Regulation of GLUT5, GLUT2 and intestinal brush-border fructose absorption by the extracellular signal-regulated kinase, p38 mitogen-activated kinase and phosphatidylinositol 3-kinase intracellular signalling pathways: implications for adaptation to diabetes

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We have investigated the role of the extracellular signal-regulated kinase (ERK), p38 and phosphatidylinositol 3-kinase (PI 3-kinase) pathways in the regulation of intestinal fructose transport. Different combinations of anisomycin, PD98059 and wortmannin had very different effects on fructose transport in perfused isolated loops of rat jejunum. Transport was stimulated maximally by anisomycin (2 μ M) and blocked by SB203580 (20 μ M), confirming involvement of the p38 pathway. PD98059 (50 μ M) alone had little effect on fructose transport. However, it had a dramatic effect on stimulation by anisomycin, diminishing the K_a 50-fold from 1 μ M to 20 nM to show that the ERK pathway restrains the p38 pathway. The K_a for diabetic jejunum was 30 nM and PD98059 had no effect. Transport in the presence of anisomycin was 3.4-fold that for anisomycin plus PD98059 plus wortmannin. Transport was mediated by both GLUT5 and

GLUT2. In general, GLUT2 levels increased up to 4-fold within minutes and with only minimal changes in GLUT5 or SGLT1 levels, demonstrating that GLUT2 trafficks by a rapid trafficking pathway distinct from that of GLUT5 and SGLT1. GLUT2 intrinsic activity was regulated over a 9-fold range. It is concluded that there is extensive cross-talk between the ERK, p38 and PI 3-kinase pathways in their control of brush-border fructose transport by modulation of both the levels and intrinsic activities of GLUT5 and GLUT2. The potential of the intracellular signalling pathways to regulate short-term nutrient transport during the assimilation of a meal and longer-term adaptation to diabetes and hyperglycaemia is discussed.

Key words: intestine, SGLT1, sugar, transport.

INTRODUCTION

The current view in the literature is that fructose is transported across the jejunal brush-border membrane in both human [1] and rat [2–5] by GLUT5, a member of the facilitative glucose transporter family that possesses a very strict stereospecificity for fructose. The basolateral membrane of rat jejunum does not contain GLUT5. Instead, fructose is transported by GLUT2, as demonstrated by immunocytochemical localization of the transporter in the basolateral membrane [6] and by the mutual and complete inhibition of glucose and fructose transport in rat basolateral membrane vesicles [7]. After 10 days of adaptation to streptozotocin-diabetes, GLUT5 levels in the brush-border membrane are enhanced almost 3-fold: in addition, GLUT2 is also readily detectable at the brush-border membrane [5]. Since GLUT2 is inhibited by both glucose and phloretin, whereas GLUT5 is not, we were able to use the difference in stereospecificity to show that both GLUT5 and GLUT2 contribute to the 1.6-fold overall enhancement in fructose transport observed in diabetes. Moreover, by selectively inhibiting the GLUT2 contribution, we were able to demonstrate that diabetes diminishes the intrinsic activity (the transport activity per molecule) of GLUT5 to 20% of its value in normal rat jejunum.

In the previous two papers [8,9], we have reported that GLUT2 is in fact present also at the brush-border membrane of normal rat jejunum, to which it trafficks in response to the activation of protein kinase C (PKC), most probably the β II isoenzyme. We have described the role of GLUT2 in mediating fructose ab-

sorption in conjunction with GLUT5 and also its role in mediating the diffusive component of glucose absorption in conjunction with SGLT1. We further explained why its role at the brush border was previously overlooked by us and others and also why we were readily able to detect it previously in diabetic but not normal jejunum [5,8]. Thus as soon as jejunum is excised for experiments *in vitro*, PKC is inactivated because of the loss of the influence of endogenous hormones. GLUT2 is therefore very rapidly lost from the membrane and is hard to detect in preparations *in vitro* from normal rats. In contrast, GLUT2 can be readily detected in the membrane of diabetic rats, because its initial level is almost seven times higher and it is lost from the membrane less quickly than in normal rats.

