Journal of Health Science, **45**(2) 59—62 (1999)

Involvement of Renal γ-Glutamyltranspeptidase in Differences in the Renal Uptake of Mercuric Mercury by Male and Female Mice of Various Strains and Ages

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(Received December 22, 1998; Accepted December 25, 1998)

Involvement of renal γ-glutamyltranspeptidase (γ-GTP) in differences in the renal uptake of Hg^{2+} by male and female mice of various ages was examined using five strains of mice, namely, BALB/cA, C57BL/6N, CBA/JN, C3H/HeN and ICR. We observed strain-related and gender-related differences in the renal accumulation of Hg^{2+} 30 min after the administration of mercuric chloride (1 μmol/kg, s.c.). Renal γ-GTP activity also varied among the tested strains, and the activity in males was about twice that in females. A significant correlation was recognized between renal γ-GTP activity and the renal accumulation of Hg^{2+}. Both renal uptake of Hg^{2+} and renal γ-GTP activity increased gradually with age in male ICR mice from 2 to 8 weeks after birth but remained relatively constant in ICR females. Significant gender-related differences in both renal accumulation of Hg^{2+} and γ-GTP activity were observed 4 weeks after birth and thereafter. Castration of male ICR mice decreased both renal accumulation of Hg^{2+} and γ-GTP activity to the levels in females. Injection of testosterone increased both renal accumulation of Hg^{2+} and γ-GTP activity in castrated male mice and in normal female mice to the levels in control male mice. These results suggest that strain-related, gender-related and age-related differences in the renal accumulation of Hg^{2+} in mice might be due to differences in renal γ-GTP activity and, furthermore, that renal γ-GTP activity might be controlled, at least to some extent, by testosterone.

Key words —— mercuric mercury, renal uptake, γ-glutamyltranspeptidase, mouse, strain-related difference, gender-related difference

INTRODUCTION

Of all mammalian organs, the kidney is the primary site of accumulation of Hg^{2+}. In mice, as much as 50% or more of an administered dose of Hg^{2+} accumulates in the kidneys within 1 hr after i.v. injection of mercuric chloride.[1)] In the kidneys of the rat,[2,3)] mouse[2–5)] and rabbit,[6)] Hg^{2+} accumulates preferentially in proximal tubule cells after the administration of mercuric chloride. Treatment of rats[7,8)] or mice[9)] with acivicin, an inhibitor of γ-glutamyltranspeptidase (γ-GTP), prior to the injection of mercuric chloride resulted in a marked increase in the urinary excretion of both glutathione (GSH) and Hg^{2+}, as well as a decrease in renal uptake of Hg^{2+}.[9)] Specific depletion of hepatic GSH, which is a major source of plasma GSH, prior to injection of mercuric chloride also substantially reduced the renal uptake of Hg^{2+} and, consequently, reduced the extent of renal damage.[9)] These observations suggest that Hg^{2+} might be transported to the kidneys as a complex with GSH, which is filtered through glomeruli, and taken up by kidney cells after degradation of the GSH moiety by γ-GTP.[9)] Recently, we found that both the renal accumulation of Hg^{2+} and renal γ-GTP activity were higher in C3H/He mice than in C57BL/6 mice.[10)] This observation suggested that renal γ-GTP activity might be one of the factors that influence strain-related differences in the uptake of Hg^{2+}

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by the kidney. Therefore, in this study, we examined the involvement of renal γ-GTP in strain-related differences in the renal uptake of Hg^{2+} as well as in gender-related and age-related differences in uptake, using five strains of mice.

MATERIALS AND METHODS

Chemicals —— [^{203}Hg]-mercuric chloride was obtained from New England Nuclear (Boston, MA). Testosterone, γ-glutamyl-*p*-nitroanilide, and glycylglycine were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Animals —— Four inbred strains of male mice of, namely, BALB/cA, C57BL/6N, CBA/JN and C3H/HeN, and of a randomly bred strain, CD-1 (ICR), and three strains of female mice, namely, BALB/c, C57BL/6N and ICR, were supplied by Charles River, Japan Inc. (Tokyo), and kept in metabolism cages (one mouse per cage) in a room with 12 h of light daily. They had access to laboratory chow (CE-2; Clea Japan) and water *ad libitum*.

