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SYMMETRICAL KINETIC PARAMETERS FOR 3-*O*-METHYL-D-GLUCOSE TRANSPORT IN ADIPOCYTES IN THE PRESENCE AND IN THE ABSENCE OF INSULIN

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Summary

3-*O*-Methyl-D-glucose transport in isolated adipocytes in the presence of insulin is symmetric in zero-*trans* experiments (transport into sugar-free solutions). $K_{zt}^{oi} = 6.10 \pm 1.65$ mM, $V_{zt}^{oi} = 1.20 \pm 0.19$ mM/s; $K_{zt}^{io} = 2.66 \pm 0.26$ mM, $V_{zt}^{io} = 1.19 \pm 0.07$ mM/s (the superscripts o and i and subscript zt refer to outside, inside and zero-*trans* conditions, respectively). In the absence of insulin $K_{zt}^{oi} = 5.41 \pm 0.98$ mM, $V_{zt}^{oi} = 0.034 \pm 0.014$ mM/s; $K_{zt}^{io} = 4.09 \pm 1.05$ mM, $V_{zt}^{io} = 0.153 \pm 0.023$ mM/s. For insulin pre-treated cells, infinite-*cis* experiments (transport into solutions of varying sugar concentrations) are also symmetric $K_{ic}^{oi} = 6.51 \pm 0.83$ mM, $V_{ic}^{oi} = 0.98 \pm 0.09$ mM/s; $K_{ic}^{io} = 3.60 \pm 1.33$ mM, $V_{ic}^{io} = 1.76 \pm 0.63$ mM/s (the subscript ic refers to the infinite-*cis* condition). In the absence of insulin, $K_{ic}^{oi} = 9.03 \pm 3.28$ mM, $V_{ic}^{oi} = 0.066 \pm 0.013$ mM/s; $K_{ic}^{io} = 4.54 \pm 1.32$ mM, $V_{ic}^{io} = 0.106 \pm 0.026$ mM/s. The infinite-*cis* parameters are shown to be technically easier to measure than the zero-*trans* parameters. The uses of integrated rate equations for studying rapid transport are demonstrated. The results show that the adipocyte sugar transport system handles 3-*O*-methyl-D-glucose symmetrically, and that insulin does not change either the internal or the external affinity constants for this glucose analogue.

Introduction

Sugar transport in the presence of insulin is a rapid process in adipocytes and low concentrations of sugar equilibrate across the plasma membrane within

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid.

30 s. The fast transport rate has hindered a complete kinetic characterization of the insulin stimulation of sugar transport and many characterizations have relied on indirect measurements of sugar uptake such as glucose oxidation. Recently, however, Whitesell and Gliemann [1] have developed a new procedure for measuring the uptake of the non-metabolised glucose analogue 3-*O*-methyl-D-glucose. This technique uses a transport inhibitor (phloretin) to block transport at short time intervals. Fast sampling is aided by the use of a metronome and cells are separated from the medium by rapid centrifugation through a light oil. Their exchange influx measurements revealed an insulin effect on V for exchange transport with no change in K_m for exchange. Following this technique we have evaluated the use of integrated rate equations to follow fast transport and have measured several additional transport parameters.

In particular, we have sought information on the kinetic parameters for the transport system at the inner membrane surface. Much indirect evidence, such as a lack of accelerated exchange, suggests that the system is symmetric with equal K_m values at inner and outer membrane surfaces, but as far as we are aware no complete characterization of net efflux has yet been published. We have measured zero-*trans* efflux (according to the nomenclature of Eilam and Stein [2]) in basal and in insulin-stimulated cells, since it was feasible that insulin might cause a redistribution of transport sites and reverse an asymmetric basal state. Illiano and Cuatrecasas [3] found a 4-fold asymmetry of K_m and V for glucose in non-insulin treated adipocyte membrane ghosts. However, we find that, providing initial rates are measured, intact adipocytes are kinetically symmetric in their transport properties in both insulin treated and non-insulin treated cells.