The striking differences in transport and transporter regulation between normal and diabetic rats are presumably caused by differences in the activities of intracellular signalling pathways. The activation of PKC by treatment of jejunum with PMA caused only a modest reduction in the intrinsic activity of GLUT5 to no less than 75% of the control value [8]; that is, very much higher than the 20% seen for diabetic rats [5]. It is therefore clear that other signalling pathways must be involved in the response to diabetes. Indeed there are some indications in the literature of the pathways that might be involved. For example, the intrinsic activity of GLUT1 in various cultured cell types, such as Chinese hamster ovary cells and 3T3-L1 fibroblasts, ranges over an order of magnitude [10], while the intrinsic activity of the GLUT2 transporter is doubled in response to experimentally maintained hyperglycaemia in rat jejunum [11].

Abbreviations used: PKC, protein kinase C; ERK, extracellular signal-regulated kinase; MAP, mitogen-activated protein; PI 3-kinase, phosphatidylinositol 3-kinase; MEK, ERK/MAP kinase kinase.

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More recently, it has been reported that stimulation of either the extracellular signal-regulated kinase (ERK) or the p38 mitogen-activated protein kinase (MAP kinase) pathways in KB cells by insulin-like growth factor-1 and interleukin respectively, almost doubles glucose transport within 1 h [12]. Similarly, activation of the p38 MAP kinase pathway by anisomycin stimulates glucose transport in 3T3-L1 adipocytes and in Clone 9 epithelial cells [13]. In each of these instances, it appeared that the intrinsic activity of pre-existing GLUT1 molecules in the plasma membrane was altered, since there were no detectable changes in the levels of GLUT1. In addition, it is well established that the trafficking of many transporters, especially GLUT4 in adipocytes, is regulated by a wortmannin-sensitive phosphatidylinositol 3-kinase (PI 3-kinase) pathway [14]. With these observations in mind, and also the fact that there are well-established connections between PKC and the MAP kinase and PI 3-kinase pathways, we decided to investigate whether these pathways might be involved in the regulation of the trafficking and intrinsic activities of GLUT5 and GLUT2.

EXPERIMENTAL

All experimental procedures have been described in detail previously [5,8,9,15].

Statistical analysis

Values are presented as means $\pm$ S.E.M. and were tested for significance using Student's *t* test.

RESULTS

Modulation of brush-border fructose transport by activators and inhibitors of intracellular signalling pathways

To establish whether the ERK and p38 MAP kinase pathways might be involved in the regulation of fructose transport across the brush-border membrane, isolated loops of rat jejunum were perfused with the following drugs, either alone or in combination: the activator of the p38 and Jun kinase pathways, anisomycin (2.0 μ M), the p38 MAP kinase inhibitor SB203580 (20 μ M), and the ERK/MAP kinase kinase (MEK) inhibitor PD98059 (50 μ M), which results in inhibition of the ERK pathway. The actions of the drugs on their target pathways are thought to be relatively specific at the stated concentrations, which are similar to those used with KB cells by Gould et al. [12]. Preliminary experiments established that maximum effects were observed with the drugs at these concentrations. Since the drugs take some time to work and isolated loops of jejunum *in vitro* have limited viability, jejunum was pre-perfused *in vivo* with anisomycin for 30 min and/or SB203580 or PD98059 for 45 min, as appropriate, before *in vitro* perfusion was commenced (see [8] for further details).

Figure 1(a) shows that the maximal stimulation by 2 μ M anisomycin increased the rate of fructose transport to 1.9-fold ($P < 0.001$) that observed with untreated control jejunum: stimulation was blocked by SB203580, whereas the drug alone had no effect on fructose transport, confirming that stimulation of transport occurred by activation of the p38 pathway. The K_a of anisomycin for fructose transport is approx. 1 μ M, as determined by interpolation of the data. Figure 1(a) also shows that there is a role for the PI 3-kinase pathway in the regulation of fructose transport; 200 nM wortmannin was an effective blocker of the stimulation of fructose transport by 2 μ M anisomycin, whereas wortmannin had no effect on the transport of 5 mM fructose in the absence of anisomycin.

