys after the injection [5]. From the results we suggested that the lesion induced by the injection of metallothionein is not due to metallothionein itself but due to the cadmium ion liberated from the degraded metallothionein in the kidneys.

The present study was intended to confirm that the injected metallothionein is degraded in the kidneys very shortly after the injection and that the liberated cadmium ion from the degraded protein induces the biosynthesis of metallothionein in the kidneys.

Cadmium-thionein-I and -II were prepared by replacing zinc in rat liver metallothionein with cadmium and separating on Sephadex G-75 and on DEAE Sephadex A-25 columns as reported already [5, 6]. The concentration of each cadmium-thionein solution was adjusted to 47.5 μg Cd/ml (Zn and Cu, less than 1 μg/ml; concentration of Tris–HCl buffer solution, 10–15 mM) by ultrafiltration on a Diaflo UM-10 membrane (Amicon). Each cadmium-thionein solution was injected intraperitoneally into 15 female rats of the Wistar strain (body wt, 135.7 ± 3.4 g, mean ± S.D.), respectively. The animals (5 rats/group) were killed 30 min, 1, and 3 days after the injection.

The metallothionein obtained from the kidneys of rats killed 30 min after the injection of cadmium-thionein-I or -II was separated into two forms on a DEAE Sephadex A-25

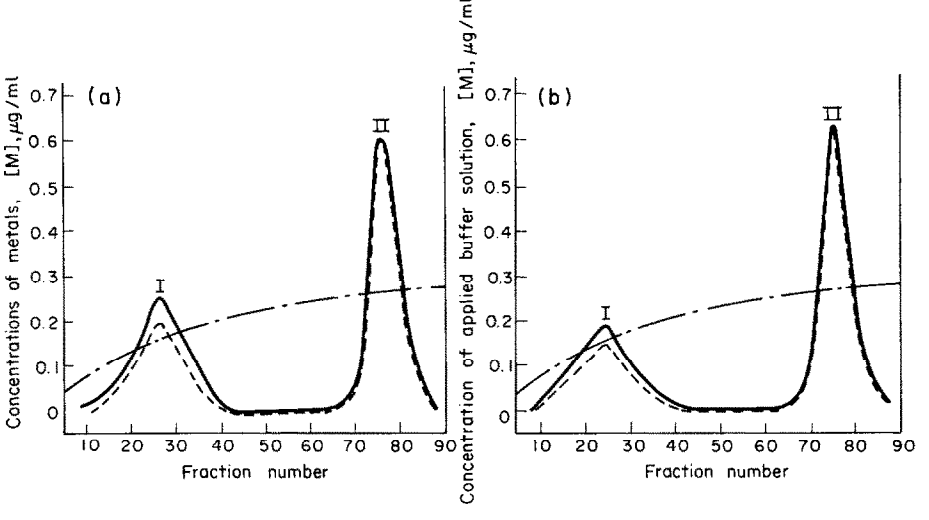

Fig. 1. DEAE Sephadex A-25 elution profiles of kidney metallothioneins obtained three days after the injection of cadmium-thionein-I (a) or -II (b). Five rats in each group were sacrificed 3 days after the injection of cadmium-thionein-I or -II. The kidneys in each group were combined, homogenized in three times the volume of 0.1 M Tris buffer solution (pH 7.4, 0.25 M glucose) using a Teflon homogenizer, and centrifuged at 105,000 *g* for 90 min at 2–4°. Each supernatant fraction was applied to a Sephadex G-75 column (5 × 80 cm) and eluted with 1 mM Tris buffer solution (pH 8.6). The metallothionein fraction was applied to a DEAE Sephadex A-25 column (1.5 × 28 cm) without concentration (100–120 ml). Two forms of metallothionein were eluted by a concentration gradient of Tris buffer solution (pH 8.6) between 1 mM (100 ml) and 300 mM (300 ml) after washing with 1 mM Tris buffer solution (pH 8.6) and collected (2.5 ml/tube). Metals were analyzed by a Hitachi 508 Atomic Absorption Spectrophotometer in each eluate. The curves are as follows: ——, Cd; — —, Zn; and — · —, applied buffer solution.

column and similar elution profiles were observed for two groups. The recovery of cadmium as metallothionein was only a few per cent of the applied amount in both groups and this suggested the possibility that the recovered cadmium as metallothionein 30 min after the injection was incorporated by exchange with zinc in the endogenous zinc-thionein. The result indicated that the injected cadmium-thioneins were degraded at least to a certain extent within 30 min after the injection.