We have confirmed conclusions based on the zero-*trans* experiments by studying infinite-*cis* transport. Infinite-*cis* entry measures V for influx but K_m at the *trans* (internal) surface. Infinite-*cis* exit measures V for efflux but K_m at the *trans* (external) surface. We find that infinite-*cis* entry and exit are equal in V and K_m in basal and in insulin-stimulated states.

The symmetry found in adipocytes is similar to that observed in isolated rat hepatocytes [4] and in rabbit erythrocytes [5], but is in marked contrast to the asymmetry found in human erythrocytes [6] and in rat thymocytes [7].

Materials and Methods

Crude collagenase (Type 1) was obtained from Worthington Enzymes, Millipore (U.K.) Ltd., London. Bovine serum albumin (fraction V) and 3-*O*-methyl-D-glucose were purchased from Sigma (London) Chemical Co., Poole, Dorset, U.K. Porcine crystalline monocomponent insulin was a gift from Novo Laboratories Basingstoke, U.K. 3-*O*-[^{14}C]Methyl-D-glucose (spec. act. > 50 mCi/mmol) was purchased from the Radiochemical Centre, Amersham, Bucks, U.K., or was synthesized from $^{14}CH_3I$ (greater than 50 mCi/mmol) (the Radiochemical Centre, Amersham) following the method of Barnett et al. [8]. The isotopes from both sources behaved identically. Dinonylphthalate and silicone oil were obtained from Hopkins and Williams, Chadwell Heath,

Essex, U.K. Phloretin was obtained from K and K Laboratories through Kodak Ltd., Liverpool, U.K.

Preparation of adipocytes. Isolated adipocytes were prepared from rat epididymal fat tissue from rats weighing 140—170 g. The method used was essentially that of Foley et al. [9]. The fat tissue was washed in 10 ml of Hepes buffer (pH 7.4) (Na^+ 140 mM; K^+ 4.7 mM; Ca^{2+} 2.5 mM; Mg^{2+} 1.25 mM; Cl^- 142 mM; $H_2PO_4^-/HPO_4^{2-}$ 2.5 mM; SO_4^{2-} 1.25 mM Hepes 10 mM; pyruvate, 0.5 mM) with 1% albumin. The tissue was chopped with a McIlwain tissue chopper (Mickle Laboratories, Gomshall, Surrey, U.K.). The tissue was then completely digested at 37°C in Hepes buffer containing 3.5% albumin, 0.5 mg/ml collagenase and 0.5 mM D-glucose. The digestion time was 30—45 min. The digested tissue was filtered through a nylon mesh (mesh size 250 μm) and the isolated cells were carefully washed five times in Hepes/1% albumin buffer. Cell suspensions were adjusted to 40 or 50% cytocrit (depending upon the transport procedure to be followed). The intracellular water space (the 3-*O*-methyl-D-glucose equilibrium space) was 1.7 μl/100 μl of packed cells.

Transport measurements. The transport characterization followed the systematic experimental tests described by Eilam and Stein [2]. The oil flotation technique developed by Whitesell and Gliemann [1] was used as the basis for the transport assays.

Zero-trans entry experiments. In this procedure, initial rates are estimated for a range of 3-*O*-methyl-D-glucose concentrations when the *trans* (internal) concentration is zero. The method adopted here was essentially that of Whitesell and Gliemann [1]. Briefly, the method is as follows: 50 μl of a 40% cell suspension were pipetted rapidly onto 15 μl of Hepes buffer containing 0.15 μCi of 3-*O*-[^{14}C]methyl-D-glucose at an appropriate 3-*O*-methyl-D-glucose concentration and at 37°C. The transport was stopped by the addition of 3 ml of Hepes buffer containing 0.3 mM phloretin using a metronome (set on 120 beats per min) to time the additions. The cells were then spun through a 1 ml layer of dinonylphthalate or silicone oil in a bench centrifuge for 1 min at 2500 × *g*. The separated cells were removed from the top of the oil with a pipe cleaner. The trapped radioactivity was extracted into 1 ml of 1% trichloacetic acid and estimated by liquid scintillation counting.

