Effects of intestinal adaptation on insulin binding to villus cell membranes

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SUMMARY  Insulin affects the expression of brush border enzymes by villus cells in vitro and in vivo. Physiological (lactation) and surgical (jejunoileal bypass) models of hyper- and hypoplasia were established so that insulin receptor characteristics could be related to villus histology, expression of sucrose and alkaline phosphatase, and plasma insulin concentrations. In lactating rats, villus height increased up to 55% (p < 0.005), and fasting plasma insulin increased 71% (p = 0.005), compared with controls. Insulin binding to villus cell membranes, and sucrose and alkaline phosphatase activities were, however, unchanged. In ileum of bypass operated rats, villus height increased 134% (p < 0.005) while insulin binding fell 68% (p = 0.025). Scatchard analysis revealed that this was largely because of reduction in binding by high affinity receptors. Sucrose and alkaline phosphatase specific activities fell 57% (p = 0.03) and 49% (p = 0.02) respectively, suggesting that ileal villus cells were hypomature. The slightly hypoplastic tissue of selfemptying loops showed normal insulin binding compared to jejenum of sham operated controls. Bypass and sham operated rats had similar fasting plasma insulin concentrations. Reduced insulin binding in markedly hyperplastic gut of bypass operated rats might reflect hypomaturity of villus cells. The reduction in insulin binding, however, might significantly modulate the effect of insulin on small intestinal mucosa and account for the fall in enzyme activity which occurs despite villus hyperplasia.

The functional capacity of the villus cell mass is an important determinant of the adequacy of the digestive/absorptive process. The differentiation of crypt cells into villus cells, while seemingly inevitable, need not guarantee differentiation into fully functioning cells. Only those older cells which have moved well up on the villus and so are well along the differentiation pathway, will fully express enzyme such as alkaline phosphatase1 or sucrase.2

Insulin is one factor which may influence differentiation. The small intestinal epithelium possesses insulin receptors3 and insulin can modify expression of brush border enzymes. It increases disaccharidase activity during organ culture of rodent small intestine.4 Furthermore, parenteral administration of insulin prevents fasting induced reduction in maltase activity.5 It is not clear, however, whether receptor status plays any significant role in modulating the effects of insulin on the villus cell, as it may in other tissues.67 In this study we examined the relationship between insulin receptor status, villus hyperplasia or hypoplasia, and expression of brush border enzymes. The lactating rat was used as a physiological model of hyperplasia. Rats with surgically induced jejunoileal bypass were studied to enable comparison of hypo- and hyperplastic tissues within the same animal.

Methods

Rats  Jejunoileal bypass surgery was performed in female Sprague-Dawley rats of 200–250 g. The jejunum was divided, 6–8 cm distal to the duodenoejunal flexure. The proximal end of the transected jejunum was then anastomosed, end-to-side, to the ileum, 10 cm proximal to caecum, and the distal end was oversewn. Insulin receptor status, histology and brush border enzymes were studied eight weeks later and the results compared with those in control rats which underwent simple jejunal transection and re-anastomosis. The rats were paired for litters, weight and sex, and were allowed to feed freely together. Four lactating rats...
were measured. Determination of width in both directions was necessary because these rats' villi were tongue-shaped with the broad side aligned against the intestinal flow. To provide a more direct measure of villus cell mass, a villus area index was derived from the formula: height \times (longitudinal base + transverse base). Measurements were made on villi and crypts in randomly selected 0.5 cm portions of histological sections from each gut segment. Measurement of villus area by a similar method has been shown to correlate well with villus cell mass.8

ASSAYS
Alkaline phosphatase, sucrase and protein1 were assayed as previously described. DNA was measured as detailed.9 Rat plasma insulin was kindly assayed by Dr Marjorie Dunlop, University of Melbourne, Department of Medicine, The Royal Melbourne Hospital. Statistical comparisons were made using Student's t test (two-tailed).10 Blood was obtained by cardiac puncture just before death.