Figure 1 Regulation of brush-border fructose transport by activators and inhibitors of the ERK, p38 and PI 3-kinase pathways

The jejunum of an anaesthetized rat was perfused with 5 mM D-fructose in the presence of anisomycin for 30 min *in vivo* and then *in vitro* for 60 min; when present, inhibitors were added to the perfusate 15 min before the anisomycin (for further detail see [8]). The concentration (in μ M) of anisomycin is given as the number underneath the data bar for each perfusion set: when present, the concentration of SB203580 (SB) was 20 μ M, that of PD98059 (PD) 50 μ M and that of wortmannin (W) 200 nM. (a) Normal jejunum, showing how control of the p38 pathway influences fructose transport; (b) normal jejunum showing how inhibition of the ERK pathway by PD98059 enhances stimulation of fructose transport by the p38 pathway; (c) 10-day streptozotocin-diabetic jejunum, showing that the K_a for activation of transport by anisomycin (without PD98059) is similar to that for normal jejunum treated with PD98059. Values are given as means $\pm$ S.E.M. ($n = 12$ for control perfusions and 3–5 for all other perfusions) and are expressed as μ mol $\cdot$ min $^{-1}$ $\cdot$ (g of dry weight) $^{-1}$. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, by unpaired Student's *t* test for comparison with the corresponding control perfusions.

Figure 2 Control of GLUT5 and GLUT2 trafficking to the brush-border membrane of rat jejunum by the ERK, p38 MAP and PI 3-kinase pathways

Brush-border membrane vesicles were prepared from normal rat jejunum perfused with 5 mM D-fructose in the presence and absence of activators and/or inhibitors of the p38 and ERK MAP kinase pathways: for further details see [8]. Vesicle protein (20 μ g) was then separated by SDS/PAGE (10% gels), transblotted on to nitrocellulose and Western blotted for GLUT5; detection was by enhanced chemiluminescence (ECL). The concentration (in μ M) of anisomycin is given as the number under each GLUT band: when present, the concentration of SB203580 (SB) was 20 μ M, that of PD98059 (PD) was 50 μ M and that of wortmannin (W) 200 nM. Top panel: representative blots of GLUT5, GLUT2 and SGLT1, showing how the ERK, p38 and PI 3-kinase pathways regulate GLUT levels in normal rat jejunum. Bottom panel: blots of GLUT5 and GLUT2, showing how inhibition of the ERK pathway by PD98059 permits low concentrations of anisomycin to achieve a stimulation of GLUT2 levels similar to those achieved by high concentrations of anisomycin in the absence of PD98059.

PD98059 (50 μ M), which blocks the ERK pathway at MEK, produced a small but significant stimulation of fructose transport on its own (28%, $P < 0.001$; Figure 1b). However, it had a dramatic effect on the K_a of anisomycin, diminishing it some 50-fold to about 20 nM. The concentration of anisomycin at which maximum stimulation of fructose transport was achieved was 0.1 μ M; stimulation at this concentration was still blocked by SB203580 (results not shown). The response of fructose transport to anisomycin in the presence of PD98059 was biphasic, since as the concentration was increased from 0.1 to 2 μ M, anisomycin was no longer able to stimulate transport. Wortmannin blocked the stimulation of fructose transport by 0.1 μ M anisomycin plus PD98059. Moreover, wortmannin diminished the rate of fructose transport with 2 μ M anisomycin plus PD98059 to 44% of the corresponding control value ($P < 0.001$). Thus the overall range in rates from maximal stimulation to maximal inhibition in this series of experiments with normal rat jejunum was some 3.4-fold.

Selected experiments with jejunum from diabetic rats are presented in Figure 1(c). Anisomycin stimulated fructose transport and was blocked by SB203580. Of particular note, maximal stimulation without PD98059 was achieved by 0.1 μ M anisomycin and the K_a for anisomycin was about 30 nM; that is, the

same within experimental error as the K_a for normal jejunum in the presence of PD98059. In contrast to normal jejunum, higher concentrations of anisomycin still stimulated transport and PD98059 had no effect under the conditions used.