The amount of radioactivity associated with the cells at zero time was measured by adding the cell suspension to the sugar immediately after the phloretin stopping solution had been added. The amount of radioactivity in the cells at equilibrium was measured by incubating the cells for an 'infinite' time of 60 min for the basal condition and 20 min for the insulin-stimulated cells. The intracellular concentration (C_t) was calculated as:

$$C_t = \frac{(\mathrm{cpm}_t - \mathrm{cpm}_0) \cdot S_o}{(\mathrm{cpm}_\infty - \mathrm{cpm}_0)}$$

where cpm_0, cpm_t and cpm_∞ are the radioactivities at times 0, t, and ∞ and where S_o is the external concentration.

Zero-trans exit experiments. In this procedure, initial rates are estimated for a range of 3-*O*-methyl-D-glucose concentrations when the *trans*(external) concentration is zero.

A 50% suspension of cells was equilibrated with 3-*O*-[^{14}C]methyl-D-glucose

at various concentrations at 37°C for 60 min. The efflux was initiated by adding 50 μl of the cell suspension to 5 ml of sugar-free Hepes buffer at 37°C. Transport was terminated by the addition of 5 ml of stopping solution (Hepes buffer containing 0.6 mM phloretin). In the presence of insulin these suspensions were mechanically stirred both during the flux period and during the addition of the stopping solution. Rapid mixing with the stopping solution is required because the volumes of efflux buffer and stopper are equal. A larger stopping volume would have been preferred but this made the isolation of the cells more difficult. In the absence of insulin manual shaking was used to produce mixing. Cells were spun through 1 ml of dinonylphthalate at 2000 × *g* for 1.5 min. The radioactivity retained in the cells was estimated as described above.

The amount of radioactivity associated with the cells at zero time and at 'infinite' time were estimated and used in the calculation of fractional efflux. Fractional efflux multiplied by the loading concentration gives the concentration remaining (C_t).

Equilibrium exchange experiments. The uptake of 3-*O*-[^{14}C]methyl-D-glucose (0.15 μCi) was determined in cell suspensions preloaded with various concentrations of 3-*O*-methyl-D-glucose. The procedure was essentially as described by Whitesell and Gliemann [1]. The incubation volumes, stopping solution and the method of cell collection for 3-*O*-[^{14}C]methyl-D-glucose estimation were the same as those used in the zero-*trans* entry experiments.

Infinite-cis entry experiments. Net influx was measured at 40 mM 3-*O*-methyl-D-glucose. Zero-*trans* influx had shown that this concentration was sufficient to produce maximal influx and, by analysis with an integrated rate equation, the measurement of a time course for backflux was used to estimate the internal K_m value. The experimental procedure was as described for zero-*trans* entry. The microcentrifuge technique described by Foley et al. [9] was also used and gave identical results.

Infinite-cis exit experiments. In this experiment, an internal concentration of 3-*O*-methyl-D-glucose is used (40 mM) which is sufficient to saturate the internal site and the efflux is measured with varying external concentrations.

A 40% cell suspension was equilibrated with 40 mM 3-*O*-methyl-D-glucose for 60 min at 37°C. The transport assay was initiated by adding 50 μl of this suspension to a volume of Hepes buffer that was calculated to give, on dilution of the extracellular sugar in the cell suspension, the desired external concentrations of 3-*O*-[^{14}C]methyl-D-glucose. For external concentrations of 1, 3, 5, 10 and 15 mM, the dilution volumes were 1.170, 0.370, 0.210, 0.090 and 0.050 ml, respectively. The transport was halted by the addition of a volume of stopping solution that brought the final sample volume to 3 ml and a constant dilution of the background or extracellular isotope was thus achieved. The estimation of the cellular 3-*O*-[^{14}C]methyl-D-glucose was as described for the zero-*trans* experiments.

Statistical treatment of the data. Experiments were always performed with two to six replicates for each condition. The coefficient of variation for replicates was approx. 10%. The kinetic parameters were determined by a weighted regression of S/V vs. S plots. Each datum point was weighted for constant percentage error as described by Cornish-Bowden [10]. Other plots were analysed with an unweighted regression.

