Effect of nephrectomy and enterectomy on plasma clearance of intravenously administered dipeptides in rats

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Summary

1. Sham-operated and bilaterally nephrectomized rats were injected intravenously with glycyl-L-leucine, glycylglycine and glycylsarcosine, and the concentrations of these dipeptides in plasma and muscle, liver, renal cortex (in the sham-operated rats) and intestinal mucosa at various intervals were determined.

2. Initially the plasma concentrations of glycyl-leucine and glycylglycine were higher in nephrectomized than in control rats but later the concentrations were similar in both groups of rats. The disappearance of these two dipeptides from plasma was almost complete within 20 min, and their plasma half-lives were not changed remarkably by nephrectomy. In contrast, nephrectomy markedly impaired disappearance of glycylsarcosine from plasma and prolonged its half-life from 7.6 min to 52.0 min.

3. Glycyl-leucine and glycylglycine were not detected in tissues of control rats injected with these dipeptides, but glycylsarcosine was recovered from all four tissues examined. Nephrectomy resulted in greater accumulations of glycylsarcosine in tissues and the appearance of glycylglycine in the remaining three tissues and glycyl-leucine in muscle.

4. Enterectomy did not have a remarkable effect on plasma half-life of glycylglycine but it allowed recovery of this dipeptide from renal cortex, liver and muscle.

5. It is concluded that kidneys and small intestine are involved in the disposition of circulating dipeptides, but in their absence other tissues may assume a greater role in this regard. However, renal clearance appears to be an important route for the disposition of dipeptides which are poorly hydrolysed by body tissues.

Key words: amino acid nutrition, dipeptide hydrolysis, dipeptide transport, renal function.

Introduction

In the preceding paper (Adibi, Krzysik & Drash, 1977), we showed that when a large load (0.5 μmol/g body weight) of dipeptides, such as glycylglycine or glycyl-L-leucine, is administered intravenously in rats, it is rapidly cleared from plasma. This clearance appears to be chiefly the result of dipeptide uptake and/or hydrolysis by body tissues. The study also demonstrated that kidney, compared with liver, muscle and intestinal mucosa, has the greatest capacity for dipeptide transport. When glycylsarcosine, which is an analogue of glycylglycine but resistant to hydrolysis, was injected intravenously, the concentration of this dipeptide accumulated in the renal cortex was over fourteen times greater than in any other tissue. In addition, Nutzenadel & Scrive (1976) have reported that the calculated maximal rate of uptake of carnosine (β-alanyl-L-histidine) was higher for renal cortical slices than for either jejunal mucosal epithelium or hemi-diaphragm in rats. Investigation of the specific activities of peptide hydrolases in tissues (muscle, liver, intestinal mucosa and kidney) with glycylglycine and glycyl-L-leucine as substrates has revealed
that activities against both substrates are considerably higher in kidney and small intestine than in the other tissues (Krzysik, Peterson & Adibi, 1975; Krzysik & Adibi, 1976). In view of these observations, it became of interest to determine the effect of nephrectomy and surgical removal of the entire small and large intestine (enterectomy) on dipeptide clearance from plasma. A preliminary communication pertaining to this study has been previously published (Krzysik & Adibi, 1976).

**Methods**

**Animals**

Male Sprague–Dawley rats weighing 270–300 g were lightly anaesthetized with ether between 09.00 and 11.00 hours. Rats had access to food (Purina Laboratory Chow) and water until 1 h before being anaesthetized. The rats were then subjected to bilateral nephrectomy, enterectomy or sham (control) operation.