Measurement of the Renal Accumulation of Mercury —— Four-week-old male mice of the five strains (BALB/c, C57BL/6N, CBA/J, C3H/HeN and ICR) and female mice of the three strains (BALB/c, C57BL/6N and ICR) were injected with [^{203}Hg]-mercuric chloride (0.37 MBq/1 μmol/kg, s.c.). Male and female ICR mice at 2, 3, 4, 6 and 8 weeks of age were also injected with the same dose of [^{203}Hg]-mercuric chloride. Mice were anesthetized with ethyl ether 30 min after injection of [^{203}Hg]-mercuric chloride, and the kidneys were removed. One kidney from each pair was used for quantitation of mercury and the other kidney was used for determination of γ-GTP activity. The amount of mercury in each kidney was determined by measuring the radioactivity of ^{203}Hg with an Auto Well gamma system (Aloka, Japan). We confirmed that administration of mercuric chloride at a dose of 1 μmol/kg had no effect on the renal γ-GTP activity of mice of each strain.

Effects of Testosterone and Castration —— To examine the effects of endogenous testosterone on the renal uptake of Hg^{2+}, we orchiectomized male 4-week-old male ICR mice under pentobarbital anesthesia (50 mg/kg, s.c.). From 7 days after castration, testosterone (5 mg/kg, s.c.), dissolved in olive oil, was injected once a day for 7 days into the castrated male mice. Female 4-week-old ICR mice were also given injection of testosterone (5 mg/kg, s.c.) daily for 14 days. Control mice were subjected to sham operation and/or injected with the same volume of olive oil. Mice treated with testosterone were injected with [^{203}Hg]-mercuric chloride (0.37 MBq/1 μmol/kg, s.c.) 24 h after the last injection of testosterone.

Determination of Renal γ-GTP Activity —— Each kidney was weighed and homogenized in 20 volumes of 50 mM Tris-HCl (pH 8.0). Homogenates were centrifuged at 800 × *g* for 10 min. Supernatants were used for determination of γ-GTP activity, which was measured as described by Tate and Meister[11], with γ-glutamyl-*p*-nitroanilide as the substrate. Concentrations of protein were determined colorimetrically by monitoring the binding of Coomassie blue (Bio-Rad Protein Assay kit, Bio-Rad Laboratories, Richmond, CA), with γ-globulin as the standard. One unit of the enzyme (U) was defined as the amount that generated one μmol of *p*-nitroaniline per min under the conditions of the reaction.

Statistical Analysis —— Student's *t*-test was used to determine the significance of differences. The relationship between the renal mercury content and γ-GTP activity was examined by linear regression analysis.

RESULTS AND DISCUSSION

We examined the accumulation of Hg^{2+} in kidneys 30 min after the administration of mercuric chloride at a non-toxic dose (1 μmol/kg) in five strains of mice. As shown in Fig. 1, there were differences among the strains with respect to the renal accumulation of Hg^{2+}. We also observed significant gender-related differences in ICR, BALB/c and C57BL/6N mice. In these three strains, males accumulated higher levels of mercury in their kidneys than the females. Renal γ-GTP activity in these mice also varied according to strain and gender, as shown in Fig. 1. The renal γ-GTP activities of these mice were significantly correlated with the levels of Hg^{2+} in their kidneys 30 min after the administration of mercuric chloride (Fig. 2).

We next examined development-related changes in the renal accumulation of mercury and γ-GTP activity in male and female ICR mice (Fig. 3). We observed gender-related differences in both renal levels of Hg^{2+} and γ-GTP activity 4 weeks after birth and thereafter. The renal uptake of Hg^{2+} and renal γ-GTP activity increased gradually with age in male mice but remained

