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The acute regulation of glucose absorption, transport and metabolism in rat small intestine by insulin *in vivo**

George L. KELLETT,† Anita JAMAL, James P. ROBERTSON and Nigel WOLLEN
Department of Biology, University of York, York YO1 5DD, U.K.

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The effect of acute changes in insulin concentrations *in vivo* on the absorption, transport and metabolism of glucose by rat small intestine *in vitro* was investigated. Within 2min of the injection of normal anaesthetized rats with anti-insulin serum, lactate production and glucose metabolism were respectively diminished to 28% and 21% of normal and the conversion of glucose into lactate became quantitative. These changes correlated with the inhibition of two mucosal enzymes, namely the insulin-sensitive enzyme pyruvate dehydrogenase, and phosphofructokinase, which was shown by cross-over measurements to be the rate-limiting enzyme of glycolysis in mucosa. The proportion of glucose translocated unchanged from the luminal perfusate to the serosal medium was simultaneously increased from 45% to 80%. All the changes produced by insulin deficiency were completely reversed with 2min when antiserum was neutralized by injection of insulin *in vivo*. The absorption and transport of 3-*O*-methylglucose were unaffected by insulin. It is concluded that glucose metabolism in rat small intestine is subject to short-term regulation by insulin *in vivo* and that glucose absorption and transport are regulated indirectly in response to changes in metabolism. Moreover, transport and metabolism compensate in such a way as to deliver the maximal 'effective' amount of glucose to the blood, whether as glucose itself or as lactate for hepatic gluconeogenesis.

Experimental diabetes produces various changes in rat small-intestinal function and structure, including enhancement of sugar transport (Crane, 1961; Levin, 1969; Caspary, 1973), increased specific activities of brush-border membrane hydrolases (Olsen & Rogers, 1971; Mahmood *et al.*, 1978; Pothier & Hugon, 1982) and of glycolytic enzymes (Anderson, 1974), depressed Ca^{2+} absorption (Schneider *et al.*, 1977), and, in the longer term, extensive morphological changes (Caspary, 1973). However, attempts to show that small-intestinal function is subject to regulation by insulin have been unsuccesful. Thus studies on the effects of insulin *in vivo* have been conflicting (Levin, 1969; Caspary, 1973), and in those few instances where the mechanism underlying the effects of insulin or insulin-deficiency has been elucidated, it has been shown to be indirect. For example, the enhancement of glucose transport in diabetic rats is thought to be caused by the synthesis of new glucose carriers in the basolateral membrane induced by hyperglycaemia (Czaky & Fisher, 1981), depressed Ca^{2+} absorption in diabetic rats by a renal defect in 1,25-dihydroxycholecalciferol synthesis (Schneider *et al.*, 1977), and depressed fatty acid esterification in normal rats injected with insulin by hypoglycaemia (Shiau & Holtzapple, 1980). Since no convincing effects of insulin on small-intestinal function *in vitro* have been demonstrated (Crane, 1961; Fromm *et al.*, 1969; Olsen & Rosenberg, 1970; Leese & Mansford, 1969, 1971), it has become widely accepted that intestine is an insulin-insensitive tissue.

We have investigated the regulatory properties of rat intestinal mucosal phosphofructokinase under two conditions of insulin deficiency, namely those of starvation and streptozotocin-diabetes (Jamal & Kellett, 1983*a*,*b*). In both states we observed that the enzyme was significantly more susceptible to inhibition by ATP than was the case for normal fed rats. Furthermore, the regulatory properties of mucosal phosphofructokinase could be restored to normal, either by the re-feeding of starved rats on a high-carbohydrate diet for 18h

* In memory of Eraldo Antonini.

† To whom reprint requests should be addressed.

overnight or by injection of insulin into streptozotocin-diabetic rats over a period of 25h. Both these observations are consistent with the idea that rat small-intestinal carbohydrate metabolism is regulated by insulin *in vivo*, and prompted us to examine the effects of acute insulin deficiency on intestinal function.