The amount of cadmium recovered as metallothionein from the kidneys one day after the injection increased more than that for 30 min after the injection. The recovered metallothioneins in both groups were mixtures of the two forms which bonded not only with cadmium but also with zinc.

Figure 1 shows the elution patterns of kidney metallothioneins obtained three days after the injection of cadmium-thionein-I or -II. The two elution profiles were similar and both forms of metallothionein bonded not only with cadmium but also with a large amount of zinc.

Although the primary amino acid sequences of both forms of rat metallothionein have not been determined, the sequences of the two forms were assumed to be different as in the case of equine kidney metallothioneins [7] and mouse liver metallothioneins [8, 9]. Therefore, the interconversion of the two forms cannot occur and the two forms cannot be obtained unless the synthesis occurs *de novo*. As the affinity of zinc to thionein is 3000 times weaker than that of cadmium [10], the replacement of cadmium in metallothionein by zinc is not possible unless the synthesis of metallothionein occurs *de novo*. Therefore, degradation of the injected cadmium-thioneins and resynthesis of metallothionein in the kidneys were confirmed. The metallothionein induced by the injection of cadmium-thionein was rich in zinc (and low in copper content) as liver metallothionein induced by injection of cadmium ion and was different from kidney metallothionein (which is rich in copper) induced by injection of cadmium ion [11].

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Beta-adrenoceptor blocking activity of 1-substituted trimetoquinol analogs

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Trimetoquinol (TMQ; 6,7-dihydroxy-1-[3′,4′,5′-trimethoxybenzyl]-1,2,3,4-tetrahydroisoquinoline) was first reported to be a potent beta-adrenoceptor stimulant by Iwasawa and Kiyomoto [1]. Later work with the stereoisomers of TMQ demonstrated that much of the pharmacological activity in beta-adrenoceptor systems resides in the *S*(−)-isomer [2–4]. TMQ has been shown to be equipotent at both $beta_1$- and $beta_2$-adrenoceptors and nearly equipotent to isoproterenol on lipolysis [2], cAMP accumulation in isolated rat adipocytes [4] and the stimulation of adenylate cyclase from isolated rat adipocyte plasmalemma.*

In an attempt to develop more potent and/or more selective adrenergic agents within the tetrahydroisoquinoline (THI) series, Miller *et al.* [5] examined the effect of increasing the size of the substituent at the 1-carbon position of TMQ by synthesizing 1-methyltrimetoquinol (1-methyl TMQ) and 1-benzyltrimetoquinol (1-benzyl TMQ). Miller *et al.* [5] found neither compound capable of inducing significant tracheal relaxation or lipolysis ($beta_2$ and $beta_1$ respectively). In the right atrium preparation, 1-methyl TMQ was a weak agonist while 1-benzyl TMQ was inactive. Of interest was the fact that, in the guinea pig, 1-benzyl TMQ was a competitive antagonist of TMQ-induced increases in chronotropy (right atria) and was ineffective in tracheal relaxation [5].

This study was performed to obtain a clearer profile of the structural aspects of the adrenoceptor activity and biochemical mechanisms possessed by 1-methyl TMQ and 1-benzyl TMQ. The adrenoceptor blocking properties of 1-methyl TMQ and 1-benzyl TMQ were characterized in three pharmacological systems (glycerol release, cAMP accumulation and adenylate cyclase activation) associated with rat adipocytes.

Experimental

General. Male Sprague–Dawley (Harlan) rats weighing between 160 and 220 g were employed in all experiments. Animals (eight to fifteen per experiment) were stunned and killed by cervical dislocation. Epididymal fat pads were removed and placed in Krebs–Ringer bicarbonate buffer. Fat cells were isolated by the method of Rodbell [6] employing crude bacterial collagenase (Worthington Biochemicals,

* M. T. Piascik and D. R. Feller, manuscript in preparation.