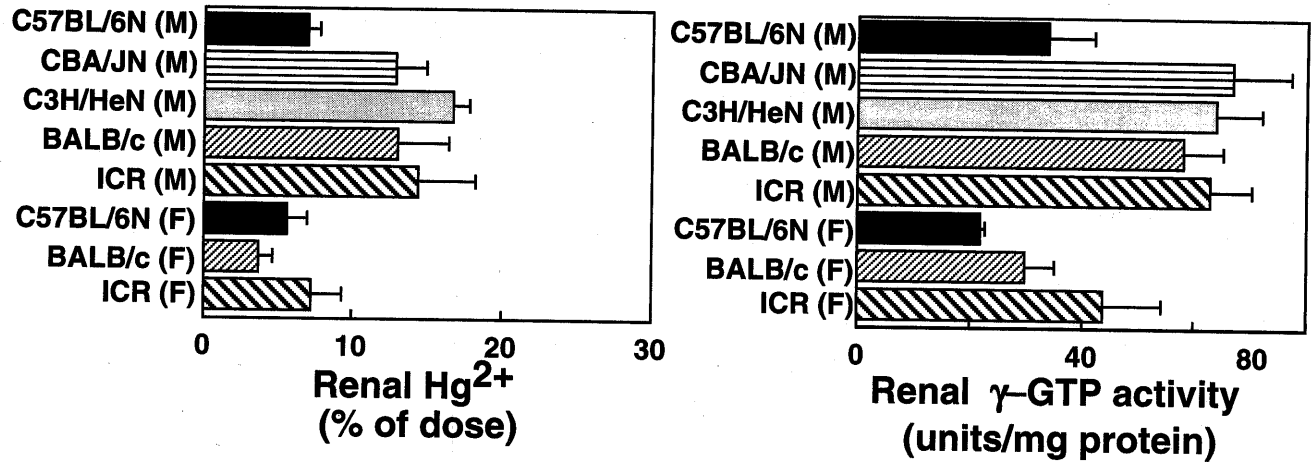

Fig. 1. Strain- and Gender-related Differences in the Renal Accumulation of Mercury and in γ-GTP Activity in Five Different Strains of Mice.

Male (M) BALB/cA, C57BL/6N, CBA/JN, C3H/HeN and ICR mice and female (F) BALB/cA, C57BL/6N and ICR mice were injected with mercuric chloride at a non-toxic dose (1 μmol/kg, s.c.). Renal concentrations of mercury and γ-GTP activities were determined 30 min after the administration. Columns and bars show means and S.D.

relatively constant in females. The pattern of these development-related and gender-related changes in renal γ-GTP activity appeared to be similar to the pattern of changes in the ability to accumulate Hg^{2+} in the kidney (Fig. 3). Moreover, there was a significant correlation ($r=0.94$, $p<0.001$) between the renal level of Hg^{2+} and renal γ-GTP activity (data not shown). These observations suggested the involvement of sex hormones in the renal accumulation of mercury and γ-GTP activity since puberty occurs in mice approximately 4–5 weeks after birth.[12]

Table 1 shows the effects of the administration of testosterone on renal uptake of Hg^{2+} and γ-GTP activity in male and female ICR mice. Treatment with testosterone significantly increased both the renal uptake of Hg^{2+} and γ-GTP

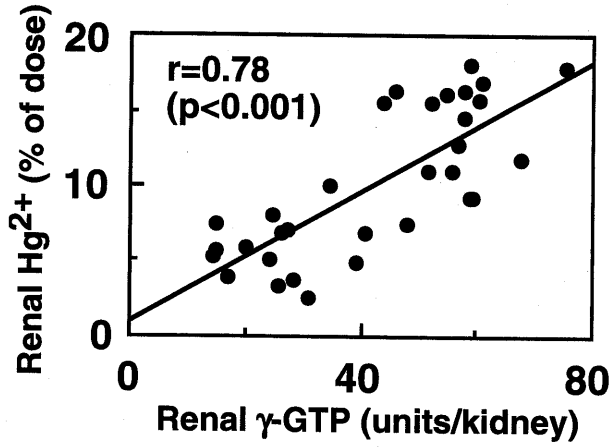

Fig. 2. Relationship between the Renal Accumulation of Mercury and Renal γ-GTP Activity in Mice 30 min after s.c. Injection of Mercuric Chloride.

Each point represents the values obtained from a single animal of one of the five mouse strains examined (see Fig. 1).