**Operative procedures**

**Bilateral nephrectomy.** A single longitudinal skin incision approximately 2 cm long was made in the mid-dorsal region. Two separate lateral body wall incisions about 0.75 cm were made 1 cm below the animal's last rib. The kidneys were exposed, and the surrounding fat and membrane were pulled away from the kidneys with forceps and returned to the peritoneal cavity. The renal veins and arteries were then ligated with a surgical suture (000), and both kidneys were excised. The body wall opening was closed by a single suture, and the mid-dorsal skin incision was closed with Michel wound clips. With the rat still under ether anaesthesia, the right jugular vein was injected with one of the following test solutions: NaCl (150 mmol/l), glycyl-L-leucine (150 mmol/l), glycglycine (1–50 mmol/l) or glycylsarcosine (1–50 mmol/l). The amount of dipeptide injected for all three dipeptides was 0·5 μmol/g body weight, and the experimental procedures described in the preceding paper (Adibi et al., 1977) for the non-nephrectomized rats (controls) were followed.

**Enterectomy.** The rat was placed under light ether anaesthesia. A single longitudinal skin incision approximately 4 cm long was made in the mid-ventral region, and a slightly smaller body wall incision was made directly beneath the skin incision. The intestine was gently brought to the outside of the body cavity, and ligatures were tied at the pylorus and rectosigmoid junction. The peritoneal membranes surrounding the intestine, including the great and lesser omentum and mesentery, were pulled away with forceps to free the entire intestine. The superior and inferior mesenteric veins and arteries were then tied off with ligatures, and the entire intestine was removed. The body wall incision was closed with sutures, and the skin incision was closed with Michel wound clips. While the rat was kept under ether anaesthesia, the right jugular vein was injected with glycylglycine as described above.

**Determination of blood volume**

Blood volume of control (sham-operated), nephrectomized and enterectomized rats was determined with 51Cr-labelled erythrocytes as described by Albert (1963). Donor rat blood (10 ml) was added to a flask containing 2·5 ml of anticoagulant solution (modified ACD solution, Squibb and Sons, Princeton, N.J.). To this mixture 50 μCi of 51Cr (sodium chromate solution, 1 mCi/ml; New England Nuclear Corp., Boston, Mass., U.S.A.) was added. After incubation at 37°C for 30 min, ascorbic acid was added. The blood mixture was centrifuged at 500 g for 5 min, plasma was decanted, and the precipitated erythrocytes were resuspended in 10 ml of NaCl solution (150 mmol/l; saline). A portion (0·5 ml) of 51Cr-labelled erythrocytes was injected into control, nephrectomized or enterectomized rats by way of a jugular vein. After 10 min blood was collected from the aorta, and radioactivity was measured on 0·5 ml samples with a gamma scintillation spectrometer. Capillary tubes were used to measure the packed cell volume of the blood. Blood volume was calculated by the commonly used equation (Albert, 1963).

**Amino acid and dipeptide analysis**

The analysis of amino acid and dipeptide concentrations in plasma and tissues of control, nephrectomized and enterectomized rats was performed by the methods described in the preceding paper (Adibi et al., 1977). The concentrations are given as mean values +1 SEM.
Clearance of dipeptide

FIG. 1. Plasma concentrations (mean±SEM, six rats) of glycyl-leucine (a), glycine (b) and leucine (c) at various intervals after the injection of glycyl-leucine in sham-operated (---; control) and nephrectomized (----) rats and the injection of saline in nephrectomized rats (△--△). Glycyl-leucine was not detected in the plasma of saline-injected rats. Significant difference (*P<0.01 and **P<0.05) between concentrations in control and nephrectomized rats.

Statistical analysis

Student’s t-test was used for the statistical analysis of the data. The half-life of each dipeptide in plasma of control, nephrectomized and enterectomized rats was determined from a straight line drawn by the least-square method (Dixon & Massey, 1969) on a semi-logarithmic graph of the mean plasma concentration (log scale) versus the elapsed time after the injection (linear scale).