Materials and methods

Female Wistar rats (220–250g) were fed *ad libitum* on standard laboratory diet (Oxoid, modified 41B) with free access to water. Antiserum to bovine insulin was raised in guinea pigs as described by Neubauer & Schone (1978). The neutralizing capacity of the antiserum was approx. 4 units of bovine insulin/ml as determined by immunochemical titration. Insulin deficiency was induced in anaesthetized rats (Sagatal, 0.1ml/100g body wt.) by the injection into the femoral vein of 1ml of anti-insulin serum, which was the volume required to produce maximal plasma glucose concentrations (approx. 20mM) after 60min. Control rats received 1ml of 0.9% NaCl. After given times, the proximal jejunum (the first 10cm from the ligament of Treitz) was removed, and mucosal samples were collected as described previously (Jamal & Kellett, 1983*a*) and frozen immediately in liquid N_2. In the subsequent preparation of extracts, mucosal samples were homogenized directly, without first being thawed.

In experiments designed to reverse the effects of anti-insulin serum, animals that had been injected with anti-insulin serum for 10min were further injected with bovine insulin either at a dose equivalent to the titre of the antiserum or at a dose of 6units/rat in excess over the titre, before removal of tissue samples. The insulin vehicle was 0.9% NaCl.

Mucosal extracts for the determination of phosphofructokinase activity were prepared and assayed as described by Jamal & Kellett (1983*a*). The regulatory properties of phosphofructokinase were expressed as the activity ratio, v/V, where v is the activity at pH7.0 in the presence of 2.5mM-ATP and a given concentration of fructose 6-phosphate and V is the maximal activity at pH8.0. Mucosal extracts for the determination of pyruvate dehydrogenase activity were prepared and assayed as described by Robertson *et al.* (1980). Only the initial activity, which reflects the proportion of active enzyme, could be assayed; total pyruvate dehydrogenase activity could not be determined, possibly because of the irreversible inactivation of the intestinal enzyme during the incubation with phosphatase, as observed by Lamers & Hulsmann (1974). *p*-(*p*-Aminophenylazo)benzenesulphonic acid used in the assay of pyruvate dehydrogenase was given by Dr. R. M. Denton (Department of Biochemistry, University of Bristol).

The concentrations of glycolytic intermediates were determined by using either frozen samples of mucosa or frozen samples of whole intestine. The latter could be frozen in liquid N_2 within seconds of removal from a rat, whereas the former took about 2min. The frozen tissue samples were ground into a powder in liquid N_2 and a 25% (w/v) homogenate was prepared in 1M-$HClO_4$ (Lamprecht & Trautschold, 1974). The homogenate was centrifuged at 2000*g* for 5min at 4°C; the supernatant was removed, neutralized with 5M-$KHCO_3$ and re-centrifuged. Samples of the supernatant were then assayed fluorimetrically for glucose 6-phosphate and fructose 6-phosphate, fructose 1,6-bisphosphate and triose phosphate, 3-phosphoglycerate, and phosphoenolpyruvate and pyruvate (Lowry & Passonneau, 1972).

Glucose absorption, transport and metabolism were studied in isolated jejunal loops *in vitro* by using the preparation described by Fisher & Parsons (1949) modified as described by Hanson & Parsons (1976) so that the recirculated luminal perfusate was segmented with bubbles of gas (O_2/CO_2, 19:1) (Fisher & Gardner, 1974); the flow rates of perfusate and gas were 25ml/min and 3ml/min respectively. The jejunal loops were perfused for 1h, beginning 10min after the injection of either anti-insulin serum or saline, with a medium consisting of Krebs–Henseleit buffer (Nicholls *et al.*, 1983) containing 5mM-glucose: the serosal side of the loop was bathed in the same medium. In the preparation, glucose is absorbed from the luminal perfusate by the mucosa; part of the absorbed glucose is then metabolized and the remainder is transported from the mucosa and appears in the serosal medium, so that the translocation of glucose from perfusate to serosal medium is an active process when glucose is present initially at the same concentration on both sides. Absorption is defined as net luminal–mucosal flux and was measured by the rate of disappearance of glucose from the luminal perfusate; transport is defined as net mucosal–serosal flux and was measured by the rate of appearance of glucose in the serosal medium; metabolism was calculated as their difference. The proportion of glucose translocated unchanged was given by transport expressed as a percentage of absorption. The conversion of glucose into lactate was given by (0.5 × total lactate production) expressed as a percentage of glucose metabolized. The difference between glucose metabolized and (0.5 × total lactate production) was assumed to represent glucose oxidation. The rates of absorption and transport were linear with time for 60min after the start of perfusions, showing that the perfusion system was

in a steady state. The absorption and transport of a non-metabolizable analogue was determined by using 3-*O*-[^{14}C]methyl-D-glucose, from Amersham International.

