Metabolism of glycine- and hydroxyproline-containing peptides by the isolated perfused rat kidney

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Isolated perfused rat kidneys removed considerable quantities of glycytyrosine, glycyhydroxyproline, tetraglycine and prolyhydroxyproline from the perfusate. The component amino acids are released into the perfusate and, in the case of the glycine-containing peptides, there is increased synthesis of serine. Removal of peptides was more than could be accounted for on the basis of filtration, so antiluminal metabolism is indicated. Metabolism of such peptides by the kidney may contribute to renal serine synthesis *in vivo*.

It has been recognized for many years that the kidney plays a specific role in the clearance of many proteins and peptide hormones from the circulation (Maack *et al.*, 1979; Carone *et al.*, 1979; Carone & Peterson, 1980; Rabkin & Kitaji, 1983). The larger proteins are cleared from the circulation after filtration by endocytosis and lysosomal degradation (Bourdeau *et al.*, 1972). Small proteins and peptide hormones, however, are cleared by a different mechanism. After filtration at the glomerulus, they are hydrolysed in the proximal tubule by enzymes of the brush border (Carone & Peterson, 1980; Rabkin & Kitaji, 1983). The small peptides so produced may then be hydrolysed to their constituent amino acids and reabsorbed by specific amino-acid-transport systems (Silbernagl & Volkl, 1977; Pullman *et al.*, 1978). An alternative route whereby proteins are first cleaved to dipeptides followed by transport and intracellular hydrolysis has been shown to exist in the intestinal mucosa (Matthews, 1975) and may also be present in the epithelial cells of the proximal tubule (Nutzenadel & Sriver, 1976; Adibi & Krzysik, 1977; Ganapathy *et al.*, 1981). Peritubular uptake of peptides may also be involved in the clearance of peptide hormones from the circulation (Katz & Rubenstein, 1973).

In addition to the sources of peptides mentioned above, the kidney may also be involved in the metabolism of di- and tri-peptides present in blood. These peptides may arise from the degrada-

tion of endogenous proteins, particularly collagen, and may be present in substantial amounts (McCormick & Webb, 1982; Powell *et al.*, 1974).

We have recently shown that the rat kidney *in vivo* has a large capacity for the synthesis of serine from a variety of precursors (Lowry *et al.*, 1985a), including glycine. As previously reported by other investigators (Pitts & McLeod, 1972; Tizianello *et al.*, 1980), we also noted the lack of stoichiometry between glycine uptake and serine release. This phenomenon persisted even in the presence of inhibitors of alternative routes of serine synthesis (Lowry *et al.*, 1985a). In experiments where we employed cysteamine, an inhibitor of the glycine-cleavage enzyme, we detected a large release of glycine from the kidney. We have suggested that these observations could be accounted for by the hydrolysis and subsequent metabolism of peptides containing glycine or hydroxyproline. The metabolism of hydroxyproline by the kidney causes a significant production of glycine (Lowry *et al.*, 1985b). In the present paper we show that the isolated perfused rat kidney is able to hydrolyse several glycine- and hydroxyproline-containing peptides and that their subsequent metabolism leads to an increase in serine production. We were also able to demonstrate a substantial peritubular uptake of peptides by the isolated perfused rat kidney.

Materials and methods

Animals

Male rats of the Sprague-Dawley strain (Charles River, Montreal, Que., Canada) were used for all

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perfusions. They were allowed free access to food and water at all times, and weighed 350–450 g when used.

Perfusions

Surgical preparation of the isolated perfused kidney was performed exactly as described by Ross *et al.* (1973). Kidneys were perfused with approx. 90 ml of Krebs–Henseleit (1932) bicarbonate-buffered saline containing 6.7% (w/v) bovine serum albumin (fraction V; Miles Laboratories, Rexdale, Ont., Canada) and 5 mM-glucose. A portion (1 μ Ci) of [*carboxy*- 14 C]inulin was added to allow the determination of glomerular filtration rate. After 40 min, peptides were added from stock solutions to achieve nominal concentrations of 5 mM for glycyl-L-tyrosine and glycylhydroxy-L-proline, 2.5 mM for tetraglycine and 3.0 mM for L-prolylhydroxy-L-proline (all products of Sigma, St. Louis, MO, U.S.A.). The perfusions were then continued for a further 45 min. Throughout the perfusions samples of perfusate and urine were collected every 15 min. With this protocol each kidney was able to serve as its own control. Sodium reabsorption by these kidneys was $95.8 \pm 2.2\%$ and $95.0 \pm 2.9\%$ during the intervals 25–40 and 40–70 min respectively (mean $\pm$ S.D., $n = 12$, not significantly different).