INSULIN BINDING STUDIES
Methods were similar to those previously described.3 Immediately after removal, gut segments were rinsed and mucosa scraped off using a glass slide. The scrapings consisted mainly (95%) of villus cells, identified by presence of a brush border, with few stromal cells or crypts. Ten volumes of 10 mmol/l Tris, 50 mmol/l mannitol, HCl (pH 7-4) were added to each volume of scrapings and homogenised for 30 seconds at speed 6 in a Waring blender. The homogenate was centrifuged for 10 minutes at 5000 g to remove nuclei and whole cells. The crude membrane preparation was then collected and washed twice by centrifugation at 27,000 gmax and finally suspended in the above buffer containing 0.1 mmol/l phenylmethylsulphonyl fluoride, aprotonin 0.01 TIU/ml, and bacitracin 100 U/ml. All the above procedures were carried out at 4°C. To triplicate 2 mg aliquots of membrane protein, 30,000 cpm of 125I-insulin was added together with varying amounts of unlabelled insulin up to 5 \times 10^{-3} mmol/l in a final volume of 0.5 ml. Membranes were incubated at 15°C for two hours. Membrane bound and free 125I was measured by gamma spectrometry after separation by rapid centrifugation through a 1-5% bovine serum albumin cushion at 27,000 gmax. Degradation of 125I-insulin was less than 5% and was corrected for as described.3 Receptor characteristics were calculated from binding curves by Scatchard analysis using the computer program 'Ligand'.11 Total insulin specific binding in each experimental condition was determined by subtraction of 125I-insulin bound in the presence of a 10,000-fold excess of unlabelled insulin from that bound per milligram of membrane protein.

CHEMICALS
Unless otherwise specified, reagents were obtained from Sigma Chemical Co, St Louis, MO, USA. Pork monocomponent insulin (Nouvo) was iodinated by the chloramine-T method to give a specific activity of 120-160 mCi/mg.

HISTOLOGY
Sagitally aligned sections for micrometric measurements were cut from paraffin embedded tissue. Paraffin blocks were sectioned in two planes, longitudinal and transverse to the long axis of the gut. Villus height, crypt depth and villus width at the base

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Fig. 1 Diagrammatic representation of gut segments studied in the various models and corresponding controls. For operated animals, segment 1-2 ('jejunum') was 5-6 cm long; 2 cm was used for histology and 3-4 cm for enzyme assay. Segment 3-4 ('self-emptying loop') was the next 20 cm; 2 cm of the most proximal end was used for histology, 3 cm for enzyme assay and the next 15 cm for receptor studies. Segment 5-6 ('ileum') was 15±3 cm long as a result of adaptation after bypass surgery; again, short proximal portions were processed for histology and enzyme assay. For non-operated rats, segments 1-2 and 5-6 were 20 cm long; proximal pieces were processed for histology and enzyme assay.

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were studied 15 days postpartum and compared with paired, weight matched, non-lactating females. All rats were killed by ether anaesthesia at 0900 to 1100 hours after an overnight fast. Segments of small intestine were removed for study as shown in Fig. 1. All test rats were > 90% body weight of controls.
in the absence of unlabelled insulin. There was insufficient material in the jejunal segment remaining in continuity after jejunoileal bypass for Scatchard analysis.

**Results**

**ANIMAL MODELS**

Lactating rats showed significant mucosal hyperplasia, p < 0.05, in both jejunum and ileum (Fig. 2). The villus area index increased by 47% in the jejunum and by 57% in the ileum. In rats subjected to jejunoileal bypass (Fig. 3) both jejunum and ileum remaining in continuity showed significant hyperplasia. The selfemptying loops showed a modest degree of atrophy, the villus area index being 90% that of the controls (p < 0.05). Plasma insulin concentrations (fasting) were 0.79 ± 0.27 ng/ml (± SD) in the rats with jejunoileal bypass, 0.70 ± 0.27 in the sham operated rats (p = NS), and 1.01 ± 0.20 in the lactating rats (p = 0.005, Student’s t test).

![Histological findings in lactating (Lac+) and nonlactating (Lac−) rats. Bars represent SE. *p < 0.05.](image)

**INSULIN BINDING STUDIES**

Binding of 125I-insulin to membranes was most stable at 15° and reached a plateau at 90 min. Insulin could be rapidly dissociated by the addition of 10−5 mol/l insulin to membranes to which 125I-insulin had been bound. Binding was proportional to the amount of membrane protein present. The amount of 125I-insulin bound to normal membranes fell from proximal to distal jejunum (p = 0.013) but fell not further in the ileum (Table 1). Insulin binding to membranes from hyperplastic ileum of bypass rats (Table 1) was substantially lower than in control ileum (p = 0.025). This difference was borne out by a significant change in the binding curves (Fig. 4). The ratio of cell protein to DNA remained constant in all models at 16.7–17.9; 1 mg protein represented cell membranes from approximately 6 × 10⁶ cells.