Biochemicals were purchased from Sigma Chemical Co. or Boehringer, and other reagents were of analytical grade.

Results

Changes in the concentrations of plasma glucose and in the activities of two enzymes, phosphofructokinase and pyruvate dehydrogenase, in the jejunal mucosa of the small intestine were determined as a function of time after the injection of anti-insulin serum. The earliest time at which measurements could be made satisfactorily was 2min, the time taken to obtain and freeze a mucosal scraping. At 2min, the initial activity of pyruvate dehydrogenase was diminished from 274 ± 50 to 138 ± 9nmol/min/g wet wt. of mucosa ($P < 0.02$, Fig. 1*b*). At the same time, the activity ratio of phosphofructokinase at 0.5mM-fructose 6-phosphate, $v_{0.5}/V$, determined at pH7.0 in the presence of 2.5mM-ATP, was diminished from 0.42 ± 0.03 to 0.24 ± 0.02 ($P < 0.001$, $n = 6$; Fig. 1*c*). The diminution reflected an increase in the susceptibility of phosphofructokinase to inhibition by ATP in insulin-deficient rats (Fig. 2*a*), resulting in an increase in K_m for fructose 6-phosphate from 0.5mM to 0.7mM (Fig. 2*b*). In contrast with the very rapid inhibition of the two enzymes, the concentration of plasma glucose was not significantly different from normal (7.4 ± 0.4mM) until more than 10min after injection of anti-insulin serum (Fig. 1*a*). The initial activity of pyruvate dehydrogenase and the activity ratio of phosphofructokinase remained depressed as the plasma glucose concentration rose to a maximal value of 20.0 ± 1.6mM at 60min, when rats were demonstrably diabetic. Thereafter the plasma glucose concentration declined rapidly, so that after 90min it had returned to normal, as had the initial activity of pyruvate dehydrogenase. In contrast, the activity ratio of phosphofructokinase was still depressed at 90min and returned to normal only by 120min (Fig. 1). Throughout the whole 120min period, no changes were observed in the concentration of plasma glucose, nor in the initial activity of pyruvate dehydrogenase and in the activity ratio of phosphofructokinase in control rats injected with 0.9% NaCl (Fig. 1).

The effect of anti-insulin serum on enzyme activity was rapidly reversed by insulin. Thus when rats injected with anti-insulin serum were again injected after 10min with insulin at a dose equivalent to the titre of antiserum, measurements made after a further 2min showed that the initial