Modulation of GLUT5, GLUT2 and SGLT1 levels by activators and inhibitors of intracellular signalling pathways

To correlate changes in fructose transport with changes in transporter density, the levels of GLUT5, GLUT2 and also SGLT1 were determined by Western blotting of highly purified preparations of brush-border membrane vesicles prepared from jejunum treated with different combinations of drugs (Figure 2). Perfusions for vesicle preparation were terminated halfway through the period over which transport measurements were made in order to provide the most direct correlation between transport and trafficking measurements. GLUT5 presented as a triplet from 62 to 57 kDa, consisting of a very closely spaced doublet and a lower-molecular-mass singlet; GLUT2 presented as a single band (Figure 2, top panel). It is immediately apparent that the brush-border membrane levels of GLUT5 change by only modest amounts over the range of drug treatments. For example, PD98059 and wortmannin increase GLUT5 1.75 ± 0.35 - and 1.53 ± 0.2 -fold respectively, compared with control levels, but anisomycin (2 μ M) and the remaining conditions have no significant effect on GLUT5 levels. The changes in SGLT1 are similar to those of GLUT5. On the other hand, GLUT2 changes under some conditions by much larger amounts (up to 4-fold). For example, anisomycin (2 μ M) and wortmannin increase GLUT2 1.66 ± 0.31 - and 1.85 ± 0.32 -fold respectively, compared with control levels; moreover, their combined effect is additive, increasing GLUT2 3.46 ± 0.66 -fold, suggesting they act through independent pathways. This pattern is repeated in the data obtained for GLUT2 with anisomycin plus PD98059 plus wortmannin (4.06 ± 0.7 -fold), since PD98059 alone has no effect compared with control.

In Figures 1(a) and 1(b), it can be seen that the sensitivity of fructose transport to stimulation by anisomycin was increased by PD98059. The blots in Figure 2 (bottom panel) show that anisomycin (0.1 μ M) plus PD98059 had little effect on GLUT5 level (less than 20% increase), whereas the rate of fructose transport is stimulated so that it is similar to that in jejunum treated with 2 μ M anisomycin in the absence of PD98059. In contrast, whereas anisomycin (0.1 μ M) and PD98059 each have no effect on GLUT2, together they increase GLUT2 1.52 ± 0.27 -fold, which is similar to the increase caused by 2 μ M anisomycin in the absence of PD98059 (1.66 ± 0.31 -fold).

Determination of the relative contributions of GLUT5 and GLUT2 and their intrinsic activities

Jejunum was perfused with fructose in the presence of phloretin in order to inhibit selectively the GLUT2 contribution to transport; the residual phloretin-insensitive contribution represents that attributable to GLUT5. Fructose does not appear in the serosal medium under any of the conditions employed in this study. Not only does this rule out any possible involvement of GLUT2 at the basolateral membrane in the response to phloretin, it also implies that metabolism is not saturated and is not rate-limiting for absorption. The ability of phloretin to inhibit transport therefore reflects absorptive events across the brush-border membrane only. In normal control jejunum, GLUT5 and GLUT2 account for 72 and 28% of fructose transport respectively (Figure 3a). When the p38 pathway is maximally stimulated by anisomycin (1.9-fold), the proportions are the

Figure 3 The relative contributions of GLUT5 and GLUT2 to fructose transport

(a) Selective inhibition of GLUT2 with phloretin permits determination of the contributions of GLUT5 and GLUT2 to the overall rate of fructose transport. Black bar, overall rate; white bar, GLUT5-mediated contribution determined in the presence of 0.2 mM phloretin. The difference between black and white bars corresponds to the GLUT2 contribution. Rates are expressed relative to that of the overall control value (taken as 1.0) for the effects of anisomycin (2.0 μ M), PD98059 (50 μ M), wortmannin (200 nM) and all three together (APDW). Note that since phloretin takes 15 min to achieve complete inhibition, the rates used in these calculations are taken over the time period 15–50 min; the figures for percentages therefore differ slightly from those given elsewhere in this paper, which are based on the full perfusion period of 5–50 min. (b) How changes in GLUT5 level and intrinsic activity determine the contribution of GLUT5 to transport. Comparison of the contributions of GLUT5-mediated rate (black bars) to overall fructose transport rate determined from the data in (a) with the levels of GLUT5 (white bars) permits calculation of the transporter intrinsic activity under a given set of conditions (shaded bars). All values are then expressed relative to the corresponding value for GLUT5 in control perfusions, taken as 1.0. (c) How changes in GLUT2 level and intrinsic activity determine the contribution of GLUT2 to transport. Calculations and key as for (b) GLUT5. Values are given as means $\pm$ S.E.M. $^{++}P < 0.01$ and $^{+++}P < 0.001$, by unpaired Student's *t* test for comparison of the overall rate values in the presence of drugs (black bars) with the control overall rate (black bar); $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, by paired Student's *t* test for comparison of GLUT5-mediated transport value in a given condition (white bar) with the overall rate value in the same condition (adjoining black bar).