Analyses

Samples of perfusate and urine were added to 0.1 vol. of 30% (v/v) HClO_4 and the precipitated proteins removed by centrifugation. The supernatant was neutralized and a portion adjusted to pH 2.2 with HCl for analysis of amino acids and peptides by automated ion-exchange chromatography (Brosnan *et al.*, 1983).

Inulin radioactivity was determined by liquid-scintillation spectrometry.

Results and discussion

In Fig. 1 are shown the results of the experiments using glycylhydroxyproline as the peptide substrate. During the initial 40 min there is a slow accumulation of glycine and serine derived from endogenous precursors. The production of these amino acids is linear and persists for at least 90 min (results not shown). Addition of glycylhydroxyproline at 40 min causes an immediate increase in the production of both glycine and hydroxyproline; however, these amino acids are produced at significantly different ($P < 0.05$) rates. The difference in the rates of production of hydroxyproline and glycine can be accounted for by the conversion of hydroxyproline into glycine and the subsequent conversion of both amino acids into serine, the production of which increased significantly after

Fig. 1. Metabolism of glycylhydroxyproline by the isolated perfused kidney

The peptide (final concn. 5 mM) was added to the perfusate at 40 min. The values show the total amounts of glycylhydroxyproline (○), hydroxyproline (■), glycine (▲) and serine (●) present in the recirculating perfusate (approx. 90 ml) at each sampling time. Results are shown as the mean $\pm$ S.D. for three experiments for which the mean kidney dry weight was 0.33 ± 0.04 g.

the addition of peptide (Table 1). Similar results were obtained when glycyl-tyrosine was used as peptide substrate (Table 1). This peptide was removed from the perfusate very rapidly, with a half-time of approx. 15 min. The removal of glycyltyrosine and production of tyrosine were stoichiometrically equivalent; however, the production of glycine was less than that of tyrosine, with the difference accounted for by the synthesis of serine. Rates of serine synthesis in the presence of glycyltyrosine were similar to those observed in the presence of 5 mM-glycine (Lowry & Brosnan, 1983).

Tetraglycine was metabolized slowly by the perfused kidney; however, the peptide must have been completely hydrolysed, since we were unable to detect significant quantities of triglycine or diglycine in either the perfusate or urine in these experiments. Similar results have been reported by Adibi & Morse (1982) for the metabolism of this peptide by rats when it was administered intravenously. The production of serine and glycine could account for all the peptide utilized (Table 1).

Metabolism of prolylhydroxyproline by the perfused kidney caused changes in the rates of release of several amino acids (Table 1). Most notable of these changes were the increases in proline, hydroxyproline and glutamine production. Significant changes were also found in the production rates of both glycine and alanine. These latter effects are similar to those observed previously

Table 1. Metabolism of peptides by the isolated perfused rat kidney

Kidneys were perfused with approx. 90 ml of medium for 40 min before the addition of peptide to the indicated concentrations. Perfusion was continued for a further 45 min, during which samples were collected every 15 min and processed as described in the Materials and methods section. Values shown are given in $\mu\text{mol/h}$ per g kidney dry wt. and are the means $\pm$ s.d. for three separate perfusions with each peptide. Dashes indicate values not measured. * $P < 0.05$; ** $P < 0.01$ for comparison with the initial control period.