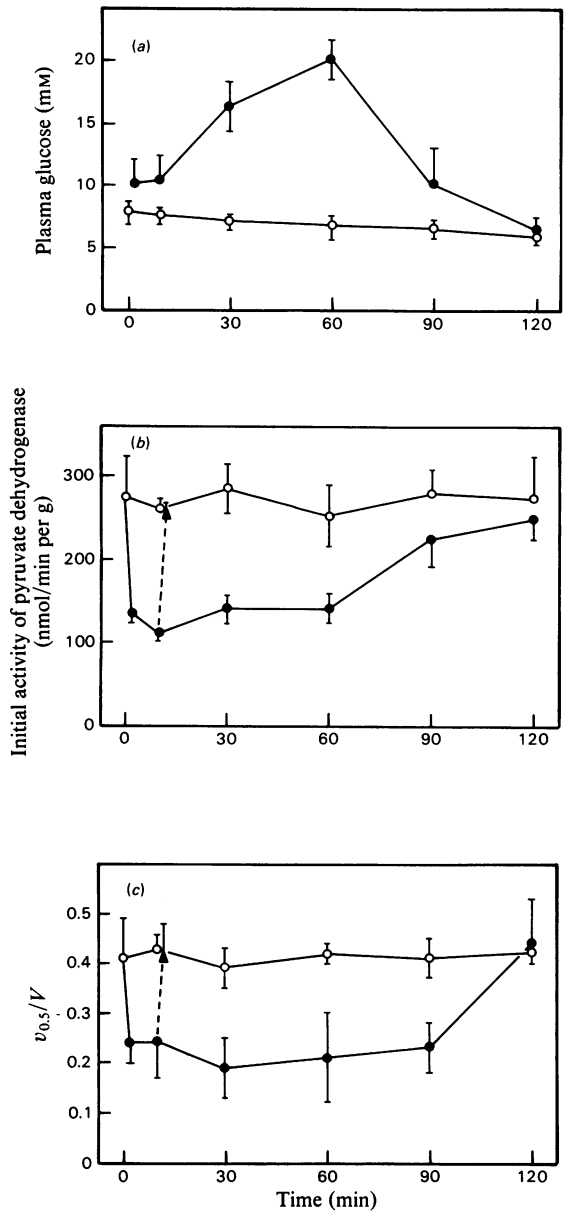

Fig. 1. *Effect of acute insulin deficiency on plasma glucose concentration and on mucosal phosphofructokinase and pyruvate dehydrogenase activities in rat small intestine*

(●) Rats were injected with 1ml of anti-insulin serum and the changes in (*a*) plasma glucose concentration, (*b*) initial activity of pyruvate dehydrogenase and (*c*) $v_{0.5}/V$ of phosphofructokinase were measured as a function of time. Control rats (○) were injected with 0.9% NaCl. If, after 10min, the antiserum was neutralized by the injection of insulin at a dose equivalent to the antiserum titre, then enzyme activities were restored to normal within a further 2min (----). For full experimetnal details see the text. Values are given as means ± S.E.M. for (*a*) six, (*b*) three and (*c*) six rats for each point.

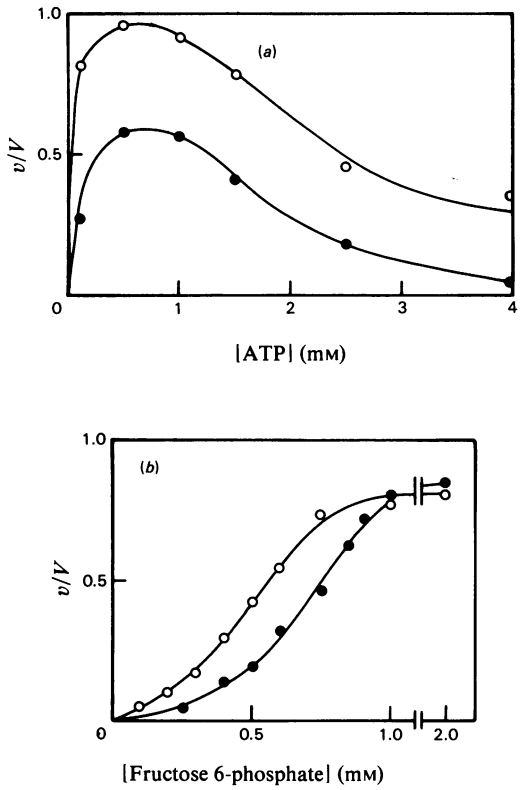

Fig. 2. *Effect of acute insulin deficiency on the regulatory properties of phosphofructokinase in the jejunal mucosa of rat small intestine*

The activity ratio at pH7, v/V, was assayed as described in the text for rats injected with anti-insulin serum (●) and control rats injected with 0.9% NaCl (○). (*a*) Dependence of activity on ATP concentration in the presence of fructose 6-phosphate (0.5mM); (*b*) dependence of activity on fructose 6-phosphate concentration in the presence of ATP (2.5mM).

activity of pyruvate dehydrogenase and the activity ratio of phosphofructokinase had already been restored to normal (Figs. 1*b* and 1*c*; broken line). The concentrations of glycolytic intermediates were determined in samples of mucosa and whole intestine taken from rats 60min after the injection of anti-insulin serum, i.e. the time at which plasma glucose concentrations were maximal and before the effects of anti-insulin serum began to decline (Table 1). In mucosa, only glucose 6-phosphate and fructose 6-phosphate were significantly altered in rats injected with anti-insulin serum compared with normal rats; the concentrations of both were increased, as expected, since phosphofructokinase was more inhibited in rats treated with anti-insulin serum. However, the concentration of fructose 1,6-bisphosphate was not affected. In whole intestine, on the other hand, the expected negative cross-over at phosphofructokinase was observed, with significant enhancement in the concentrations of glucose 6-phosphate and fructose 6-phosphate and significant diminution in that of fructose 1,6-bisphosphate. No changes in the concentration of other metabolites were observed (Table 1).