other way round, with GLUT5 and GLUT2 accounting for 35 and 65 % of transport respectively. We then compared how the GLUT5-mediated rate, the GLUT5 amount and the GLUT5 activity changed when the p38 pathway was maximally stimu-

lated, relative to the corresponding control values; similar calculations for GLUT2 were also made and the results for GLUT5 and GLUT2 are presented in Figures 3(b) and 3(c). For example, because anisomycin stimulates the overall rate to 1.9-

fold, the components of the overall rate mediated separately by GLUT5 and GLUT2 in the presence of anisomycin are 0.8- and 3.7-fold of their corresponding control values (black bars in Figures 3b and 3c). In this instance the GLUT5 level is unaltered, while the GLUT2 level is increased to 1.66-fold of control values (Figures 3b and 3c, white bars). From these data, it is a straightforward matter to calculate the relative intrinsic activity of each transporter, which is defined as the transport activity per molecule of transporter, given by the rate of transport divided by the transporter density and expressed relative to that in the control preparations. We find that the relative intrinsic activities for GLUT5 and GLUT2 in the presence of 2 μ M anisomycin are 0.7 ± 0.04 and 2.23 ± 0.18 respectively (Figures 3b and 3c, shaded bars). In round terms, then, we can say that the activation of the p38 pathway causes a 1.9-fold increase in fructose transport; this is caused by a doubling of GLUT2 level and of GLUT2 intrinsic activity, with at best minor changes in the level and intrinsic activity of GLUT5.

Similar analyses have been performed to look at the effects of PD98059 and wortmannin alone and of anisomycin plus PD98059 plus wortmannin (Figure 3). Comparison of the different treatments reveals immediately that it is the GLUT2-mediated rate which alters most and so contributes most to regulation under the different conditions. The GLUT2-mediated rate ranges from being unchanged to increasing up to 3.7-fold: in contrast, the GLUT5-mediated rate is unaltered except for anisomycin plus PD98059 plus wortmannin, where it is diminished to one-third of the control value. Similarly the GLUT2 level changes to a much greater extent than the GLUT5 level. Again, GLUT5 intrinsic activity is either unchanged or diminished to as little as 0.29-fold compared with the control value (anisomycin plus PD98059 plus wortmannin), whereas GLUT2 intrinsic activity ranges from 2.5-fold (PD98059) to 0.27-fold (anisomycin plus PD98059 plus wortmannin). In the latter case, the intrinsic activities of both GLUT5 and GLUT2 are extremely inhibited, accounting for the fact that fructose transport is one-third that seen with anisomycin.

DISCUSSION

GLUT2 at the brush-border membrane

We have previously established a role for GLUT2 in the transport of fructose [8] and glucose [9] across the brush-border membrane under the control of PKC. Thus GLUT2 was present in highly purified preparations of brush-border membrane vesicles from normal rat jejunum at levels comparable with highly purified preparations of basolateral membrane vesicles. GLUT2 was functional and therefore accessible to fructose in the luminal perfusate. One part of fructose absorption was selectively blocked by phloretin, which inhibits GLUT2 [5,7,8]. The other, phloretin-insensitive, part of absorption was mediated by GLUT5. PKC-dependent trafficking of brush-border GLUT2 was dynamic and changed markedly in a consistent pattern with different drug treatments, which had no significant effect on the levels of GLUT5 and SGLT1 [8]. There was a direct correlation between changes in fructose absorption and in GLUT2 level, because there were no significant changes in the intrinsic activities of either GLUT2 or GLUT5 [8]. Similar behaviour was seen in the control of GLUT2 trafficking by glucose, although GLUT2 intrinsic activity was increased by low concentrations of glucose at which the GLUT2 level was unaltered [9].