Results

Effect of bilateral nephrectomy

The plasma concentrations at various intervals after injections of glycyl-leucine, glycylglycine and glycylsarcosine in control and bilaterally nephrectomized rats are compared in Figs. 1–3. Nephrectomy resulted in higher initial concentrations of glycyl-leucine and glycylglycine (Fig. 1 and Fig. 2). For glycylglycine at 5 min, and for glycyl-leucine at 15 min, and at subsequent intervals after the injection, there were no longer any significant differences between plasma concentrations of the above dipeptides in control and nephrectomized rats. The post-nephrectomy plasma disappearance pattern of glycylsarcosine (Fig. 3) differed from that of glycylglycine and glycyl-leucine (Fig. 1 and Fig. 2). During the initial 5 min there was no significant difference between concentrations of glycylsarcosine in plasma of control and nephrectomized rats. However, the differences in plasma concentrations of the two groups of rats became more pronounced as the interval between injection and blood collection was prolonged. As shown in Fig. 4, the half-lives of glycylglycine and glycyl-leucine were not changed remarkably by nephrectomy, but that of glycylsarcosine was markedly increased (over fourfold) by nephrectomy.

The plasma concentrations of leucine or glycine after the injection of saline, glycyl-leucine, glycylglycine or glycylsarcosine are also shown in Figs. 1–3. In the experiment with glycyl-leucine (Fig. 1), except for concentrations of glycine at 15 min and of leucine at 2 min, there were no significant differences between
FIG. 2. Plasma concentrations (mean±SEM, six rats) of glycylglycine (a) and glycine (b) at various intervals after the injection of glycylglycine in sham-operated (---; control) and nephrectomized (----) rats. Significant difference (*P<0.01 and **P<0.05) between concentrations of glycylglycine or glycine in control and nephrectomized rats.

FIG. 3. Plasma concentrations (mean±SEM) of glycylsarcosine (a) and glycine (b) at intervals after the injection of glycylsarcosine in six sham-operated (---; control) and four nephrectomized (----) rats. Concentration of glycylsarcosine or glycine was significantly greater (*P<0.01 and **P<0.05) in the nephrectomized than in control rats. Sarcosine was not detected at any interval in plasma.
Clearance of dipeptide

Concentrations of leucine or glycine in control and nephrectomized rats. With glycylglycine (Fig. 2), the glycine concentration was significantly greater at 2 min in the control than in the nephrectomized group, but glycine concentrations became significantly greater at 15 and 20 min in the nephrectomized group than in the control group. Sarcosine was not detected in plasma of either control or nephrectomized rats. During the initial 15 min the plasma concentrations of glycine after glycylsarcosine injections were slightly greater in the control rats than in the nephrectomized rats (Fig. 3). To determine whether the initial higher plasma concentrations of glycylglycine or glycyl-leucine were related to a decrease in the blood volume by nephrectomy, the blood volume was determined in six control and four nephrectomized rats with $^{51}$Cr-labelled erythrocytes. The data showed that about 6.4% of the total blood volume was lost upon removal of the kidneys (blood volume $17.4 \pm 0.6$ vs. $16.3 \pm 0.3$ ml respectively). This decrease in volume, however, was not statistically significant.

The liver, muscle and intestinal mucosal concentrations of glycyl-leucine, glycylglycine and glycylsarcosine at 5 and 30 min after the injection of each of these dipeptides in control (sham-
TABLE 1. Tissue concentrations of injected dipeptides in sham-operated (control) and bilaterally nephrectomized rats
Results are expressed as μmol/g of tissue: mean values ± SEM for four rats. *Significant difference (P < 0.01) for nephrectomized versus sham-operated rats.