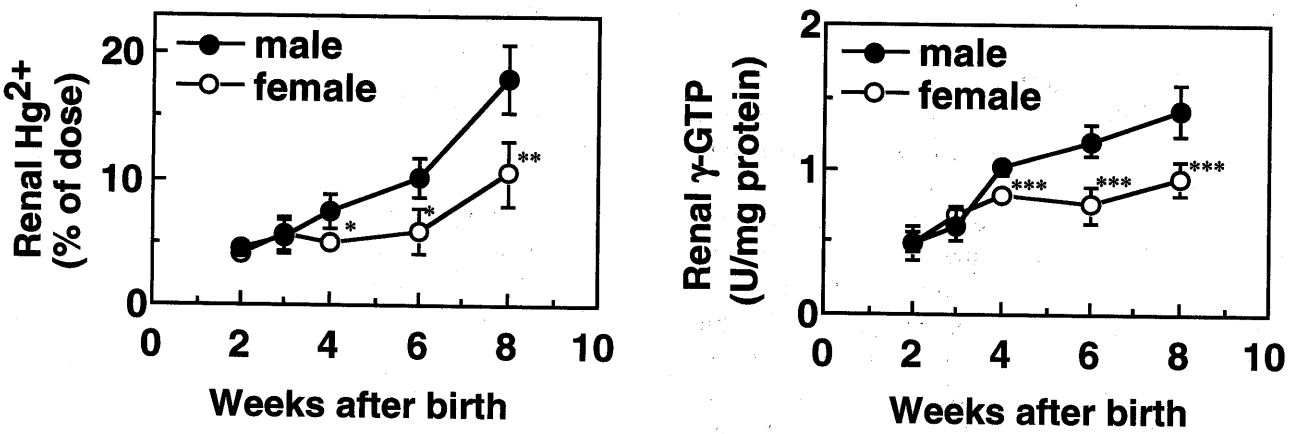

Fig. 3. Age-dependent Changes in the Renal Accumulation of Mercury and in γ-GTP Activity in Male and Female ICR Mice.

Mice were injected with mercuric chloride (1 μmol/kg, s.c.). Renal concentrations of mercury and γ-GTP activities were determined 30 min after administration of mercuric chloride. Values are means ± S.D. *, **,*** Significantly different from male mice ($*p<0.05$, $**p<0.01$, $***p<0.005$; Student's t-test).

Table 1. Effects of Testosterone on the Renal Accumulation of Hg^{2+} and γ-GTP Activity

Sex	Treatment	Renal Hg (% of dose)	Renal γ-GTP (U/mg protein)
Male	Olive oil	14.9±1.8	0.78±0.11
	Testosterone	15.2±3.6	0.94±0.14
Female	Olive oil	8.0±1.5	0.45±0.06
	Testosterone	15.3±2.3*	0.95±0.05*

Male and female ICR mice ($n=4$ in each group) were injected with testosterone dissolved in olive oil (5 mg/kg/day, s.c.) for 14 days. Renal γ-GTP activity was determined 24 h after the last injection of testosterone. [^{203}Hg]-mercuric chloride (1 μmol/kg, s.c.) was injected 24 h after the last injection of testosterone. Renal levels of ^{203}Hg were determined 30 min after administration of [^{203}Hg]-mercuric chloride. Values are means ± S.D. *Significantly different from olive oil-treated female mice ($p<0.005$; Student's t-test).

Table 2. Effects of Castration and Subsequent Administration of Testosterone on Renal Accumulation of Hg^{2+} and γ-GTP activity

Operation	Treatment	Renal Hg (% of dose)	Renal γ-GTP (U/mg protein)
Sham operation	Olive oil	15.3±0.8	0.79±0.15
	Testosterone	15.2±4.0	1.00±0.13
Castration	Olive oil	7.8±1.7	0.41±0.03
	Testosterone	14.8±1.8*	0.70±0.13*

Male ICR mice ($n=4$ per group) were castrated or sham-operated on day 0. Testosterone dissolved in olive oil (5 mg/kg/day, s.c.) was injected daily from day 7 to day 13. Renal γ-GTP activity was determined 24 h after the last injection of testosterone. [^{203}Hg]-mercuric chloride (1 μmol/kg, s.c.) was injected 24 h after the last injection of testosterone. The renal level of ^{203}Hg was determined 30 min after injection of [^{203}Hg]-mercuric chloride. Values are means ± S.D. *Significantly different from olive oil-treated castrated mice ($p<0.005$; Student's t-test).

activity in female mice but not in male mice. Renal levels of Hg^{2+} and γ-GTP activity in males fell to the levels observed in females after castration but returned to control levels after subsequent treatment with testosterone (Table 2).

Our data suggest that strain-, gender- and age-related differences in the renal uptake of Hg^{2+} might be regulated, at least to some extent, by renal γ-GTP. The results obtained in the present study are consistent with those in a previous study[9] which suggested that Hg^{2+} forms a complex with GSH in the liver or plasma and is translocated as part of this complex to the kidney, where it is incorporated into kidney cells after degradation of the GSH moiety by γ-GTP located in the brush border membranes of renal proximal tubules.[13] Renal γ-GTP might be one of the factors that are essential for incorporation of Hg^{2+} into kidney cells in mice. Changes in the activity of this enzyme might affect not only the renal uptake of Hg^{2+} but also the renal toxicity of Hg^{2+}. Although the involvement of renal γ-GTP in strain-related,[14] gender-related[14,15] and age-related[15] differences in the renal uptake of methylmercury has been reported previously, the present results might help in the development of future studies of the renal toxicity of Hg^{2+} since it is Hg^{2+} and not methylmercury that inflict severe damage on the kidney.

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