Glucose-induced Acceleration of Deoxyglucose Transport in Rat Adipocytes

EVIDENCE FOR A SECOND BARRIER TO SUGAR ENTRY*

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The transport of trace (a concentration much less than \( K_m \) for transport) 2-deoxyglucose (deoxyglucose) and 3-0-methylglucose (methylglucose) into rat adipocytes was measured using 1- to 2-s pulses at 37°C in the absence of glucose and in the presence of glucose with and without pre-exposure to glucose. Pre-exposure of insulin-stimulated adipocytes to 10 mm glucose for 30 min resulted in an acceleration of trace deoxyglucose transport above that seen in the absence of glucose. In contrast, when glucose was added together with deoxyglucose, an inhibition of transport was observed. There was no effect up to 5-min pre-exposure to 10 mm glucose, but acceleration appeared thereafter and reached a steady state by 30 min, i.e. a 3-fold higher transport rate than that seen when deoxyglucose and glucose were added together. Glucose (3.5 mm) appears to be a threshold concentration for this effect. A smaller effect was seen in the absence of insulin (1.5-fold) using 10 mm glucose and in the presence of insulin using 10 mm mannose (2.7-fold). No effect was seen using 10 mm galactose, fructose, pyruvate-lactate, deoxyglucose, and methylglucose. There was no effect of glucose pre-exposure on insulin-stimulated methylglucose transport. We propose that entry occurs across two independent barriers in series separated by an aqueous pore, that transport across the second barrier is more rate limiting for deoxyglucose than for methylglucose, and that metabolites of glucose decrease the resistance of the second barrier to deoxyglucose transport.

Measurement of glucose transport and its control by insulin in adipocytes is of widespread interest. Since measurements of glucose metabolism cannot always be taken as a measure of glucose transport, nonmetabolizable analogues of glucose such as methylglucose, 1-arabinose, and D-allose (1-6) have been utilized in an attempt to assess transport activity. In addition, another analogue of glucose, deoxyglucose, which is transported and phosphorylated but not further metabolized, has been utilized for this purpose (7-10).

In a previous study, we have shown that trace methylglucose is transported at least two times faster than trace deoxyglucose in adipocytes even though the ability of deoxyglucose to inhibit trace methylglucose transport is slightly greater than that of methylglucose (11). This suggested a partially rate-limiting step for deoxyglucose uptake between the initial interaction of the sugars with the transport system and phosphorylation. In the present communication, we provide more direct evidence that such a barrier does indeed exist and that the resistance of the barrier is regulated. In addition, we propose a model for sugar transport in adipocytes which is consistent with the findings.

METHODS

Isolated adipocytes were prepared as described previously from epididymal fat pads of \( ad\ libitum \) fed male Wistar rats weighing 150 to 180 g (11). Hepes buffer (pH 7.4 at 37°C) with and without bovine serum albumin, 10 mg/ml, was prepared as previously (1). Collagenase (type 1) was from Worthington, pig insulin was from Nordic Insulin, and phloretin was from K & K Laboratories. 3-O-\[^{14}C\]methyl-\(\beta\)-d-glucose (59 to 60 mCi/mmol) and 2-deoxy-\[^{14}C\]glucose (54 to 60 mCi/mmol) were from the Radiochemical Centre, Amersham. Other chemicals were analytical grade.

The uptake of 50 to 60 \( \mu \)l labeled \[^{14}C\]methylglucose or \[^{14}C\]deoxyglucose was measured as described by Whitesell and Gliemann (11) with some modifications (11). Transport was stopped by the addition of 400 \( \mu \)l of phloretin (0.1 mM) to the 52 \( \mu \)l of incubation and a 400-\( \mu \)l aliquot of the mixture was added to a microtube (550 \( \mu \)l) containing 100 \( \mu \)l of silicone oil (D = 0.90). The tube was spun in a Beckman microfuge for 30 s and cut through the oil phase, and the cell pellet was added to a 3-m1 vial with 2.5 ml of scintillation fluid. Zero time was determined by adding 400 \( \mu \)l of phloretin solution to the isotope solution before adding cells. Under these conditions, the extracellular space (i.e. zero time uptake) constituted 6% of the intracellular waterspace in the cell pellet. The coefficient of variation on 1-s measurements (total counts minus extracellular counts) was 5 to 10%. These measurements were made in a room maintained at 37°C. Intracellular \[^{14}C\]deoxyglucose and \[^{14}C\]deoxyglucose phosphate was determined as previously described in detail (see "Methods" in Ref. 11).