<table>
<thead>
<tr>
<th>Operation</th>
<th>Dipeptide injected</th>
<th>Time after injection (min)</th>
<th>Liver</th>
<th>Muscle</th>
<th>Intestinal mucosa</th>
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<tr>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>0</td>
<td>0</td>
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<td>Glycyl-leucine</td>
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<td>0</td>
<td>0.08 ± 0.01</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>0.11 ± 0.06</td>
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<td></td>
<td></td>
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<td>0.01 ± 0.01</td>
<td>0.03 ± 0.02</td>
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<td>0.54 ± 0.05</td>
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<td></td>
<td></td>
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<td>0.24 ± 0.02*</td>
<td>0.21 ± 0.01*</td>
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<tr>
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<td></td>
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<td>30</td>
<td>0.24 ± 0.02*</td>
<td>0.21 ± 0.01*</td>
<td>0.37 ± 0.06*</td>
</tr>
</tbody>
</table>

Enterectomy Glycylglycine 5 0.07 ± 0.01 0.17 ± 0.06 0.18 ± 0.05
30 0.01 ± 0.01 0.01 ± 0.01 0.08 ± 0.02

**FIG. 5.** Plasma concentration (mean ± SEM) of glycylglycine (a) and glycine (b) at intervals after the injection of glycylglycine in six sham-operated (----; control) and four enterectomized (-----) rats. Concentration of glycylglycine or glycine was significantly greater (*P < 0.01 and **P < 0.05) in the enterectomized than in control rats.
Clearance of dipeptide

Operated) and bilaterally nephrectomized rats are summarized in Table 1. Although neither glycyl-leucine nor glycylglycine was detected in the above tissues of control rats, glycylglycine was recovered in the muscle, liver and intestinal mucosa, and glycyl-leucine in the muscle of nephrectomized rats. Concentrations of glycylsarcosine in the intestinal mucosa, muscle and liver at 30 min after the injection were significantly greater in nephrectomized than in control rats (Table 1).

Effect of enterectomy

Plasma concentrations of glycylglycine at various intervals after the injection of this dipeptide in sham-operated (control) and in enterectomized rats are compared in Fig. 5. Although the blood volume was slightly reduced by enterectomy (less than 10%), except at 5 min, there was no significant difference between plasma concentrations of glycylglycine at any interval in these two groups of rats. As shown in Fig. 4, the half-life of glycylglycine in plasma was not remarkably changed by enterectomy. Except at 5 min after the glycylglycine injection, there were no significant differences between glycine concentrations in the plasma of control and enterectomized rats (Fig. 5). In contrast to control rats, glycylglycine was recovered from all the tissues (muscle, liver, renal cortex) examined in enterectomized rats (Table 1).

Discussion

Renal epithelial cells, like intestinal mucosal cells, have a brush-border luminal membrane containing carrier systems for free amino acids (Bergeron & Morel, 1969; Foulkes, 1972; Wilson & Scriver, 1967), peptide hydrolases (George & Kenny, 1973) and possibly carrier systems for small peptides (Benoit & Watten, 1968; Nutzenadel & Scriver, 1976). These and other physiological and biochemical features have rendered the kidney a key organ in amino acid homeostasis (Adibi, 1971). Although there is continuous glomerular filtration of circulating amino acids and possibly small peptides, there is very little loss of these substances in the urine (Adibi, 1971; Adibi et al., 1977).

Kidneys, because of their relatively high rates of blood perfusion, dipeptide transport (Adibi et al., 1977; Nutzenadel & Scriver, 1976) and dipeptide hydrolysis (Krzysik et al., 1975), and their unique function in continuous filtration and reabsorption of plasma constituents, may be considered the principal organ for the disposition of circulating dipeptides. They may participate in this function by three different mechanisms: (a) there could be extensive filtration of circulating dipeptides followed by renal tubular absorption and extensive hydrolysis within these cells; (b) there could be hydrolysis of filtered dipeptides by the brush-border hydrolases on the surface of renal tubular cells, followed by the absorption of liberated amino acids, and (c) there could be uptake of circulating dipeptides by antiluminal membrane of renal epithelium followed by intracellular hydrolysis. Based on the above considerations, it was expected that the removal of kidneys would markedly impair the disappearance of dipeptides. This expectation was not entirely fulfilled by the results of experiments in nephrectomized rats, since the plasma half-lives of glycylglycine and glycyl-leucine were not remarkably changed by this procedure (Fig. 4). However, within the initial 5 min after the injection, the plasma concentrations of both of these dipeptides were greater in the nephrectomized than in control rats (Fig. 1 and Fig. 2), and there was no sharp decrease in the elevated plasma concentration of glycylsarcosine for as long as 60 min after the injection of this dipeptide (Fig. 3). Indeed, the plasma half-life of glycylsarcosine was increased sevenfold after nephrectomy (Fig. 4). Furthermore, not only did the concentration of glycylsarcosine in rat tissues become greater after nephrectomy, but glycylglycine and glycyl-leucine were also detected in tissues after this procedure (Table 1).