When jejunum taken from rats 10min after the injection of anti-insulin serum was perfused luminally for 60min *in vitro* with glucose (present on both the luminal and the serosal sides at an initial concentration of 5mM), glycolysis was profoundly inhibited: compared with control values, the rates of glucose metabolism and lactate production were diminished to as little as 21% and 28% respectively, and the conversion of glucose into lactate became quantitative (Table 2). Absorption was also diminished to 58%, although transport was unaffected, so that the proportion of glucose translocated unchanged was increased from 45% to 80%. The injection of insulin at a dose equivalent to the antiserum titre 10min after the injection of antiserum

Table 1. *Concentrations of glycolytic intermediates in mucosa and in whole intestine after the production of acute insulin deficiency in rats*

Rats were injected with 1ml of anti-insulin serum or 0.9% NaCl, and metabolites were determined after 60min. For full experimental details see the text. Concentrations are expressed in nmol/g wet wt. of tissue and are given as means ± S.E.M. for six rats. *P* values are given for the comparison of rats treated with anti-insulin serum and control rats: ***$P<0.001$; *$P<0.05$.

	Mucosa			Whole intestine		
Intermediate	Control	Anti-insulin serum	Change (%)	Control	Anti-insulin serum	Change (%)
Plasma glucose	7.4±0.2	18.8±0.9***	+154±12	6.9±0.3	21.6±2.1***	+213±31
Glucose 6-phosphate	52.8±2.5	82.9±4.9***	+57±10	50.7±2.3	68.1±2.5***	+34±7
Fructose 6-phosphate	13.6±0.8	24.9±3.8*	+83±28	11.9±0.9	16.8±2.2*	+41±20
Fructose 1,6-bisphosphate	127.5±0.8	127.3±4.3	-	128.9±4.7	97.7±11.1*	−24±9
Triose phosphates	35.0±2.4	39.6±4.4	-	48.9±6.1	43.2±3.6	-
Phosphoenolpyruvate	25.7±2.9	28.5±4.8	-	27.7±3.8	30.3±5.6	-
Pyruvate	340.0±19.3	329.5±61.7	-	220.3±11.3	187.9±20.8	-

Table 2. *Regulation of glucose absorption, transport and metabolism in rat small intestine by insulin in vivo*

Rats were injected with 1 ml of 0.9% NaCl or anti-insulin serum, and jejunal segments were removed after 10 min. In experiments designed to reverse the effects of anti-insulin serum, insulin was injected after 10 min either at a dose equivalent to the titre of antiserum (titre) or a dose of 6 units/250 g rat in excess over antiserum (titre + 6 units); jejunal segments were then removed after times stated. Jejunal loops were perfused for 60 min *in vitro* with a recirculated perfusion medium segmented with gas; glucose was present on both the luminal and serosal sides at a concentration of 5 mM. For full experimental details see the text. Values are given as means ± S.E.M. for *n* rats. *P* values are given for the comparison of rats treated with anti-insulin serum and control rats: ***$P<0.001$, **$P<0.01$, *$P<0.05$.

			Rate (μmol/h per g dry wt.)							
			Glucose			Lactate production				
Treatment of rat	Insulin (units/rat)	*n*	Absorption	Transport	Metabolism	Luminal side	Serosal side	Total/2	Glucose translocated (%)	Glucose utilized converted into lactate (%)
Normal	–	4	413 ± 9	185 ± 8	229 ± 3	67 ± 10	270 ± 15	169 ± 6	45 ± 1	74 ± 3
Anti-insulin serum	–	6	240 ± 13***	193 ± 13	48 ± 2***	41 ± 1	51 ± 2***	47 ± 1***	80 ± 1***	97 ± 3***
Anti-insulin serum	Titre	4	370 ± 16	137 ± 10*	233 ± 7	111 ± 14*	237 ± 12	174 ± 10	36 ± 6	75 ± 5
Normal	6 units for 2 min	5	320 ± 19**	115 ± 11***	206 ± 17	45 ± 5	152 ± 14***	98 ± 9***	35 ± 3	48 ± 5**
Anti-insulin serum	Titre + 6 units for 2 min	4	400 ± 60	38 ± 22***	362 ± 63***	72 ± 8	266 ± 33	169 ± 20	10 ± 6***	51 ± 10**
Anti-insulin serum	Titre + 6 units for 10 min	4	438 ± 68	98 ± 9***	340 ± 65	46 ± 6	253 ± 29	150 ± 17***	24 ± 4***	46 ± 5***