A typical Scatchard analysis of insulin binding to jejunal villus cell membranes is shown in Fig. 5. Computer analysis by ‘Ligand’ gave a significantly better fit with a two-site rather than a one-site model, consistent with two classes of receptors, one of high and one of low affinity. Binding site characteristics in lactating animals, which showed mild to moderate

![Fig. 3 Histological findings in jejunoileal bypass rats and in sham operated controls. 'Blind loop' refers to the selfemptying loop. Villus area index was calculated as detailed in methods. *p < 0.05.](image)
Table 1  Quantification of specific insulin binding to cell membranes

| Physiological Model: |  
|----------------------|-------|
| Jejunum – lactating (3) | 11914 ± 1446 |
| – nonlactating (3) | 11210 ± 506 |
| Ileum – lactating (4) | 5021 ± 685 |
| – nonlactating (4) | 4848 ± 627 |
| Jejunoileal Bypass Model: |  
| S-E loop – bypass (4) | 4173 ± 994 |
| – sham † (3) | 5316 ± 124 |
| Ileum – bypass (4) | 2414 ± 312 |
| – sham (3) | 7534 ± 1114 |

* Total ¹²⁵I-insulin bound in absence of nonradioactive insulin less nonspecific binding in presence of 10000 fold excess of unlabelled insulin. Results shown as t ± SE. † Probability, Student’s t test (two tailed). Number of animals shown in brackets. ‡ This was actually distal jejunum which corresponded to the gut segment taken from the self-emptying (S-E) loop in bypass animals.

hyperplasia, were not significantly different from those of nonlactating animals (Table 2). In the markedly hyperplastic ileum of jejunoileal bypass rats, however, substantial changes were seen (Table 2), in particular, there were many less high affinity receptors (p < 0.01). Such changes reflected the lower total binding in these tissues. Insulin binding to the mildly atrophic tissue of the self-emptying loops was similar to that in control tissue (Table 2).

ENZYME ASSAYS
Sucrase and alkaline phosphatase activities, characteristically, were highest in jejunum. Ileal hyperplasia in jejunoileal bypass rats was accompanied by a significant fall in specific activity of these brush border enzymes (Table 3). There was no significant

Fig. 4 Insulin binding isotherms for ileal membranes prepared from sham operated rats (closed symbols) and bypass operated rats (open symbols). The solid line represents the computer compiled fit of the data for each experimental condition.

Fig. 5 Composite Scatchard plot of insulin binding to jejunal epithelial membranes from three non-lactating rats. Specific B/F: ratio of specifically bound ¹²⁵I-insulin to free (unbound) insulin.

Table 2 Details of insulin binding to cell membranes in the models of intestinal adaptation

<table>
<thead>
<tr>
<th></th>
<th>Class 1 receptor</th>
<th>Class 2 receptor</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Affinity (I/mol × 10⁻⁹)</td>
<td>Binding capacity (mol/mg × 10⁹)</td>
</tr>
<tr>
<td>Non-lactating v Lactating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum – non-lactating</td>
<td>4.50 ± 0.79*</td>
<td>5.55 ± 0.45</td>
</tr>
<tr>
<td>– lactating</td>
<td>5.83 ± 1.70</td>
<td>7.49 ± 1.38</td>
</tr>
<tr>
<td>Ileum – non-lactating</td>
<td>2.19 ± 0.24</td>
<td>6.46 ± 1.25</td>
</tr>
<tr>
<td>– lactating</td>
<td>2.30 ± 0.51</td>
<td>3.50 ± 0.72</td>
</tr>
<tr>
<td>Bypass v. Sham Operated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum – sham</td>
<td>5.85 ± 1.30</td>
<td>1.31 ± 0.27</td>
</tr>
<tr>
<td>– self-emptying loop</td>
<td>6.07 ± 1.55</td>
<td>2.57 ± 0.23</td>
</tr>
<tr>
<td>Ileum – sham</td>
<td>2.18 ± 0.12</td>
<td>6.59 ± 1.01</td>
</tr>
<tr>
<td>– bypass</td>
<td>6.36 ± 0.41</td>
<td>0.92 ± 0.18</td>
</tr>
</tbody>
</table>

* t ± SE Values given are for the compiled Scatchard plot prepared from individual rat data in each group. †Scatchard plots were compared by Ligand using an F-test criterion on the residual variances and by the Runs Test.
change in brush border enzyme activity (data not shown) in hypoplastic tissue of selfemptying loops or in hyperplastic tissue of lactating rats.