RESULTS

We have previously observed that uptake of trace deoxyglucose alone is linear from 2 to 30 s but that the uptake rate of trace deoxyglucose in the presence of 10 mm glucose is progressively reduced until a new constant rate is obtained after about 20 s (11). For comparison, these data are shown in Fig. 1 with a new observation that, when the cells are preincubated for 30 min with 10 mm glucose, there is an acceleration of the deoxyglucose uptake rate above that measured in the absence of glucose during the first 4 s. In contrast, an inhibition of uptake is seen when glucose is added with the trace deoxyglucose. Although the rate of uptake in cells preincubated with glucose is initially accelerated, it decreases progressively until a new constant rate is obtained after about 15 s. This final rate is the same as that attained when glucose is added with
the trace deoxyglucose (Fig. 1, inset). We have previously shown that the final rate reflects that of phosphorylation and that the decrease in rate of uptake is due to a progressive shift of the rate-limiting step from transport to phosphorylation (11). Since the final rate in the presence of 10 mM glucose (30 s to 4 min (Fig. 1, inset)) is less than 2% of the initial velocity of the accelerated deoxyglucose uptake, one would expect very little accumulation of deoxyglucose phosphate during short incubations when cells were pre-exposed to glucose. This hypothesis was tested in four experiments and the content of deoxyglucose phosphate was undetectable, i.e. less than 2% of the accumulated radioactivity following a 2-s pulse. We therefore conclude that the glucose-induced acceleration is on transport and not phosphorylation of deoxyglucose.

The time course of the acceleration effect of 10 mM glucose on trace deoxyglucose transport is shown in a representative experiment (Fig. 2). There is no change in the inhibition induced by 10 mM glucose for up to 5-min preincubation; by 15 min significant acceleration appears, and by 30 min the effect appears to have reached a constant level.

The concentration dependence of this effect is shown in Fig. 3. Neither 1 nor 2 mM glucose is capable of eliciting the effect.

Glucose (3.5 mM) appears to be near threshold, eliciting a significant response in some experiments and no response in others. Five millimolar glucose consistently yields a significant response which is half of that obtained with 10 mM. There is no significant increase from 10 to 30 mM glucose indicating that the effect is approaching a maximum. The data thus far...
suggest that accumulation of glucose or some metabolite of glucose is responsible for the acceleration.

This idea is further substantiated by data shown in Table I. First, there is a smaller acceleration effect of glucose in the absence of insulin. Since there is a significantly greater accumulation of glucose and its metabolites in the presence of insulin than in its absence (12, 13), a smaller effect would be expected in the absence of insulin if accumulation of glucose or its metabolites is necessary for the effect. In addition, there is no significant acceleration using methylglucose, which is transported two times faster than glucose (14) but not phosphorylated (1). Mannose, which is transported approximately 20% slower than glucose,3 phosphorylated and further metabolized to glucose intermediates (15), has a slightly smaller effect than glucose. These findings suggest that phosphorylation and therefore accumulation of metabolites of glucose are necessary for the effect. No acceleration is evident using 10 mM pyruvate + 10 mM lactate, which can either be catabolized or converted to fructose 1,6-diphosphate (in the absence of glucose) (16), suggesting that neither ATP nor accumulation of metabolites beyond phosphofructokinase is eliciting the effect. No effect is seen with fructose which is transported so slowly (17) that accumulation of metabolites would not be expected, and galactose, which is transported at nearly the same rate as glucose,3 but phosphorylated very slowly (18). The results with galactose and methylglucose appear to eliminate the possibility that the accumulation of sugars themselves elicits the effect. In addition, there is no effect using deoxyglucose as a substrate so that not just phosphorylation but conversion to a glucose intermediate appears to be necessary for the effect.

This acceleration phenomenon is not generally applicable to the transport of all sugars since no acceleration of tracer methylglucose was observed. In three experiments, the ratio of methylglucose transport (1-s pulse) in cells pre-exposed to 10 mM glucose to that obtained with glucose added at zero time was 1.0 ± 0.1 (S.D.). This suggests that there is a regulated step in deoxyglucose transport not present for methylglucose transport.

Comparison of the relative transport rates of tracer glucose and methylglucose and their inhibition constants on tracer methylglucose transport reveals that glucose is transported at a rate relative to methylglucose which is proportional to its relative affinity for the transport system (14). However, tracer deoxyglucose has a slower rate relative to methylglucose (Table II) even though its affinity for the transport system is actually 10% higher (11). This indicates a lower Vmax for deoxyglucose than for methylglucose and glucose.4 However, the accelerated transport rate of tracer deoxyglucose is the same as the transport rate of tracer methylglucose (Table II), indicating that glucose can increase Vmax for deoxyglucose to a level similar to that of methylglucose and glucose.

**DISCUSSION**

The glucose transport system in adipocytes permits specific monosaccharides to move passively across the cell membrane at rates several orders of magnitude greater than those found for simple diffusion across a lipid bilayer. The hexose transport exhibits saturation kinetics, competitive inhibition, and countertransport as expected for a carrier-facilitated process (1–5, 12). No difference has been detected between the Vmax for net uptake and equilibrium exchange of methylglucose (no trans-effect) so that it has not been possible to exclude a simple, symmetrical, equilibrating transport system (1). In contrast, large trans-effects have been observed for hexose transport in red blood cells (19), and it has therefore been necessary to postulate more complicated, asymmetric transport models for this system.