Table 3. *Effect of insulin deficiency on the absorption and transport of 3-O-methyl-D-glucose by rat small intestine*
Rats were injected with 1ml of 0.9% NaCl or anti-insulin serum, and jejunal segments were removed after 10min. Insulin was injected as described in Table 2. Loops were perfused with 3-*O*-methyl-D-glucose present on both the luminal and serosal sides at a concentration of 5mM. For full experimental details see the text. Values are given as means ± S.E.M. for rats. The only significant differences observed were between the absorption and transport of rats injected with insulin at a high dose of 6 units/rat in excess over antiserum: †$P<0.02$.

Treatment of rat	Insulin (units/rat)	n	Rate (μmol/h per g dry wt.) Absorption	Transport
Normal	–	5	112±13	108±12
Anti-insulin serum	–	5	94±14	94±15
Normal	6 units for 2min	4	140±13	97±8†
Anti-insulin serum	Titre+6 units for 2min	4	143±14	98±11†

restored all functions to normal within 10min, except for transport, which was depressed to about 74% (Table 2). In contrast, the injection of insulin at dose of 6units/rat in excess over antiserum more than reversed the effects of antiserum on metabolism; indeed, glucose metabolism was increased more than 7-fold to a value 158% of normal. Absorption was restored to normal, so that transport was diminished to 20% of normal. In this instance, $v_{0.5}/V$ for mucosal phosphofructokinase was 0.50±0.02 ($n=4$), but was not significantly above normal; the initial activity of pyruvate dehydrogenase, on the other hand, was greater than normal (341±45 compared with 274±50nmol/min per g wet wt., $P<0.01$), and the conversion of glucose into lactate was diminished from 74% to 51%. The properties of intestine from antiserum-treated rats that had been injected with the high insulin dose did not match those of normal rats injected with the same dose. The latter showed only a normal rate of glucose metabolism, whereas absorption and transport were diminished to 77% and 62% of normal. Although glucose metabolism was not affected, the proportion of glucose converted into lactate was diminished to 48% (Table 2).

The absorption of the non-metabolizable analogue, 3-*O*-methyl-D-glucose, matched its transport throughout the perfusion period, and anti-insulin serum was without effect. The injection of either normal rats or antiserum-treated rats with a high dose of insulin (6units/rat in excess over antiserum) was also without significant effect on the absorption and transport of the analogue; however, absorption was significantly greater than transport, by some 45% ($P<0.02$; Table 3).

Discussion

Short- and long-term regulation of rat small-intestinal enzyme activities by insulin in vivo

Pyruvate dehydrogenase exists in active (dephosphorylated) and inactive (phosphorylated) forms whose interconversion is controlled by insulin (Denton *et al.*, 1975); the initial activity measured in the present study reflects the proportion of enzyme in the active form. The production of insulin deficiency in rats by the injection of anti-insulin serum causes the proportion of active pyruvate dehydrogenase to be halved within 2min and to remain at that value until 90min after injection, when the effects of anti-insulin serum have been overcome by the secretion of insulin and rats are no longer diabetic (Fig. 1). These changes, together with the observation that the neutralization of antiserum by injection of insulin into rats treated with anti-insulin serum restores the proportion of active pyruvate dehydrogenase to normal within 2min (Fig. 1), provide firm evidence therefore that intestinal mucosa is subject to short-term regulation by insulin *in vivo*.

In general, the changes in the proportion of the active form of pyruvate dehydrogenase are paralleled by changes in the activity ratio of mucosal phosphofructokinase (Fig. 1), which is more susceptible to inhibition by ATP in rats treated with anti-insulin serum that in normal rats (Fig. 2). It is therefore apparent that mucosal phosphofructokinase is also regulated by insulin *in vivo*. The inhibition of phosphofructokinase in rats injected with anti-insulin serum precedes the enhancement of plasma glucose concentrations, in confirmation of our previous conclusion that hyperglycaemia is not responsible for the depressed activity ratio seen in streptozotocin-diabetic rats (Jamal & Kellett, 1983*b*).