Although bilateral nephrectomy resulted in detection of glycylglycine in the intestine, muscle and liver tissues, glycyl-leucine was recovered only in the muscle (Table 1). The relatively high peptide hydrolase activities in the liver and intestine could have accounted for the failure to find unhydrolysed glycyl-leucine in these tissues of nephrectomized rats. The cytoplasmic peptide hydrolase activity in tissues is greater against glycyl-leucine than against glycylglycine and, furthermore, cytoplasmic peptide hydrolase activity in the muscle is lower than those in the intestine and in the liver (Krzysik et al., 1975; Krzysik & Adibi, 1976).

These data indicate that kidneys are involved
in the disposition of circulating dipeptides, but in their absence other tissues may assume a greater role in this regard. Glycylsarcosine is not a naturally occurring dipeptide, and the fact that its clearance from plasma is markedly impaired by nephrectomy may not be physiologically relevant. However, there are naturally occurring dipeptides which, like glycylsarcosine, are not efficiently hydrolysed by tissue peptide hydrolases. For example, hydroxyproline-containing, β-aspartyl and γ-glutamyl dipeptides have been recovered in the urine of man or rat after either a protein meal or increased breakdown of endogenous proteins (Milne, 1971). Therefore the kidney appears more important for the disposition of dipeptides which are relatively resistant to hydrolysis by other tissues.

The same mechanisms (a, b and c) discussed above for the kidney could also be considered in relation to the possible role of the small intestine in the disposition of circulating dipeptides. However, one important difference distinguishes the potential of the kidney from that of the intestine to hydrolyse circulating dipeptides. Although there is probably extensive exposure of luminal membrane of renal tubular cells to filtered dipeptides, such exposure by the luminal membrane of intestinal mucosal cells is unlikely. Although the movement of dipeptides from plasma to the lumen of the intestines has not yet been studied, such movement is quite small for amino acids (Adibi & Gray, 1967; Christensen, Feldman & Hastings, 1963). Therefore a more likely mechanism for the participation of the small intestine in the disposition of circulating dipeptides is the entry of the dipeptides through the antiluminal membrane of mucosal cells and hydrolysis by cytoplasmic peptide hydrolases, which make up 80–95% of the cell dipeptidase activity (Kim, Birtwhistle & Kim, 1972; Peters, 1970). Results of the present experiments indicate that loss of this mechanism is not critical in plasma disappearance of dipeptides such as glycyglycine (Fig. 5), since other tissues may compensate for the loss of the small intestine (Table 1).

The plasma disappearance curves (concentration versus time) obviously represent the sum of the different processes involved in the removal of dipeptides from the circulation, e.g. distribution in the extravascular spaces, renal excretion and hydrolysis of dipeptides in various sites in different organs. Nevertheless, in the present experiments, plasma concentrations plotted on a semi-logarithmic scale as a function of time closely approximated a straight-line relationship (Fig. 4). Therefore calculation of half-lives appeared to be a convenient way of showing the overall effect of nephrectomy and enterectomy on plasma dipeptide disappearance rates. It is perhaps important to emphasize that even without the calculated half-life values the observed data permit the conclusion that nephrectomy does not affect remarkably the plasma disappearance of glycy-leucine and glycyglycine, but it does impair that of glycylsarcosine.

Acknowledgments

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References


