Mucosal peptide hydrolase and brush-border marker enzyme activities in three regions of the small intestine of rats with experimental uraemia

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SUMMARY

1. The activities of nine peptide hydrolases and three non-peptidase brush-border marker enzymes have been quantified in crude homogenates prepared from the proximal, mid and distal regions of small-intestinal mucosa for sham-operated (n = 9) and uraemic (n = 14) rats. Abnormalities in enzyme activities were observed in all regions studied in the uraemic group, although no reduction in food intake occurred.

2. The proximal region of the small intestine from uraemic rats showed a general fall in enzyme activities associated with the brush-border. This fall was combined with a decline in mucosal protein content. In contrast, the mid and distal regions showed increased activity against the dipeptide tyrosyl-glycine.

3. It is proposed that the fall in brush-border enzyme activities in the proximal small intestine of uraemic rats is a response to the increased water intake associated with this, and presumably other, rat models of uraemia. The increased enzyme activity against tyrosyl-glycine found in the mid and distal regions of the small intestine of uraemic rats may be caused by an increased small-intestinal transit rate, but could be an attempt to maximize tyrosine absorption in response to decreased plasma tyrosine levels.

4. This study casts doubt on specific activities being the most useful units of enzyme activity, when measured in crude homogenates prepared from the proximal small intestine of uraemic rats. It also demonstrates that enzyme activities measured at a single site in the small intestine of uraemic rats may not be representative of the enzymatic changes occurring in the small-intestinal mucosa as a whole.

Key words: enzymes, intestinal mucosa, kidney failure, peptide hydrolases, small intestine, uraemia.

INTRODUCTION

Despite many reports of morphological changes in the gastrointestinal tract in patients with chronic renal failure [1-10], very little information is available regarding the functional integrity of the small intestine in this condition. Information on this subject could be of considerable value, as dietary treatment, specifically a reduction in dietary protein content, is of major importance in the treatment of the syndrome during its early stages. In addition, malabsorption of specific amino acids has been suggested as one possible mechanism bringing about some of the changes in plasma amino acid concentrations associated with uraemia [11-13].

Previous literature regarding direct measurement of amino acid or peptide absorption from the small intestine in uraemia is scant [11, 14, 15]. As mucosal brush-border and cytosolic peptidases are believed to be functionally important during the terminal digestion of protein, and are also reported to correlate well with morphological changes [16], quantification of these enzymes has been used previously to give an indication of potential malabsorption of protein in uraemia [5, 17-21]. Due to the problems of obtaining human tissue, virtually all work of this type has been performed with tissue provided by animal models of the human condition. These animal studies have, however, produced conflicting results, possibly due to the differences in intestinal regions studied and the differences in the animal models used [17-21].

In this study we have measured the activities of several peptide hydrolases and three non-peptidase brush-border marker enzymes in crude homogenates prepared from proximal, mid and distal small-intestinal mucosa obtained from uraemic and sham-operated rats. In the rat model of
chronic renal failure used, the level and duration of the induced uraemia has been well defined and the food intake of the uraemic and sham-operated animals was comparable [22].

METHODS

Animals

Male Sprague-Dawley rats, weighing approximately 300 g (range 273–347 g), were obtained from the University of Nottingham and were used for all experiments. Uraemia was induced by a two-stage surgical procedure as previously described [22]. Food with a protein content of 22.7% (w/w) and tap water were available ad libitum throughout the protocol. Blood samples were taken at 14-day intervals, animals were weighed regularly and food intake was estimated over regular intervals. Eighty-four days after the initial surgical step the small intestine was removed from animals while they were under heavy methoxyflurane anaesthesia. The small intestine was cooled and cleaned in situ by flushing the contents into the caecum with ice-cold saline (150 mmol/l NaCl) before removal.

Determination of plasma creatine and urea

The plasma creatinine concentration was determined in all blood samples by a modified Jaffé reaction which has been described previously [22]. In addition, the plasma urea concentration was measured pre-operatively and at the time of killing by using a Jeol JLC 6AH amino acid analyser.

Regions of intestine investigated

A proximal sample of small intestine was taken, starting 2 cm from the pylorus. A mid sample was taken from halfway down the length of the small intestine and a distal sample from the extreme distal end. All samples were approximately 7 cm in length. The proximal 4 mm of each sample were removed and sent for histological examination, whilst the remainder was blotted dry and stored in sealed tubes at −70°C until needed. Enzyme activities, in both uraemic and control samples, were found to be stable under these conditions for up to 1 month.