We see here exactly the same features in the regulation of fructose transport by the ERK MAP kinase, p38 MAP kinase and PI 3-kinase pathways. The trafficking patterns in particular are even more strikingly illustrated by the Western blots in

Figure 2, which demonstrate up to a 4-fold range of GLUT2 levels under conditions where GLUT5 increases no more than 1.75-fold (PD98059) and often does not change significantly; SGLT1 also changes only slightly. It is therefore clear that GLUT2 trafficks by a different pathway from GLUT5 and SGLT1. The principal difference now is that correlations between transport and transporter levels are much less direct, because the intrinsic activities of GLUT2 and GLUT5 are altered by as much as 9-fold between certain drug treatments (Figure 3).

Cutting a monolayer culture of intestinal IEC-6 cells with a scalpel results in the activation within just 2 min of ERK and p38 MAP kinases at the edges of the wound [16]. The first step in measuring transporter trafficking by Western blotting was to collect mucosa in the standard way by scraping, which represents mucosal wounding on a grand scale. An important experimental point in our present work was therefore to flush the jejunum with ice-cold buffered mannitol, even before it was removed from the perfusion apparatus, so as to arrest the trafficking of GLUT2 (and also GLUT5), to maintain the low temperature while opening and scraping the jejunum and to homogenize the mucosa under ice-cold conditions immediately. We have noted that similar considerations are perhaps even more important with respect to the control of brush-border membrane GLUT2 levels by PKC [8,9].

The role of intracellular signalling pathways in the regulation of fructose transport: implications for adaptation to diabetes

Activation of the p38 pathway stimulates fructose transport 1.9-fold. This is achieved with little effect on the level or intrinsic activity of GLUT5, but rather with a doubling of the brush-border membrane level of GLUT2 and also a doubling of its intrinsic activity (Figure 3). In contrast, the ERK or the PI 3-kinase pathways have little effect on fructose transport; nevertheless, our studies show that significant regulatory events can occur without an effect on transport. Thus as demonstrated by the converse effects of PD98059 alone, which inhibits the ERK pathway at MEK, the ERK pathway inhibits the trafficking of GLUT5 to the membrane but doubles its intrinsic activity, whereas it has no effect on the trafficking of GLUT2 but more than halves its intrinsic activity. Similarly, the converse effects of wortmannin alone reveal that the PI 3-kinase pathway inhibits the trafficking of both GLUT5 and GLUT2 to the membrane as well as doubling their intrinsic activities.

The above experiments refer to conclusions drawn from independent modulation of the different pathways. There is, however, considerable interaction or cross-talk between the pathways. The most marked example of such cross-talk is seen when inhibition of the ERK pathway with PD98059 increases the sensitivity of fructose transport to activation by anisomycin some 50-fold, lowering the K_a from 1 μ M to 20 nM. Such experiments imply that an important function of the ERK pathway in jejunum is to restrain the activity of the p38 pathway.

It might be argued that such large changes in transport brought about by extreme modulation of pathway activity by a powerful inhibitor have little physiological significance. However, this is not so: rather, such experiments demonstrate the range of what is possible and what we might look for in physiological situations. This is shown clearly in diabetic rats. The ERK pathway is stimulated by insulin and therefore should be inhibited in diabetic compared with normal jejunum. Indeed, we found that the sensitivity of fructose transport to stimulation of the p38 pathway by anisomycin is similar ($K_a \approx 30$ nM) in diabetic jejunum without PD98059 to that in normal jejunum when the ERK pathway is inhibited by PD98059 ($K_a \approx 20$ nM; Figure 1).