Peptide	Initial concn. (mM)	Time interval (min)	Production or utilization of:							
			Peptide	Serine	Glycine	Tyrosine	Glutamine	Alanine	Proline	Hydroxyproline
Glycyltyrosine	4.1 $\pm$ 0.3	10-40 40-85	— -1327 $\pm$ 86	25.9 $\pm$ 3.2 78.1 $\pm$ 14.4*	13.2 $\pm$ 6.5 947 $\pm$ 90**	10.2 $\pm$ 7.7 1192 $\pm$ 262*	17.4 $\pm$ 0.9 12.0 $\pm$ 2.3*	11.5 $\pm$ 3.5 11.8 $\pm$ 3.4	— —	— —
Glycylhydroxyproline	5.0 $\pm$ 0.2	10-40 40-85	— -290 $\pm$ 57	26.7 $\pm$ 1.9 55.2 $\pm$ 4.7**	10.7 $\pm$ 1.3 119 $\pm$ 25*	7.2 $\pm$ 1.3 4.7 $\pm$ 0.4	20.5 $\pm$ 4.8 24.3 $\pm$ 1.0	11.4 $\pm$ 0.7 —	5.8 $\pm$ 1.7 1.5 $\pm$ 1.6**	— 76.4 $\pm$ 11.9**
Tetraglycine	2.1 $\pm$ 0.2	10-40 40-85	— -158 $\pm$ 80	25.1 $\pm$ 4.2 50.1 $\pm$ 16.8	11.1 $\pm$ 1.2 444 $\pm$ 173*	6.9 $\pm$ 0.6 4.6 $\pm$ 0.5	13.6 $\pm$ 2.3 4.3 $\pm$ 2.5*	10.4 $\pm$ 1.0 6.4 $\pm$ 1.6*	— —	— —
Prolylhydroxyproline	3.4 $\pm$ 0.1	10-40 40-85	— —	36.2 $\pm$ 4.9 35.9 $\pm$ 6.2	11.9 $\pm$ 1.7 20.6 $\pm$ 2.5*	8.1 $\pm$ 1.8 4.1 $\pm$ 1.2*	29.3 $\pm$ 3.0 54.6 $\pm$ 13.3*	15.8 $\pm$ 1.6 -4.9 $\pm$ 1.2**	3.5 $\pm$ 2.4 60.3 $\pm$ 3.7**	— 48.9 $\pm$ 8.2**

during the synthesis of serine from hydroxyproline by both the perfused kidney and isolated cortical tubules (Lowry *et al.*, 1985b) and are consistent with the metabolism of hydroxyproline via 4-hydroxy-2-oxoglutarate aldolase and alanine: glyoxalate transaminase. The absence of an increase in serine synthesis from prolylhydroxyproline may be due to the low concentration of glycine produced during the metabolism of this peptide. The production of glutamine in the presence of prolylhydroxyproline most likely represents its net synthesis from glutamate produced during the metabolism of proline. The amido nitrogen of glutamine may also be derived from glutamate in a manner analogous to that reported by Forissier & Baverel (1981) for the synthesis of glutamine from alanine by guinea-pig kidney-cortex tubules. Glutamine release occurred at low rates in all perfusions; however, in those perfusions where glycine was produced at high rates, i.e. in the presence of glycyltyrosine or tetraglycine, the rate of release of glutamine was significantly decreased. This indicates that the glutamine release is due to synthesis from endogenous precursors, as it has been shown that glycine is an inhibitor of glutamine synthetase (Tate & Meister, 1971; Deuel *et al.*, 1973).

The marked differences in the utilization of glycyltyrosine and glycylhydroxyproline indicate a distinct substrate specificity of the enzyme(s) involved in their metabolism. Several studies have shown that the peptidases present in the brush border vary with respect to their affinity towards peptide substrates (for a review see Kenny & Booth, 1978). Further Ganapathy *et al.* (1981) have demonstrated that brush-border-membrane vesicles are able to transport glycylproline without hydrolysis and have characterized an Na^+ -independent peptide carrier in these vesicles (Ganapathy & Leibach, 1983). It is therefore possible that the differences in the rates of metabolism of glycyltyrosine and glycylhydroxyproline could be a function of their site of hydrolysis. Table 2 compares the filtration and excretion of these two peptides with their rate of removal from the perfusate. Two conclusions are apparent from these results. First, the amount of peptide filtered is less than the renal uptake, indicating that there must be a large antiluminal uptake of the peptides. In the case of glycyltyrosine, this uptake may account for as much as 95% of the total uptake. In the case of glycylhydroxyproline, antiluminal uptake could account for about 30% of total uptake. Secondly, there are quantitative, and possibly qualitative, differences in the handling of filtered peptides. There are distinct differences in the excretion rates of the two peptides when similar filtered loads are compared. During the 40-50 min