Discussion

Expression of brush border enzymes is the hallmark of the differentiating villus cell. Measurement of enzyme activity along the villus/crypt gradient has shown that young villus cells express disaccharidases less well than the more mature cells and that alkaline phosphatase is most active in the mature cells situated at the tip of the villus. Luminal factors such as bacteria, bile salts and pancreatic proteases reduce brush border enzyme activity by affecting the villus cell life span and/or turnover of brush border enzymes. In contrast, certain ingested nutrients can induce enzyme activity—for example, sucrase is induced by sucrose and fat induces alkaline phosphatase. Hormones such as corticosteroids also modify brush border enzyme activity.

Insulin appears to play a role in modifying the expression of brush border enzymes by the differentiated villus cell. Insulin added to small intestine in organ culture causes an increase in brush border disaccharidases. When added to suckling rat small intestine in organ culture, it leads to a precocious appearance of sucrase, an increase in glucoamylase, a decrease in lactase and stimulation of DNA synthesis. When administered to suckling rats in vivo insulin has the same effect as in organ culture and is synergistic with thyroxine. Little is known of its effect when given to adult rats, except that parenteral administration seems to prevent the fall in maltase which is normally induced by fasting. These effects of insulin would suggest that the insulin binding sites present on villus cells are of physiological significance in that they promote or facilitate differentiation of the cell. Interestingly, insulin does not stimulate amino acid absorption by intestine even though it stimulates amino acid uptake by a range of other cell types.

The presence of high and low affinity binding sites for insulin corresponds to observations in other tissues. It is not yet clear if the two classes of binding site normally present in mammalian cells subserve different functions—for example, stimulation of growth by one class and of cellular metabolic functions by the other. It is also not clear if the differences we observed in binding site characteristics between proximal and more distal small intestine, are related to differences in functional (digestive/absorptive) capacity, to differences in degree of cell differentiation or to differences in rate of cell turnover between these segments of small intestine. Certainly, the modest hyperplastic changes seen in lactation, and the mild hypoplasia seen in the selfemptying loop, are not associated with major alterations in insulin binding or expression of brush border enzymes.

A substantial reduction in insulin binding did occur, however, in the markedly hyperplastic tissue of the rats with jejunoileal bypass. Mouse crypt cells have fewer insulin binding sites than villus cells. It is unlikely though that a proportional increase in crypt cells caused the reduction, as in the jejunoileal bypass model villus height increased in the same proportion as crypt depth (Fig. 3). Plasma insulin concentrations were the same in bypass and sham operated animals and so the reduction could not have been because of the down-regulation of binding sites. In such a case, down regulation would also have been expected in the selfemptying loop. It seems probable that the substantial fall in insulin binding in hyperplastic jejuno of jejunoileal bypass rats may be analogous to what is seen in cultured adipocytes where insulin binding site density increases with increasing morphological differentiation. In the jejunoileal bypass animals, the low levels of brush border enzymes (expressed relative to DNA) in hyperplastic intestine indicate that the villus cells are relatively hypomature. It remains to be shown, however, whether this hypomaturity is the cause or result of the decrease in insulin receptors.

Whatever the explanation, there are substantial changes in insulin binding sites in the jejunoileal bypass model of hyperplasia. As hormone action can be modulated by receptor status these changes in insulin receptor status could modify the effect of insulin on the maturing villus cell.

Table 3 Relationship between villus area and brush border enzyme activity.

<table>
<thead>
<tr>
<th></th>
<th>Villus area (μm² × 10⁻²)</th>
<th>Sucrase (mU/mg DNA)</th>
<th>Alkaline phosphatase (mU/mg DNA)</th>
<th>Leucine aminopeptidase (mU/mg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ileum - bypass</td>
<td>83 ± 5*</td>
<td>25 ± 2*</td>
<td>1058 ± 102</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Ileum - sham</td>
<td>194 ± 11</td>
<td>51 ± 6</td>
<td>2108 ± 225</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>P = †</td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*± SE, n = 4; enzyme activities expressed/mg cellular DNA.
† Student's t test.
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References


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