Results and Discussion

The net influx parameters of 3-*O*-methyl-D-glucose transport into adipocytes have been previously determined by a number of groups [1,11,12]. However, a variety of different time points have been chosen to measure the apparent initial rate. The purpose of measuring the zero-*trans* influx parameters reported here was to provide a direct comparison with the infinite-*cis* parameters for similarly treated cells. We also wished to evaluate, using an integrated rate equation, the longest single-point time interval that could be used to evaluate the true initial rate. This was considered to be particularly important when permeability was maximal as in insulin-treated cells at low sugar concentrations. The influx of 1 mM 3-*O*-methyl-D-glucose at 1, 2, 3 and 4 s is shown in Fig. 1a. To calculate the initial rate, Eqn. 1 is used:

$$(A + BS_o)\left[\frac{\ln(1 - C/S_o) + C/S_o}{C}\right] - \frac{A}{S_o} = -\frac{t}{C} \qquad (1)$$

where $A = KR_{oo} + R_{21}S_o$, $B = KR_{oo}/\pi + R_{12} + (R_{12}/\pi + R_{21}/\pi + R_{ee}/K)S_o + R_{ee}S_o^2/K\pi$, and π is the concentration of osmotically active but impermeant material. S_o is the external concentration, C is the internal concentration, R_{ee} is the reciprocal of the maximum velocity in the equilibrium exchange experiment, R_{12} is the reciprocal of the maximal velocity in the zero-*trans* efflux experiment and R_{21} is the corresponding quantity in the influx direction. $R_{oo} = R_{12} + R_{21} - R_{ee}$.

A plot of $1/v$ (t/C) vs. $-(\ln(1 - C/S_o) + C/S_o)/C$ (Fig. 1b) will give the reciprocal of the initial influx rate $(1/v_o)$ on the ordinate. [2,13]. The calculated initial rate for 1 mM 3-*O*-methyl-D-glucose entry into insulin-stimulated cells (Fig. 1b) is 0.21 mM/s. The apparent initial rate calculated as C/t at 1 s is 0.16 mM/s. Hence, even at 1 s, the true initial rate is slightly (24%) underestimated. At 2 s, the initial rate is underestimated by 43%.

A comparison of the kinetic parameters for insulin-treated cells determined

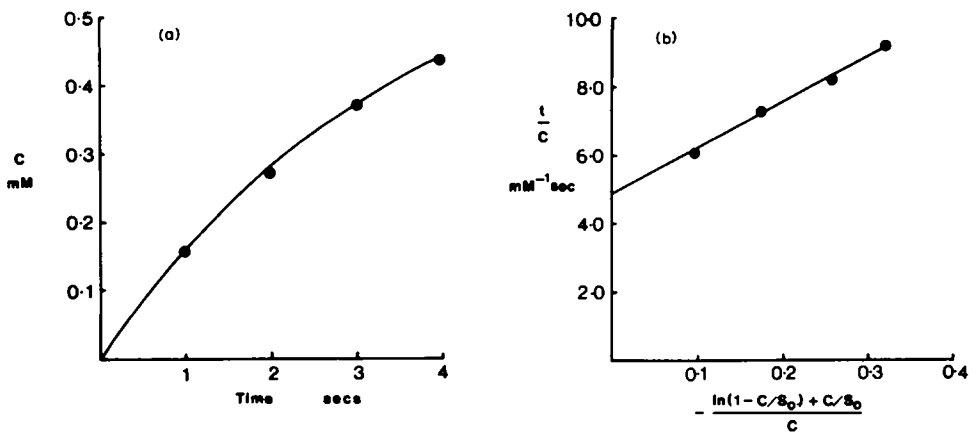

Fig. 1. (a) A time course for net entry of 1 mM 3-*O*-methyl-D-glucose in cells pre-treated with 10 nM insulin at 37°C (34 observations). (b) An integrated rate equation replot of 1 mM 3-*O*-methyl-D-glucose net entry. The initial rate V_0 = 0.210 mM/s.