Table 2. Clearance of peptides by the isolated perfused rat kidney
Kidneys were perfused with approx. 90 ml of medium for 40 min before the addition of peptide. Perfusion was continued for a further 45 min, during which samples were collected every 15 min and processed as described in the Materials and methods section. Values shown are given in $\mu\text{mol/g}$ kidney dry wt. and are the means $\pm$ S.D. for three separate perfusions with each peptide.

Clearance parameter		Time interval (min) ...		
		40-55	55-70	70-85
(a) Glycyl-L-tyrosine	Perfusate change			
	Filtration			
	Peptide	-616 $\pm$ 50	-267 $\pm$ 22	-134 $\pm$ 20
	Glycine	407 $\pm$ 66	220 $\pm$ 50	91 $\pm$ 39
	Tyrosine	467 $\pm$ 49	232 $\pm$ 43	47, 155
	Excretion			
	Peptide	32.3 $\pm$ 13.8	11.3 $\pm$ 4.1	4.6 $\pm$ 1.4
	Glycine	8.1 $\pm$ 3.7	15.0 $\pm$ 7.1	16.6 $\pm$ 6.3
	Tyrosine	9.3 $\pm$ 4.1	16.8 $\pm$ 7.3	19.4 $\pm$ 5.8
	Perfusate change			
	Peptide	2.1 $\pm$ 1.6	2.2 $\pm$ 1.6	1.6 $\pm$ 0.5
	Glycine	2.5 $\pm$ 1.3	3.3 $\pm$ 1.5	3.3 $\pm$ 1.6
	Tyrosine	2.4 $\pm$ 1.8	3.8 $\pm$ 0.9	3.4 $\pm$ 1.6
(b) Glycylhydroxy-L-proline	Perfusate change			
	Filtration			
	Peptide	-98 $\pm$ 30	4 $\pm$ 63	-146 $\pm$ 30
	Glycine	22.7 $\pm$ 1.3	34.0 $\pm$ 7.9	29.3 $\pm$ 10.6
	Hydroxyproline	13.6 $\pm$ 0.5	21.7 $\pm$ 4.2	20.7 $\pm$ 4.6
	Excretion			
	Peptide	78.3 $\pm$ 19.7	49.4 $\pm$ 9.4	41.4 $\pm$ 8.3
	Glycine	1.1 $\pm$ 0.2	2.1 $\pm$ 0.4	3.0 $\pm$ 0.9
	Hydroxyproline	0.4 $\pm$ 0.1	1.0 $\pm$ 0.2	1.7 $\pm$ 0.5
	Perfusate change			
	Peptide	13.6 $\pm$ 6.5	19.1 $\pm$ 4.9	16.5 $\pm$ 9.3
	Glycine	0.2 $\pm$ 0.1	0.5 $\pm$ 0.2	0.7 $\pm$ 0.4
	Hydroxyproline	0.1 $\pm$ 0.0	0.3 $\pm$ 0.1	0.4 $\pm$ 0.2

interval 32.2 μmol of glycytyrosine/g kidney dry wt. are filtered and only 6.5% is excreted. A similar filtered load of glycyhydroxyproline is present during the 70–85 min interval when 41.4 μmol /g kidney dry wt. are filtered, of which 40.1% is excreted. These results suggest that the two peptides are handled quite differently by the brush-border enzymes. It is probable that glycytyrosine is hydrolysed before being reabsorbed, whereas the reverse may be true for glycyhydroxyproline. In support of this hypothesis is the observation (Ganapathy & Leibach, 1982) that glycyhydroxyproline is an inhibitor of carnosine uptake by brush-border membrane vesicles, and thus may also be a substrate for this transporter protein.

In conclusion we have demonstrated that the perfused kidney is able to metabolize large quantities of small peptides and that this metabolism can lead to the production of amino acids other than those which are constituents of the peptides. In particular the synthesis of serine is increased when glycine- or hydroxyproline-containing peptides are presented to the kidney.

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