Homogenate preparation

Homogenates were prepared from the scraped and weighed mucosa of each intestinal segment in 2 mmol/l Tris–HCl buffer containing 50 mmol/l mannitol, pH 7.1 at 4°C. Homogenization was performed on ice, using a Teflon/glass homogenizer, at a concentration of 50 μl of buffer/mg of scraped mucosa.

Protein determination

Homogenate protein concentration was determined by a scaled-down version of the method of Lowry et al. [23].

Assays of brush-border marker enzyme activities

The activities of the brush-border marker enzymes, zinc-resistant α-glucosidase (EC 3.2.1.20), p-chloromercuribenzoate-resistant β-galactosidase (EC 3.2.1.23) and alkaline phosphatase (EC 3.1.3.1), were determined fluorimetrically, in the presence of 0.1% (w/v) Triton X-100, by a scaled-down version of the method described by Peters [24]. Substrates were 4-methylumbelliferyl-α-o-glucopyranoside, 4-methylumbelliferyl-β-D-galactopyranoside and 4-methylumbelliferyl-phosphate, respectively.

Assays of aminopeptidase activities

The activities of four peptidases, aminopeptidase N (microsomal aminopeptidase, EC 3.4.11.2), aminopeptidase A (EC 3.4.11.7), dipeptidyl peptidase IV (EC 3.4.14.5) and γ-glutamyltransferase (EC 2.3.2.2), were determined fluorimetrically, in the presence of 0.1% (w/v) Triton X-100, by a scaled-down method of that described by Peters [24]. Substrates were L-leucine-β-naphthylamide, L-α-glutamic acid-β-naphthylamide, glycyl-L-proline-β-naphthylamide and L-γ-glutamic acid-β-naphthylamide, respectively.

Peptide hydrolase activities against specific dipeptide substrates


Intestinal histology

Histological specimens were fixed in 10% (v/v) phosphate-buffered formalin before undergoing haematoxylin/eosin staining by the procedure routinely used at the Central Pathology Laboratories, Stoke-on-Trent.

Statistical analysis

Normally distributed data are presented as means ± 95% confidence interval, whereas abnormally distributed data are presented as medians with the interquartile ranges and means in parentheses. Statistical analysis of data was performed by using one-way analysis of variance or the Wilcoxon rank-sum test depending on data distribution.

RESULTS

Weight gain and food intake

The uraemic group showed a transient fall in body weight after each surgical step, but comparison of weight gain and food intake throughout the protocol showed no significant difference between the uraemic and sham-operated groups. These data are presented in detail elsewhere [22].
Plasma creatinine and urea concentrations

The mean plasma creatinine concentrations of the sham-operated (n = 9) and uraemic (n = 14) groups at the time of killing were 102.0 ± 21.3 μmol/l and 36.9 ± 8.7 μmol/l, respectively, and were significantly different. The plasma urea concentrations of the uraemic group showed a similar degree of change. These data are presented in detail elsewhere [22].

Mucosal protein content

Of the three regions of the small intestine studied, only the proximal region showed a significant change in protein concentration with uraemia. This change consisted of a substantial fall in mucosal protein content in the uraemic group (Tables 1 and 2).

Table 1. Enzyme activities and protein content in the proximal, mid and distal regions of the small intestine from the sham-operated animals (controls)

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Proximal region</th>
<th>Mid region</th>
<th>Distal region</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Glucosidase</td>
<td>68.48 ± 4.86</td>
<td>58.18 ± 10.18</td>
<td>10.58 ± 3.52</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>21.35 ± 301</td>
<td>165.0 ± 62.4</td>
<td>22.62 ± 5.0</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>0.328 ± 0.022</td>
<td>0.397 ± 0.102</td>
<td>0.142 ± 0.020</td>
</tr>
<tr>
<td>Aminopeptidase N</td>
<td>94.56 ± 7.36</td>
<td>138.4 ± 29.40</td>
<td>50.12 ± 8.68</td>
</tr>
<tr>
<td>Aminopeptidase A</td>
<td>7.136 ± 0.478</td>
<td>15.03 ± 2.29</td>
<td>10.77 ± 2.01</td>
</tr>
<tr>
<td>Dipeptidyl peptidase IV</td>
<td>22.82 ± 1.03</td>
<td>31.28 ± 2.90</td>
<td>17.40 ± 1.84</td>
</tr>
<tr>
<td>γ-Glutamyltransferase</td>
<td>19.47 ± 2.85</td>
<td>18.76 ± 2.96</td>
<td>9.234 ± 0.886</td>
</tr>
<tr>
<td>Gly-Leu hydrolase</td>
<td>4462 ± 1260</td>
<td>6526 ± 2468</td>
<td>3000 ± 1274</td>
</tr>
<tr>
<td>Leu-Gly hydrolase</td>
<td>1924 ± 276</td>
<td>2580 ± 618</td>
<td>1651 ± 346</td>
</tr>
<tr>
<td>Pro-Leu hydrolase</td>
<td>1395 ± 562</td>
<td>1384 ± 551</td>
<td>819 ± 257</td>
</tr>
<tr>
<td>Phe-Gly hydrolase</td>
<td>1365 ± 40</td>
<td>1647 ± 190</td>
<td>683 ± 77</td>
</tr>
<tr>
<td>Tyr-Gly hydrolase</td>
<td>1340 ± 172</td>
<td>1410 ± 368</td>
<td>677 ± 178</td>
</tr>
<tr>
<td>Protein content (μg/mg)</td>
<td>132.8 ± 6.5</td>
<td>103.7 ± 8.3</td>
<td>76.04 ± 4.26</td>
</tr>
</tbody>
</table>

