Effect of insulin induced hypoglycaemia on in vitro uptake of 3-O-methylglucose by rat jejunum

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Summary Acute hypoglycaemia (<2.0 mmol/l) selectively increased in vitro 3-O-methylglucose uptake by rat jejunum. In contrast, uptake of 3H-phenylalanine or 59Fe (III) was unchanged in the hypoglycaemic animals. The hypoglycaemia was accompanied by decreased tissue adenosine nucleotide energy charge. The increased uptake was phlorrhizin-inhibitable and therefore reflected increased Na+ dependent glucose carrier activity. Cycloheximide did not block the increased 3-O-methylglucose uptake, implying that the mechanism is not the result of increased de novo carrier protein synthesis.

Rats rendered chronically diabetic show various changes in small intestinal function and structure including enhanced sugar transport, increased activities of brush border hydrolases and of glycolytic enzymes, depressed calcium absorption and, in the longer term, morphological changes.1-4 Early studies in alloxan induced diabetes in rats indicated that glucose absorption was accelerated in diabetes and this effect was prevented by insulin.5 3-O-methyl D-glucose absorption by isolated perfused jejunal segments was also increased in alloxan induced diabetes and in rats where diabetes was induced by anti-insulin serum.6 Another study confirmed increased intestinal glucose absorption in streptozotocin induced diabetes, but with a less marked rise in glucose absorption in anti-insulin serum induced diabetes.7 There was no change in glucose absorption when insulin was added to the media.

A study carried out in man also showed that intestinal glucose absorption was significantly greater in diabetic than in normal subjects. Insulin administration did not alter glucose absorption in either patient group, even though it always depressed the blood glucose.8 In contrast, starvation induced hypoglycaemia in the rat produced accelerated glucose absorption by everted sacs of rat upper ileum.9 Little is known, however, of the mechanism of the hypoglycaemic response. We have therefore characterised and used an in vitro technique for determining 3-O-methylglucose transport by isolated jejunal tissue slices and investigated the changes after insulin induced hypoglycaemia.

Methods

Animals
Five week old male Sprague-Dawley rats (140-160 g) were used throughout. They were fed a standard rodent diet (Labsure LAD 1 diet Lavender Hill, Manea, Cambridgeshire).

In vitro uptake
The technique used has been fully validated for use with a variety of nutrients.10 In particular, 59Cr EDTA has been shown in several studies to be an excellent choice for an extracellular marker when measuring 3-O-methylglucose uptake.11-14 In brief, a piece of intestinal tissue was removed 3 cm distal to the duodenojejunal junction, cut longitudinally and sectioned into fragments (5-15 mg wet weight). After a brief rinse in buffered HEPES (N-2-hydroxyethaneperipperazine N-2 ethane sulphonic acid)-oxygenated medium (16 mmol/l HEPES-KOH, 125 mmol/l NaCl, 3-5 mmol/l KCl, 1 mmol/l CaCl2, 10 mmol/l MgSO4, pH 7.4), the fragments were trans-
ferred to a similar medium containing [3H] 3-O-methylglucose with 51Cr-EDTA as the extracellular fluid marker. After incubation, the reaction was terminated by blotting and rinsing the fragments in ice cold HEPES medium. The tissue samples were rebotted, weighed and counted for 51Cr in a LKB ‘Wallac 1280’ gamma counter. Samples were then oxidised in a Packard ‘Tri-Carb’ sample oxidiser and the [3H]2O counted in a ‘Wallac 8100’ liquid scintillation counter. Glucose uptake, corrected for the 51Cr extracellular space was expressed as nmol/mg tissue/min. For 55Fe (III) and [3H] phenylalanine uptakes, the same methods were used except that the medium contained 55Fe (III)-(nitrilotriacetate acid), or 4[3H]-phenylalanine.

**Insulin Administration**

Insulin 0.5 U/kg or 5 U/kg was administered through the tail vein and the animals killed 15–60 min later. Tail vein glucose concentrations were monitored at two min intervals with BM sticks.