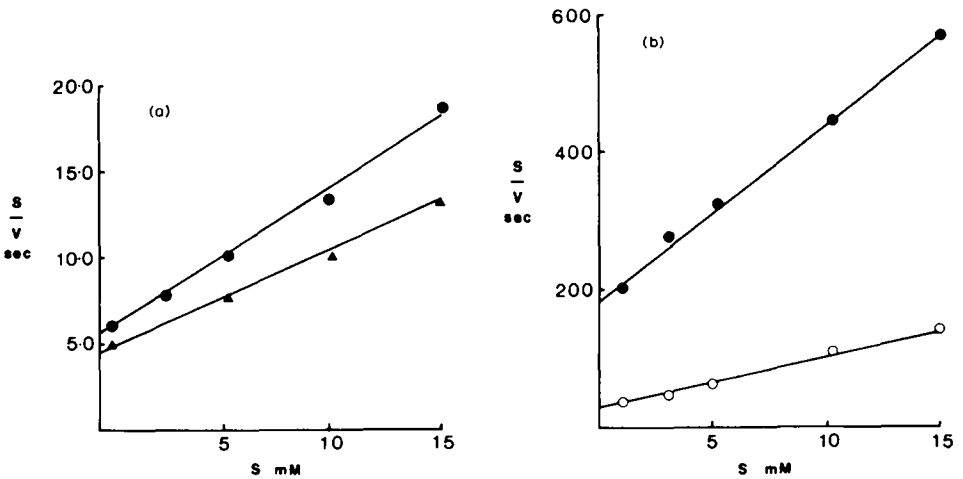

Fig. 2. (a) (•) Zero-*trans* influx of 3-*O*-methyl-D-glucose in cells pretreated with 10 nM insulin, 1 s, 37°C (45 observations). K_{zt}^{oi} = 6.10 ± 1.65 mM and V_{zt}^{oi} = 1.20 ± 0.19 mM/s. (▲) Zero-*trans* influx of 3-*O*-methyl-D-glucose at 37°C in cells pretreated with 10 nM insulin. Initial rates are calculated from five time points in triplicate at each concentration. (b) (•) Zero-*trans* entry of 3-*O*-methyl-D-glucose in basal cells at 37°C. K_{zt}^{oi} = 5.4 ± 0.98 mM, V_{zt}^{oi} = 0.034 ± 0.014 mM/s (24 observations). (○) Zero-*trans* exit of 3-*O*-methyl-D-glucose at 37°C. K_{zt}^{io} = 4.09 ± 1.05 mM, V_{zt}^{io} = 0.153 ± 0.023 mM/s (22 observations).

from rates calculated as C/t at 1 s and rates calculated using Eqn. 1 is shown in Fig. 2a. For 1 s uptakes, the zero-*trans* influx parameters (K, V) are K_{zt}^{oi} = 6.11 ± 1.66 mM, V_{zt}^{oi} = 1.21 ± 0.19 mM/s, and for initial rate plots K_{zt}^{oi} = 7.49 ± 0.59 mM, V_{zt}^{oi} = 1.79 ± 0.08 mM/s. Hence, the main effect of underestimating the initial rate is to underestimate the maximum velocity V.

Preliminary experiments on basal as opposed to insulin-stimulated cells showed that the transport is sufficiently slow for the underestimation of rates from a single time point not to be a major problem. Zero-*trans* uptake at 1—15 mM 3-*O*-methyl-D-glucose was therefore studied at 15 s (Fig. 2b): K_{zt}^{oi} = 5.4 ± 0.90 mM and V_{zt}^{oi} = 0.034 ± 0.014 mM/s. The results show that insulin raises V_{zt}^{oi} 30-fold. The result is similar to the effect of insulin on exchange uptake and efflux reported by others [1,14] in that V rather than K_m is increased by insulin. Siegel and Olefsky [11] and Czech [12] have also reported an insulin-dependent change in V for 3-*O*-methyl-D-glucose net uptake but much longer time points were chosen for estimations of initial rates and undoubtedly some underestimate of V may have occurred with a consequent underestimate of the overall magnitude of the insulin response.