Table 2. Enzyme activities and protein content in the proximal, mid and distal regions of the small intestine from the uraemic animals expressed as a percentage of the values for the sham-operated animals

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Proximal region</th>
<th>Mid region</th>
<th>Distal region</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Glucosidase</td>
<td>66***</td>
<td>92</td>
<td>101</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>61****</td>
<td>64</td>
<td>75</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>60****</td>
<td>97</td>
<td>128</td>
</tr>
<tr>
<td>Aminopeptidase N</td>
<td>75**</td>
<td>101</td>
<td>118</td>
</tr>
<tr>
<td>Aminopeptidase A</td>
<td>60****</td>
<td>91</td>
<td>122</td>
</tr>
<tr>
<td>Dipeptidyl peptidase IV</td>
<td>70**</td>
<td>102</td>
<td>120</td>
</tr>
<tr>
<td>γ-Glutamyltransferase</td>
<td>77</td>
<td>99</td>
<td>93</td>
</tr>
<tr>
<td>Gly-Leu hydrolase</td>
<td>99</td>
<td>99</td>
<td>134</td>
</tr>
<tr>
<td>Leu-Gly hydrolase</td>
<td>81</td>
<td>94</td>
<td>114</td>
</tr>
<tr>
<td>Pro-Leu hydrolase</td>
<td>101</td>
<td>132</td>
<td>149*</td>
</tr>
<tr>
<td>Phe-Gly hydrolase</td>
<td>83**</td>
<td>108</td>
<td>124</td>
</tr>
<tr>
<td>Tyr-Gly hydrolase</td>
<td>96**</td>
<td>142**</td>
<td>160**</td>
</tr>
<tr>
<td>Protein content</td>
<td>85***</td>
<td>102</td>
<td>109</td>
</tr>
</tbody>
</table>

Values are percentages calculated from results expressed in m-units/mg wet weight tissue for enzyme activities and in μg/mg for protein content. Statistical significance: *P<0.02, **P<0.01, ***P<0.001, ****P<0.0001 for the comparison between the sham-operated (n = 9) and uraemic (n = 14) groups.
The majority of these changes in enzyme activity occurred in the proximal region of the small intestine from uraemic animals. Relating enzyme activities to wet weight revealed several significant differences between the sham-operated and uraemic groups in this region. These differences consisted of a significant fall in the activities of all three brush-border marker enzymes, three of the four aminopeptidases and in the hydrolase activity against phenylalanyl-glycine in the intestine from uraemic animals. y-Glutamyltransferase was the only aminopeptidase measured not to be affected. The majority of these reductions in enzyme activities in the proximal region of the small intestine of uraemic rats were still apparent when related to protein content, although the level of significance was some 10-100-fold lower. When proximal activities were related to a-glucosidase activity, hydrolase activities against the dipeptides glycyl-leucine, phenylalanyl-glycine and tyrosyl-glycine were significantly elevated in the uraemic group as was the activity of aminopeptidase N.

The mid and distal regions of the small intestine showed far fewer changes than the proximal region, the most consistent finding being an increase in activity against the dipeptide tyrosyl-glycine in the distal and/or mid regions whether related to tissue wet weight, protein content or a-glucosidase activity. Alkaline phosphatase activity, however, was depressed in both mid and distal regions when related to a-glucosidase activity. In addition, y-glutamyltransferase activity appeared to be depressed in the distal region when related to protein content, whereas hydrolase activity against prolyl-leucine in the same region was increased when related to tissue wet weight.

**Intestinal histology**

Histological examination, by light microscopy, of intestinal segments taken from areas adjacent to those studied enzymologically revealed no gross differences in morphology between the sham-operated and uraemic groups.