Figure 4 Essential features of the regulation of brush-border intestinal fructose transport by ERK, p38 and PI 3-kinase intracellular signalling pathways

The diagram shows the essential features by which fructose transport, GLUT5 and GLUT2 levels and their relative intrinsic activities at the brush-border (BB) membrane are regulated by the ERK, p38 and PI 3-kinase pathways in response to insulin, growth factors and stress stimuli. The sites of action of activators and inhibitors of the pathways are shown: note that the ERK pathway restrains the activity of the p38 pathway. The effects on fructose transport and transporter levels at the brush-border membrane are indicated: $\leftrightarrow$, no effect; $\uparrow$, increase; $\downarrow$, decrease. The listed effects are those revealed by experiments in which only a single pathway in rat jejunum is perturbed by inhibitors or activators. Since the regulation of fructose transport is determined by the balance between the activities of the three pathways (and possibly others), the outcome may be very different from that when only one pathway is perturbed; see text for detailed examples.

We already know that insulin regulates jejunal glucose transport both at pharmacological [17,18] and physiological concentrations [19]. Similar considerations apply to experiments with cultured cells, as the ERK pathway is stimulated by insulin and inhibits p38 MAP kinase in cultured foetal neurons [20], an observation which parallels directly our finding that the ERK pathway restrains the activity of the p38 pathway.

A further example of how our data suggest possible explanations or avenues of investigation for established observations in diabetic rats is provided by the fact that maximal activation of the p38 pathway by anisomycin results in a fructose transport rate that is 3.4-fold that seen in the presence of anisomycin plus PD98059 plus wortmannin. As noted, anisomycin doubles GLUT2 intrinsic activity; stimulation of the p38 pathway, which is a stress-activated pathway regulated by, among other factors, hyperosmolarity, could therefore in principle afford a mechanism for the doubling of the intrinsic activity of GLUT2 at the basolateral membrane in response to the stress of experimentally maintained hyperglycaemia [11,21]. In this situation, plasma insulin levels are high and both the ERK and the insulin-sensitive PI 3-kinase pathways will be activated.

In diabetes, however, both pathways will be comparatively inhibited. Thus we observed that perfusion with anisomycin plus PD98059 plus wortmannin results in an intrinsic activity for GLUT2 of 0.27 and for GLUT5 of 0.29 compared with normal, and that GLUT2 levels at the brush-border membrane were 4-fold those in normal jejunum. These findings suggest a potential mechanism to explain the observation that the intrinsic activity of GLUT5 in diabetic rats is about 0.2–0.33 of its normal value: it would also explain why GLUT2 is present at high levels in the brush-border membrane of diabetic rats [5]. The range of GLUT2 intrinsic activity ranges 9-fold from perfusion with anisomycin to perfusion with anisomycin plus PD98059 plus wortmannin. Establishment of the exact mechanisms involved in adaptation of fructose transport to diabetes will require investigation of the effects of similar combinations of drugs on diabetic jejunum and detailed characterization of the activities of individual signalling pathways and the cross-talk between them.

Regulation of fructose transport after a meal

All the changes in fructose transport reported here take place on a short time scale, between 30 min and 1 h. The ERK, p38 and PI 3-kinase pathways therefore have the potential to regulate fructose transport during the period of digestion and assimilation of a meal. Figure 4 summarizes the mechanisms underlying the changes in fructose transport that occur when the activity of the ERK, the PI 3-kinase or the p38 pathway is manipulated separately by drugs in isolated loops. In the animal, of course, each of the three pathways will be activated to different extents, depending on hormonal and dietary status, and the rate of fructose transport will be determined overall by the balance of the activities of the pathways in any condition. Moreover, the ERK MAP kinase and PI 3-kinase pathways have upstream and downstream connections respectively, to PKC (not shown in Figure 4). Analysis of adaptive changes must now include detailed investigation of the signalling pathways and the key enzymes involved, the cross-talk between the pathways, identification of the hormones that control those pathways and elucidation of the molecular mechanisms of regulation of transporter levels and intrinsic activities determined by those pathways. The different possible combinations are effectively infinite and there are many compensatory changes, so that significant regulatory events altering the level or activity of transporters may occur without any change in transport rate (Figure 4). In order to isolate specific regulatory events, it will be necessary to adopt strategies similar to those applied here.

This research was supported by the Wellcome Trust.

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Received 12 April 2000; accepted 13 June 2000