**Glucose Clamping**

In order to investigate whether hyperinsulinaemia or the insulin induced hypoglycaemia was responsible for the increased 3-O-methylglucose uptake, the blood glucose of some animals was clamped by intravenous perfusion of glucose through the tail vein post insulin injection. An initial priming dose of 5 ml 50% (w/v) glucose in water was administered followed by 0.5 ml/min for the duration of the experiment.

**Cycloheximide Studies**

Cycloheximide was administered as a bolus dose through the tail vein (3 mg/kg body weight) in three groups of experiments: (i) concurrently with insulin as an intravenous injection; (ii) 30 min before the insulin injection; (iii) inhibitor alone into control animals. The dose of cycloheximide administered was shown by previous investigators to inhibit protein synthesis.

**Effect of Phlorrhizidin**

Phlorrhizidin was added to the buffer medium to block glucose uptake Na+ dependent by the mucosal fragments. The concentration used was 10 mmol/l which has been shown to block 95% of active hexose uptake in the small intestine.

**Nucleotide Analysis**

Intestinal tissue collected as above was freeze clamped in liquid nitrogen. The weighed, frozen intestine was thawed in trichloroacetic acid, centrifuged and the supernatant collected. Sodium bicarbonate was added to adjust the pH to pH 7.4. ATP was determined by the hexokinase reaction. ADP

![Graph](image-url)
jejunal fragments for time The Results insulin was injected at time 0 and values show mean (SE) for six animals in each group. Animal groups are: (○) 0-15 mol/l NaCl; (■) insulin 0-5 U/kg; (▲) insulin 5 U/kg.

Fig. 2  Direct linear plots by method of Eisenhall and Cornish-Bowden of [H]-3-O-methylglucose uptake by jejunal fragments for varying medium 3-O-methylglucose concentrations. Results are mean (SE) for: (□) control animals; (■) animals injected with 5 U insulin/kg body weight 30 min before study; (●) as previous experiment but incubation medium contained ouabain 1 mmol/l.

and adenosine monophosphate (AMP) were determined by the pyruvate kinase and adenylate kinase reactions, respectively. All reagents were from Sigma Chemicals (Poole, Dorset) or BDH Chemical Co Ltd (Poole, Dorset). Radiochemicals were obtained from Amersham International (Amersham, Bucks). Actrapid porcine insulin was obtained from Novo Laboratories (Novo Industrials, Denmark).

Results

In vitro 3-O-methylglucose (3 MG) uptake The time course for 3 MG uptake for varying 3 MG concentrations (up to 200 mmol/l) are shown in Figure 1a. 3 MG uptake was directly proportional to wet weight of jejunum for a range from 1 to 25 mg (r=0-83; p<0-01) and at varying medium concentrations showed Michaelis-Menten kinetics (Fig. 1b). Calculation of $K_m$ and $V_{max}$ was by direct linear plots (Fig. 2). After 20 minutes incubation in the buffer medium, the tissue slices undergo progressive deterioration and ultimately cellular death. Accordingly, the method is reliable for incubation periods of up to 20 minutes. Five minutes was chosen as the standard incubation time for the experiments reported here as (from Fig. 1a and b) it will be seen that, first, it is on the linear slope of the time course curves, and therefore is a more reliable measure of cellular kinetics and, second, there will be minimal risk of any cellular degeneration at this stage in the experiment.

Effect of insulin infusion

The blood glucose in control and insulin treated rats are shown in Figure 3. Both the low and high doses of insulin produced a fall in the blood glucose over the first 10 to 15 min followed by recovery phase. In vitro jejunal glucose uptake was assayed at 15, 30, and 60 min after the insulin injections and showed Michaelis Menten kinetics (Fig. 4). Only the 30 minute values are shown as the effect is greatest at this time with 5 U/kg insulin injection. At 15 minutes and 60 minutes there was no significant difference in uptakes with insulin injection versus controls: however, ouabain and phlorhizin inhibitor was seen at both these times. The apparent kinetic parameters were shown in Table 1. Addition of insulin to the incubation medium did not affect the in vitro 3 MG uptake. In separate experiments in vitro 3 MG uptake was deter-
Hypoglycaemia and jejunal glucose uptake

Fig. 4 3-O-methylglucose uptake by jejunal mucosa. Uptakes performed 30 min after injection of 0-15 (moll) NaCl (●) or 5 U/kg insulin (○) in standard medium, in media containing ouabain after injection of 0-15 moll NaCl (▲) or after injection of 5 U/kg insulin (△) or in media containing phloretin after injection of 5 U/kg insulin (■). Results show mean (SE) for six animals in each group.

