Effects of Exercise, Cold, and Immobilization Stresses on \(\gamma\)-Glutamyltransferase Activity in Rat Kidney

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Summary Effects of acute and chronic stresses (exercise, cold, and immobilization) on \(\gamma\)-glutamyltransferase (\(\gamma\)GT) activity in the rat kidney were investigated. In the extramicrosomal fraction there were significant decreases in acute-exercised rats but significant increases in cold-adapted and immobilized rats. In the microsomal fraction, on the other hand, the \(\gamma\)GT activity of acute-exercised rats increased definitely. The results suggest that different types of stresses have different effects on \(\gamma\)GT activity in the rat kidney.

Key words: acute and chronic stresses, \(\gamma\)-glutamyltransferase, rat kidney.

\(\gamma\)-Glutamyltransferase [EC 2.3.2.2] (\(\gamma\)GT) is widely distributed in animal tissues. In particular, the highest activity is found in the kidney where it is concentrated in the brush border of the proximal convoluted tubules. The enzyme, which is present in large amounts in the microsomal fraction, catalyzes the initial transfer of the \(\gamma\)-glutamyl residue from glutathione and other \(\gamma\)-glutamyl compounds to acceptor amino acids or peptides (Meister et al., 1981). In the kidneys, \(\gamma\)GT takes part in the process of reabsorption of amino acids from the primary urine to the blood.

It has recently been demonstrated that \(\gamma\)GT activity in both the extramicrosomal and microsomal fraction of the rat liver varies substantially under exercise and cold stresses (our unpublished observation). The results suggested that the \(\gamma\)GT-release ability of the microsomal membrane is increased under acute exercise but decreased under swimming training and long-term cold exposure. To our knowledge, there is no report on the changes of renal \(\gamma\)GT under such stresses. It is well known that physical effort causes proteinuria even in healthy subjects (Poortmans and Jeanloz, 1989).
Increased urinary protein excretion during exercise has been thought to be due in large measure to the leakage of the glomerular barrier (POORTMANS and JEANLOZ, 1968) but impaired tubular reabsorption has also been suggested as the causal factor in exercise-induced proteinuria (POORTMANS, 1972). In addition, exogenous heavy metals show higher toxicity to kidneys in the cold environment than in the thermoneutral environment (NOMIYAMA et al., 1978).

The present study attempts to determine the effects of several stresses (exercise, cold, and immobilization) on γGT activity in the rat kidney.

Seventy-seven male Wistar strain rats (7 weeks old) were divided into the following seven groups: 1) Group EX (n = 15): acute-exercised rats which were forced to swim in water at 35°C to exhaustion with a load of 3 g/100 g body weight (a mean period of 125 min); 2) Group TR (n = 10): rats trained to swim in water at 35°C up to 3 h/day for 10 weeks according to the protocol of HARRI and KUUSELA (1986); 3) Group CE (n = 10): acute-cold-exposed rats at ambient temperature (Ta) of -5°C for 1 h; 4) Group CA (n = 10): cold-acclimated rats at Ta of 5°C for 4 weeks; 5) Group CG (n = 6): cold-adapted rats reared at Ta of 5°C for 40 successive generations; 6) Group ST (n = 15): repetitively stressed rats for 4 weeks by daily 3-h immobilization on a wooden board in a recumbent position at Ta of 25°C as described previously (KUROSHIMA et al., 1984); and 7) Controls (n = 11): non-stressed rats reared at Ta of 25°C. They were all placed under artificial lighting for 12 h from 07:00 to 19:00 in individual cages and given the standard diet (Oriental MF, Oriental Yeast Co., Ltd., Tokyo) and tap water ad libitum. The animals were cared for in accordance with the guiding principles in the care and use of animals based upon the Helsinki Declaration. The average weight was 188 ± 1 and 247 ± 3 g (mean ± S.E.M.) at the beginning and the end, respectively, of each experiment. As previously reported (KUROSHIMA et al., 1984), the body growth was definitely suppressed in CA and ST rats. However, there was no significant difference among the seven groups in the rates of kidney weight to body weight (data not shown).

Twenty-four h after chronic stresses (Groups TR, CA, CG, and ST) and immediately after acute stresses (Groups EX and CE), rats were decapitated with a guillotine. The kidneys were quickly removed and washed with ice-cold sucrose-Tris-EDTA buffer (0.25 M sucrose, 10 mM Tris-HCl, 1 mM EDTA, pH 7.4) and used for assays of γGT and total protein; the tissues were homogenized in the same buffer with a Potter Elvehjem homogenizer, and then centrifuged at 12,000 × g for 10 min. The supernatant obtained was centrifuged at 105,000 × g for 60 min. This supernatant contained the extramicrosomal (EM) fraction, while the pellet comprised the microsome-rich (M) fraction. The pellet was used in part for the release experiment and further homogenized in the presence of 0.2% Triton X-100 to extract microsomal enzyme, γGT.