**DISCUSSION**

Previous investigations of small-intestinal peptide hydrolase activities in rat models of chronic renal failure suffer from the fact that weight gain, and presumably food intake, of uraemic animals is markedly reduced [17-21]. As intestinal mucosal enzymes have been shown to change in response to decreased food intake and variation in dietary composition [26-28], the results obtained from such studies are questionable. In contrast, the rat model used in this study produced animals which maintained normal food intake and weight gain [22].

The enzymes studied here can be divided into three groups: non-peptidase brush-border marker enzymes, which are found almost exclusively in the brush-border, the aminopeptidases, which are also located in the brush-border, and the peptide hydrolase activities against dipeptides, which are predominantly cytosolic.

Of the three regions of the small intestine examined in this study, the proximal region showed the most noticeable changes. The general fall in enzymes associated with the brush-border, including the peptidase against phenylalanyl-glycine which has approximately 60% of its activity residing here [29], was accompanied by a fall in mucosal protein content. As most of these reductions in proximal brush-border enzyme activities are still apparent when related to protein content, it becomes evident these changes are not due to a simple increase in mucosal water content. It also becomes apparent that the fall in the brush-border enzyme activities is greater in scale than the overall fall in protein content. This may indicate that the proximal fall in mucosal protein content is caused by a general fall in brush-border enzymes with the cytosolic enzymes being unaffected. If this is the case, it casts doubt upon specific activities being the most useful units of enzyme activity in whole homogenates from this region, as falls in brush-border enzyme activities would be masked and increases in cytosolic enzyme activities would be enhanced.

It does not appear that these proximal reductions in brush-border enzyme activities are a direct result of uraemia as similar changes are not found in the mid and distal regions of the small intestine. The most likely explanation seems to be an adaptive response to the increased water intake associated with this, and presumably other, models of chronic renal failure. Increased water intake would dilute the substrates from these enzymes in the proximal intestine and could also increase the rate of transit of ingested material through this region. The resultant fall in substrate availability would in turn provoke the enterocytes to reduce brush-border enzyme synthesis. This autoregulation theory has been proposed by Kim et al. [26] after their study of the response of rat small-intestinal mucosal enzymes to starvation and is supported by the finding that activities of brush-border peptidases and disaccharidases change in response to isocalorific diets of different composition [27].

The increase in hydrolase activity against tyrosyl-glycine in both the mid and distal regions appears to be due to a tyrosine-specific aminopeptidase, as the glycine component of this dipeptide is common to two of the other dipeptides which were studied and found to be unchanged. This increased activity may be a response to increased concentrations of tyrosine-containing peptides in these regions, which would be conducive to an increased transit rate through the proximal small intestine proposed above and could point to a distal shift in the intestinal regions concerned with tyrosine assimilation. Further investigation of absorption of peptides containing tyrosine from the small intestine would seem to be indicated if only to exclude the possibility of malabsorption of tyrosine contributing to the low plasma levels of this amino acid consistently reported in uraemia [13, 22, 30, 31].

It is unlikely that the changes in mucosal enzyme activity described in the uraemic animals are functionally significant in this study, as the weight gain of the uraemic group was very close to that of the sham-operated group.
The renal insufficiency induced in these animals was, however, relatively mild in comparison with other models and it is conceivable that more pronounced changes occur in more severely uraemic animals. If this is the case, these changes could contribute to the reduction in weight gain observed in uraemic animals when compared with pair-fed controls [14].

The findings from the proximal region of the small intestine in this study are in general agreement with those of Grimmel et al. [17, 18], who studied a region of the intestine 10–20 cm from the pylorus [17, 18]. They also found that peptidase activities against glycyl-leucine, leucyl-glycine and prolly-leucine were unaffected by uraemia and in a later study also reported a fall in maltase activity in the same region. The results of our study are, however, in contrast with those of Sterner et al. [20, 21], who found an increase in peptidase activity against glycyl-leucine and no change in maltase activity in a slightly more distal region to that studied by Grimmel et al. [17, 18].

In conclusion, mucosal peptide hydrolases of the small intestine do change in experimental chronic renal failure, even when food intake is not impaired. The response of the proximal region differed from the other regions of small intestine studied. This suggests that investigations of terminal digestion/absorption from a single small section of the small intestine in uraemia may prove to be unrepresentative of terminal digestion/absorption from the small intestine as a whole. It would seem therefore, that in uraemia at least, studies of terminal digestion/absorption from the whole of the small intestine are likely to provide the most valuable information on the functional integrity of this organ.

ACKNOWLEDGMENTS

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REFERENCES