Mineral with 1 mmol/l ouabain in the incubation medium and the results are shown in Figure 4: in both control and insulin treated animals uptake was fully inhibited by ouabain. Intestinal 3 MG uptake in insulin treated animals in whom blood glucose levels were 'clamped' also showed saturation kinetics but the kinetic parameters did not differ from control animals (Table 1).

The specificity of the response to insulin infusion was investigated by estimating 3Fe (III) and [3H]-phenylalanine uptake in vitro. As seen in Table 2 no significant differences were found between the control and the insulin treated groups. Intestinal 3 MG uptake in insulin treated and control animals treated with cycloheximide are shown in Table 1. The increased 3 MG uptake in the hypoglycaemic animals receiving cycloheximide were similar to that seen in animals receiving insulin alone. When cycloheximide was administered to control animals no blood glucose changes were seen within the first two hours. Insulin treated rats had reduced ATP levels and energy charge with increased ADP and AMP compared with controls. The total nucleotide level was unaffected (Table 3).

Discussion

This study shows that marked hypoglycaemia increases absorption of 3-O-methylglucose by the rat jejunum. The effect is maximal 30 minutes after the injection of the hormone and is not seen with lower doses of insulin and is prevented by maintenance of normal blood glucose. The kinetic studies show that the effect is the result of an increase in the apparent \( V_{\text{max}} \) for 3 MG uptake – that is, increased carrier number or activity. The range of medium glucose concentrations used was from 5 to 250 mmol/l. The lower limit of the range was chosen to be well below \( K_m \) to allow accurate determination of the \( K_m \).

The upper limit did pose problems regarding the osmolarity of the incubation medium: this was partly compensated by up to a 30% reduction in the NaCl content of the buffer at the higher concentrations of 3 MG. The spurious controversy between real and apparent \( K_m \) does not apply, although Debnam and Levin\(^a\) claimed that the true \( K_m \) for active absorption for a variety of sugars is about three times less than that of the apparent \( K_m \), the \(^{3}C\) permeability probe has been shown in many subsequent studies to correct for this.\(^a\)

The results in the insulin+glucose treated rats which show no change in the 3 MG uptake indicates that hypoglycaemia per se and not the increased plasma insulin level is the mediator of the enhanced 3 MG uptake. To confirm this it would be necessary to carry out further studies to exclude other factors – for example, hyperglucagonaemia, as concentrations of pancreatic glucagon would be expected to rise during acute hypoglycaemia.\(^b\) When glucagon (1 μg) was administered iv in control animals, however, no change in intestinal glucose uptake was observed.

No changes in 3 MG uptake were seen when jejunal slices were incubated with the medium containing 1000 U/l insulin. This also suggests that the

Table 1  Intestinal 3-O-methylglucose uptake 30 minutes after 5 U/kg insulin under various experimental conditions.

<table>
<thead>
<tr>
<th>Control</th>
<th>Experimental</th>
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<tbody>
<tr>
<td>( K_m )</td>
<td>( V_{\text{max}} )</td>
</tr>
<tr>
<td>30 min after insulin</td>
<td>60±0.3 (3-0)</td>
</tr>
<tr>
<td>30 min after insulin with glucose clamping</td>
<td>59±0.1 (2-1)</td>
</tr>
<tr>
<td>30 min after insulin and 60 min after cycloheximide</td>
<td>57±1 (3-2)</td>
</tr>
<tr>
<td>30 min after insulin with phloretin in incubation medium</td>
<td>58±0 (2-9)</td>
</tr>
</tbody>
</table>