The rapidity of the changes in the activities of pyruvate dehydrogenase and phosphofructokinase in response to acute changes in insulin concentrations *in vivo* suggests that glucose metabolism in intestine is regulated directly by insulin. Although such a contention can ultimately only be proved by experiments *in vitro*, it is consistent with reports that epithelial cells possess specific binding sites for insulin, although such sites have yet to be shown to be functional receptors (Bergeron *et al.*, 1979; Forgue-Lafitte *et al.*, 1980).

The administration of insulin to streptozotocin-

diabetic rats is also able to restore the diminished activity ratio of mucosal phosphofructokinase to normal (Jamal & Kellett, 1983*b*). However, in contrast with rats treated with anti-insulin serum, insulin was ineffective in the short term; indeed it was only partially effective after 17h, did not become fully effective until 25h, and its action was blocked by the protein synthesis inhibitor cycloheximide. The half-life of mucosal glycolytic enzymes is of the order of 7–10h (in the starved condition; Jones & Mayer, 1973). Hence changes in protein synthesis will not be involved in the response to anti-insulin serum, a fact confirmed by the observation that no changes in total phosphofructokinase activity were observed over the 120min period of the experiment (results not shown). It is apparent, then, that the effect of insulin deficiency on the control of mucosal phosphofructokinase activity, and therefore of glycolysis (see below), proceeds in two stages: firstly, rapid inhibition, via an allosteric or covalent modification mechanism that is readily reversible by insulin, and, secondly, longer-term changes in protein synthesis that block the rapid re-activation by insulin and that must themselves be first reversed by long-term administration of insulin.

Control of glycolysis in rat small intestine

We have previously proposed that phosphofructokinase, rather than hexokinase, is the principal rate-limiting enzyme of glycolysis in rat small intestine (Jamal & Kellett, 1983*a*,*b*). The demonstration in whole intestine of a negative cross-over in the concentrations of glycolytic intermediates from glucose 6-phosphate and fructose 6-phosphate to fructose 1,6-bisphosphate when insulin-deficient rats were compared with normal rats (Table 1), together with the simultaneous inhibition of mucosal phosphofructokinase activity and diminution in glucose metabolism, confirms our proposal. Leese & Bronk (1975) have shown that the potential glycolytic capacity of the mucosa is far higher than that of the muscularis, so that the properties of the whole intestine largely, though not completely, reflect those of mucosa. This may also account for the observation that, except for pyruvate, there were no significant differences in the concentrations of glycolytic intermediates between control samples of mucosa and whole intestine (Table 1). That mucosa does not show a complete negative cross-over may be a proper reflection of changes in glycolytic flux in mucosa. Alternatively it may be an artifact from the occurrence of changes in the concentrations of intermediates in the 2min required to obtain and freeze the mucosal samples; this is unlikely, however, since the concentrations of intermediates in control samples were very similar in mucosa and whole intestine and the latter was frozen within seconds of removal. No cross-over was observed at the level of pyruvate kinase in either case.

The demonstration that phosphofructokinase is the rate-limiting enzyme of glycolysis in rat small intestine affords a ready explanation for the existence of a Pasteur-type effect, in which glucose metabolism is inversely related to oxygen concentration in vascularly perfused preparations of rat small intestine (Lamers & Hulsmann, 1972; Hanson & Parsons, 1976; Porteous, 1978). work from our laboratory has shown that fructose 2,6-bisphosphate and the enzyme responsible for its formation, phosphofructokinase-2, are not present in intestinal mucosa (Jamal *et al.*, 1984). Some other mechanism must therefore exist for the mediation of the short-term effects of insulin reported here.

The initial activity of pyruvate dehydrogenase in normal rats was 76 ± 14 μmol/h per g dry wt. (calculation based on a dry-wt./wet-wt. ratio for mucosa of 0.215; Leese, 1974). This activity is similar to that reported by Pritchard & Porteous (1977) and is not much less than the rate of glucose oxidation, 120 ± 14 μmol/h per g dry wt. (Table 2). Moreover, the conversion of glucose metabolized into lactate is quantitative in insulin-deficient rats, and, although a proportion of pyruvate dehydrogenase is still present in the active form, the latter must be completely inhibited, possibly by acetyl-CoA derived from endogeneous triacylglycerol. Thus pyruvate dehydrogenase catalyses the rate-limiting step of oxidation. The simultaneous inhibition of phosphofructokinase accounts for the inhibition of glucose metabolism in insulin-deficient rats.