The difference observed in the present study between the apparent Vmax for methylglucose and deoxyglucose is incompatible with a simple carrier model in adipocytes. One might modify this model by postulating different turnover rates of different sugars on an otherwise simple carrier system. However, the absence of a marked trans-effect for methylglucose implies that the turnover is nearly the same for empty carriers and those loaded with methylglucose. It seems therefore unlikely, although not impossible, that carriers loaded with different sugars exhibit different turnover rates.

The models for the human red blood cell transport system, which is not insulin responsive, were designed to explain transport phenomena not present in adipocytes, and there is therefore no a priori reason to use any of those as a framework to understand the present findings. In addition, none of the currently proposed models (asymmetric carrier, tetramer, two carriers in parallel and introverting hemiport (20–23)) seem to adequately encompass the differences in kinetic behavior of methylglucose and deoxyglucose. In addition, a possible locus for the action of a glucose metabolite is not readily pointed out in the existing hexose transport models.

For these reasons, we propose the following three-step model which is consistent with the experimental results: 1) transport of sugar between the extracellular water and an intramembrane aqueous pore, 2) diffusion through the pore, and 3) transport between the pore and the intracellular water.

\[ V_{max} = (K_m \cdot V_{intra}) \cdot (S) \]

1 J. E. Foley, R. Foley, and J. Gliemann, unpublished observation.
Transmembrane transport occurs only when Steps 1 to 3 are connected in series as a unit. Step 2 is not rate-limiting. Step 3 is partially rate-limiting for deoxyglucose, but not (or at least to a lesser extent) for methylglucose. Accumulation of glucose intermediates increases either the affinity or the capacity for deoxyglucose at Step 3. Only in the presence of insulin can the key metabolite(s) accumulate in concentrations sufficient to elicit a near maximal acceleration effect.

The transport system is thought of either as an intrinsic protein with subunits at Steps 1 and 3 or as an assembly of independent proteins. Sugar molecules would bind to segments of membrane protein at both the extracellular and the intracellular interface (Steps 1 and 3) and these segments would function as simple symmetrical "microcarriers." The presence of a pore in the model eliminates the necessity of a very large protein carrier moving the sugar molecule across the entire membrane. When Step 3 is not assumed to be rate-limiting, that is with deoxyglucose in the presence of glucose metabolites and with methylglucose under all conditions, the kinetics observed is not different from that of a simple symmetrical carrier.

The discrepancy between the inhibition constant of glucose measured on trace sugar transport rates (about 10 mM (1, 3, 4, 11)) and that measured on cytochalasin B binding (about 30 mM (24)) would, according to the model, be explained in the following way. The inhibition constant on trace sugar transport is a reflection of the affinity at Step 1, whereas that on cytochalasin binding is a reflection of the affinity at Step 3. The latter assumption seems reasonable since cytochalasin B has been reported to inhibit hexose transport in erythrocytes from the inside (25). Assuming that all sugars have the same or lower affinity at Step 3 than at Step 1, it follows that Step 3 must have a higher capacity than Step 1.

Insulin increases \( V_{\text{max}} \) of hexose transport in adipocytes (1, 2) and it is likely that this effect is primarily brought about by an increase in the number of operational transport units (26). This notion is further supported by the recent finding that glucose-inhibitable cytochalasin B binding sites are distributed between a microsomal and a plasma membrane fraction and that insulin increases the proportion associated with the membrane fraction (27). According to the model, this effect of insulin might reflect an increase in the total number of membrane-associated transport units (Steps 1 to 3) or an attachment of Step 3 subunits to previously nonfunctioning Steps 1 and 2 subunits. In either case, the result would be an increase in operational transport units. This hypothesis is in agreement with our previous finding that the effect of insulin (fold stimulation) is nearly the same on methylglucose and deoxyglucose transport in the absence of glucose in cells metabolically similar to those used in the present study (14). This finding implies that insulin cannot cause major modifications of the transport units, i.e. the concentration of substrate in the pores (Step 2) in basal and insulin-stimulated cells must be nearly the same.

The two carriers in series model proposed here implies that the transport system exhibits asymmetry for sugars which have to overcome a resistance at Step 3. Under those circumstances the system would be quite similar to the model suggested by Regen and Tarpley for red blood cells (20), i.e. a hexose carrier followed by an unstirred layer on the cytoplasmic interface. It should also be noted that the introverting hemiportal model, developed for red blood cells, is physically similar to the model proposed here in that there are two barriers in series separated by a pore (23). However, this model is conceptually different from ours because it defines equal kinetic constants of the two barriers. Asymmetry and trans-effect for a given sugar is explained as a result of hexose-induced allosteric modifications at either barrier, whereas differences between the behavior of methylglucose and deoxyglucose like those found in adipocytes are not readily explained.

It is conceivable that the transport systems of red blood cells and adipocytes share some basic features apart from the fact that they both transport hexose by facilitated diffusion. The differences between the two transport systems may in some way be a reflection of the ability of the adipocyte to respond to insulin. It remains to be elucidated whether the model proposed here will be applicable to other cell types, particularly those whose hexose transport systems are regulated by insulin.

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REFERENCES