In the experiment on the release of γGT, 1.0 ml of the pellet was incubated at 37°C in the isotonic sucrose buffer or distilled water at pH 7.4 for 1 h, as described by TONCSEV and FRENKL (1984). Both extracts were used for determination of γGT activity.
activity. The activity of the enzyme was measured at 37°C by the method of Taniguchi et al. (1974). In vivo and in vitro release abilities of microsomal membranes for γGT activity were calculated by the following formulae:

\[
\text{In vivo release ability (\%)} = \frac{\text{EM fraction} \times 100}{(\text{EM fraction} + \text{M fraction})},
\]

and

\[
\text{in vitro release ability (\%)} = \frac{\text{hyposmotic condition}}{\text{isosmotic condition}} \times 100.
\]

Protein content was assessed by a Bio-Rad Protein Assay kit (Richmond).

The analysis of variance and Dunnett's t-test were applied to the data. In all statistical analyses, the 0.05 level of significance was used. The values in all figures were in the percentage to those of controls (mean ± S.E.M.).

Figure 1 shows significant increases in protein content in the EM fraction of the kidneys of EX and CG rats, whereas a significant decrease was noted in the M fraction of EX rats. In addition, the protein content in the EM fraction of TR rats

![Fig. 1. Protein content in the extramicrosomal (EM) and microsomal (M) fractions of the rat kidney. EX, acute exercise; TR, swimming training; CE, acute cold exposure; CA, cold acclimation; CG, cold adaptation; ST, immobilization. The values in the EM and M fractions of controls were 23.2 ± 1.5 and 24.6 ± 1.3 mg/g wet tissue (mean ± S.E.M.), respectively. *Significantly different from controls: p < 0.05.](image)
tended to elevate \( (p < 0.10) \). The result on the EM fraction in EX rats was in sharp contrast to those reported previously in the liver (KASPEREK et al., 1982; our unpublished observation), although the result on the M fraction was in agreement with those in the same studies (KASPEREK et al., 1982; our unpublished observation). KASPEREK et al. (1982) suggested that the autophagolysosomal system is responsible for the exercise-induced hepatic protein loss. In the present study, however, there were no definite changes in renal activities of lysosomal enzyme (β-glucuronidase, arylsphatase A and B, and cathepsin D) (data not shown). OH et al. (1978) observed that in contrast to the findings on hepatocytes, only small responses were noted in metallothionein synthesis in kidney under various stresses including exercise and cold, being in approximate agreement with the findings of the recent (unpublished) and present studies on protein content.

Figure 2 shows γGT activity in both fractions. In the EM fraction there was a significant decrease of γGT activity in EX rats but significant increase in CG and ST rats. In the M fraction, on the other hand, the γGT activity in EX rats increased definitely and that in CA rats tended to elevate \( (p < 0.10) \). The results of γGT activity in both fractions of EX rats were also in marked contrast to liver, which showed
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A significant increase in the EM fraction but a downward tendency in the M fraction under similar conditions (our unpublished observation). As compared to the EM fraction, relatively small responses of \( \gamma \)-GT activity to all the stresses investigated, except acute exercise, were noted in the M fraction. In accordance with the results shown in Fig. 2, in vivo release ability estimated by \( \gamma \)-GT activity was reduced in EX and TR rats but increased in ST rats, whereas no significant changes in in vitro release ability were noted in all groups of animals (Fig. 3). The in vivo release ability in CA rats tended to decrease (\( p < 0.10 \)). The results of the in vitro release ability indicate that the \( \gamma \)-GT release ability of the renal microsomal membrane is not easily affected by hypotonic conditions, even in the distilled water, in agreement with the findings in the hepatic microsomal membrane (our unpublished observation). Unlike in the other tissues such as skeletal and cardiac muscles (Haibach and Hosler, 1985) and liver (Ohno et al., 1988; our unpublished observation), why the in vivo release ability decreased in the kidneys of EX rats remains to be elucidated. There appeared to be a discrepancy between the changes in \( \gamma \)-GT activity in CA and CG rats, which is in contrast to the results on liver; namely, \( \gamma \)-GT activity in the M fraction of the liver of both groups increased markedly, with resulting significant decreases in the in vivo \( \gamma \)-GT release ability (our unpublished observation). However, the results of CG and ST rats in all experiments showed the consistent trend, as shown in Figs. 1–3.

It seems unlikely that even during extreme types of exertion, kidney is affected.

Fig. 3. In vivo and in vitro release abilities—estimated by \( \gamma \)-glutamyltransferase activity—of the membrane of the kidney microsomes in rats. Abbreviations are the same as in Fig. 1. * Significantly different from controls: \( p < 0.05 \).
to a degree such as to lead to increased release of γGT in the bloodstream (Haralambie, 1976). Therefore, investigations of the effects of exercise, cold, and immobilization stresses on the release of the enzyme in the urine seem warranted. Inoue et al. (1986) have indicated that since γGT activity of the intact rat kidney is extremely high, even a few percent of γGT activity may be sufficient for the transport and degradation of glutathione. Thus, the physiological significance of the stresses-induced, relatively small but significant changes in γGT activity in the present study awaits further study.

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REFERENCES


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