Regulation of glucose absorption and transport and their relationship to metabolism in response to insulin in vivo

The perfusion experiments measured active sugar absorption, since glucose was present at the same low concentration (5mM) on both sides of the intestine and utilization was measured as the difference between absorption and transport. The modified Fisher & Parsons (1949) preparation (recirculated, segmented flow) used in the present study appeared to be very efficient, for the proportion of glucose translocated unchanged was some 45%; indeed, this value is comparable with even the highest value of 44% reported for vascularly perfused preparations at similar low concentrations of glucose in the lumen alone (Hanson & Parsons, 1977; Porteous, 1978; Nicholls *et al.*, 1983). In response to acute insulin deficiency, glucose absorption and metabolism were diminished to 58% and 21% of normal respectively, whereas transport was unaffected (Table 2). The diminution in absorption appeared to be contingent on the

diminution in metabolism, for the absorption of 3-*O*-methyl-D-glucose was unaffected by acute insulin deficiency (Table 3); this analogue was not capable of being metabolized, and transport matched absorption for the whole of the perfusion (Table 3). The restoration of glucose metabolism to normal by the injection of insulin-deficient rats with insulin at a dose equivalent to the antiserum titre restored absorption to normal. The dependence of absorption and transport on metabolism is further emphasized by their relationship in an insulin-replete state in which metabolism was increased to 158% of normal by the injection of insulin into antiserum-treated rats at a dose of 6 units/rat in excess over antiserum. In this instance glucose absorption increased as before, but not beyond the normal value of 413 μmol/h per g dry wt. Thus this value appeared to represent the maximal possible at 5 mM-glucose, determined by the kinetic characteristics of the glucose carrier of the brush border. Because absorption was limited and metabolism greatly enhanced by insulin repletion, transport was diminished to as little as 20% of normal. Although control experiments in which normal rats were injected with the same dose of insulin suggest that a small part of this decrease might be caused by a direct effect independently of metabolism (line 4, Table 2), no change in the transport of 3-*O*-methyl-D-glucose was observed (Table 3), and it is clear that the predominant factor determining the diminished glucose transport is the enhanced metabolism. It is thus apparent that metabolism has priority over transport and that, had metabolism been increased still further until it matched absorption, then transport would have been completely abolished. This conclusion is supported by the observation of Nicholls *et al.* (1983), using vascularly perfused jejunum from normal fed rats, that at concentrations of luminal glucose below a threshold value of 2 mM all glucose absorbed was metabolized; net transport became detectable only above 2 mM and increased with increasing glucose concentrations.

It is clear then that the absorption and transport of glucose are intimately related to its metabolism and that the regulation of absorption and transport by insulin occurs primarily as the indirect consequence of the regulation of metabolism. A potential mechanism that provides a simple rationale for the relationship between absorption, transport and metabolism is that changes in metabolism produce changes in the intracellular glucose concentration, so altering the concentration gradients that determine the active absorption and passive transport of glucose. Whether absorption or transport is affected depends on which of the two processes is rate-limited and on the direction of change in metabolism.

Role of the intestine in inter-organ metabolism

Shapiro & Shapiro (1979) have reported that in rats in which the liver was cut off from the blood circulation by a porto-caval shunt about 10–15% of glucose was translocated unchanged when present initially in the lumen at a concentration of 33 mM, implying that in normal rats 'effective' glucose transport occurs *in vivo* via intestinal lactate production and subsequent hepatic gluconeogenesis. Indeed, Remesy *et al.* (1978) have further reported that intestine is the principal source of lactate for gluconeogenesis. As far as the whole animal is concerned, then, 'effective' glucose transport is given in our terms by [transport + (total lactate)/2] (assuming that luminal lactate is reabsorbed). In normal and insulin-deficient rats the cellular requirement for ATP either is low or is satisfied by the metabolism of endogenous substrate, such as triacylglycerol, so that the rate of pyruvate oxidation is low. The data in Table 2 (lines 1 and 2) show that, in such circumstances, 'effective' transport is 86% and 100% respectively of that absorbed, despite an almost 5-fold difference in the rates of glucose metabolism between normal and insulin-deficient rats. The elegance of the intestinal function lies in the compensation of metabolism and transport in such a way as to deliver the maximal 'effective' transport of glucose to the blood, whether as glucose itself or as lactate for use by the whole animal.

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