Results show mean (SE) where \( K_m \) (mmol/l), \( V_{\text{max}} \) (nmol/5 min/mg tissue) and \( n=6 \) for each experiment. Statistical analysis by Student's t-test: *\( p<0.01 \).
Table 2  Effect of hypoglycaemia on the in vitro uptake of [3H]-phenylalanine and "Fe by rat jejunum

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypoglycaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( V_{max} )</td>
<td>( K_m )</td>
</tr>
<tr>
<td>'H-Phe</td>
<td>(0-01) 4-90</td>
<td>0-24</td>
</tr>
<tr>
<td>&quot;Fe(III)</td>
<td>0-035 (0-003)</td>
<td>0-110 (0-004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0-113 (0-004)</td>
</tr>
</tbody>
</table>

Results show mean (SE) for five animals in each group. \( K_m \) (mmol/l) and \( V_{max} \) (nmol/min/mg tissue).

phenomena is not a direct insulin effect on the gut. An alternative explanation could be that in a preparation that has no vascular clearance, insulin failed to penetrate the muscle layer, and did not reach the enterocyte receptors.

The specificity of the response for glucose was confirmed by the absence of any effect of hypoglycaemia on intestinal "Fe (III) or phenylalanine uptake in the hypoglycaemic animals. The results of ouabain studies would indicate that the increased glucose uptake is mediated by the Na\(^+\) dependent carrier. Phlorrhizin, a specific competitive inhibitor of glucose uptake at the brush border, is a more useful reagent and the results provide strong evidence that a Na\(^+\) dependent carrier is indeed involved.

The results of the nucleotide analysis show that the mucosal energy charge of the hypoglycaemic animals is reduced compared with the controls and presumably is caused by the hypoglycaemia. It is worth noting that the excised intestinal tissue was freeze clamped so that nucleotide levels in the incubated tissue itself could be determined. This accounts for the lower energy change values compared with in situ freeze clamped tissue.\(^{17}\)

The failure of cycloheximide to prevent the increased glucose uptake in the hypoglycaemic animals suggest that de novo synthesis of glucose carrier was not responsible for the increased 3 MG uptake. Possible mechanisms therefore would include increased recruitment of glucose carriers to the brush border membrane or increased carrier activity (turnover number).

The acute regulation of glucose absorption, transport and metabolism in rat small intestine has been studied in vivo but these studies have mainly investigated the hyperglycaemic state.\(^{16-20}\) These changes appeared to correlate with the inhibition of two mucosal enzymes, pyruvate dehydrogenase (EC 1.2.2.2) and phosphofructokinase (EC 2.7.1.11).

Table 3  Effect of hypoglycaemia on jejunal nucleotide content

<table>
<thead>
<tr>
<th>Animals</th>
<th>Control</th>
<th>Hypoglycaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>77-9 (9-0)</td>
<td>47-2 (6-2)(^+)</td>
</tr>
<tr>
<td>ADP</td>
<td>80-2 (8-7)</td>
<td>62-3 (7-5)*</td>
</tr>
<tr>
<td>AMP</td>
<td>63-6 (7-7)</td>
<td>85-1 (9-3)*</td>
</tr>
<tr>
<td>Total nucleotide</td>
<td>222 (25)</td>
<td>195 (23)*</td>
</tr>
<tr>
<td>Energy charge</td>
<td>0-53 (0-09)</td>
<td>0-40 (0-07)(^+)</td>
</tr>
</tbody>
</table>

Results show mean (SE) nucleotide content (pmol/mg tissue) for 4 animals in each group. Assays performed on tissue collected 30 min after either 0-15 (mol/l) NaCl (control) or 5 U/kg insulin (hypoglycaemic). Statistical analysis: \(*p<0.05; \pm p<0.01.\) Energy charge (EC) calculated as ref \(^+\): \( EC = \frac{\text{ATP} + \frac{1}{2} \text{ADP}}{\text{ATP} + \text{ADP} + \text{AMP}} \)

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