The 10-fold insulin stimulation of exchange flux found by Vinten et al. [14] and Whitesell and Gliemann [1] is similar to our results on net flux (Table I). Our results for exchange flux measured at 1 s (insulin), 15 and 30 s (basal), show a similar insulin response (Table I). The experiments were repeated here to provide a direct comparison with the net flux data.

The measurement of zero-*trans* efflux of 3-*O*-methyl-D-glucose has rather severe experimental difficulties. To achieve zero-*trans* conditions, cell suspensions must be diluted into a large volume of efflux buffer in order to reduce backflux of extracellular label. This means that mixing in order to stop the reaction is awkward for short time points. Long time intervals have the dis-

TABLE I

KINETIC PARAMETERS FOR 3-*O*-METHYL-D-GLUCOSE TRANSPORT IN RAT ADIPOCYTES AT 37°C

Results are the mean ± S.E. (from regression).

Experiment	+Insulin (10 nM)		Basal	
	K_m (mM)	V (mM/s)	K_m (mM)	V (mM/s)
Zero-*trans* entry	6.10 ± 1.65 (n = 45)	1.20 ± 0.19	5.41 ± 0.98 (n = 24)	0.034 ± 0.014
Zero-*trans* exit	2.66 ± 0.26 (n = 130)	1.19 ± 0.07	4.09 ± 1.05 (n = 22)	0.153 ± 0.023
Infinite-*cis* entry	6.51 ± 0.83 (n = 51)	0.98 ± 0.09	9.03 ± 3.28 (n = 49)	0.066 ± 0.013
Infinite-*cis* exit	3.60 ± 1.33 (n = 47)	1.76 ± 0.63	4.54 ± 1.32 (n = 60)	0.106 ± 0.026
Equilibrium exchange	4.45 ± 0.26 (n = 42)	0.84 ± 0.002	4.22 ± 1.24 (n = 63)	0.058 ± 0.0001

advantage that a significant backflux occurs (possibly exhanced by the accumulation of radioactively labelled sugar in unstirred layers) and this tends to give an apparently slow efflux.

To overcome these problems we found it necessary to vary the time courses studied for different sugar concentrations. The most reliable time points are those giving 10—60% exit (Fig. 3a). The estimation of initial rates from these points clearly requires an integrated rate equation because of the rapid and non-linear fall in the internal concentration. The equation used is:

$$\frac{-\ln\frac{S_t}{S_o}}{S_o - S_t} = \frac{V \cdot t}{K_m(S_0 - S_t)} - \frac{1}{K_m}$$

where S_0 and S_t are the internal concentrations at time zero and t, respectively. $t/(S_0 - S_t)$ is plotted against $-\ln(S_t/S_0)/(S_0 - S_t)$ and $1/K_m$ is estimated from the intercept on the ordinate [15]. Although not strictly necessary for estimating K_m, initial rates at each concentration can also be calculated separately from this plot. When $-\ln(S_t/S_0)/(S_0 - S_t) = 1/S_0$, then $t/(S_0 - S_t) = 1/v_o$ (the initial rate). The calculated initial rates for zero-*trans* efflux have been used in S/V vs. S plots and confirm the estimates of kinetic parameters using the integrated rate plot. From the integrated rate plot (Fig. 3b), $K_{zt}^{io} = 2.66 \pm 0.26$ mM and $V_{zt}^{io} = 1.19 \pm 0.07$ mM/s in the presence of insulin. In the absence of insulin (Fig. 2b), $K_{zt}^{io} = 4.09 \pm 1.05$ mM and $V_{zt}^{io} = 0.153 \pm 0.023$ mM/s. Thus, insulin gives a large increase in V for zero-*trans* efflux with only a small decrease in K_m. In the insulin-stimulated condition, the kinetic parameters for net efflux and influx are approximately equal but the K_m value for efflux is slightly lower than that for influx. This may be a reflection of the technical difficulties involved in measuring exit, but the possibility of a slight intrinsic difference in the transporter at inner and outer membrane surfaces cannot be completely eliminated. The V value for efflux

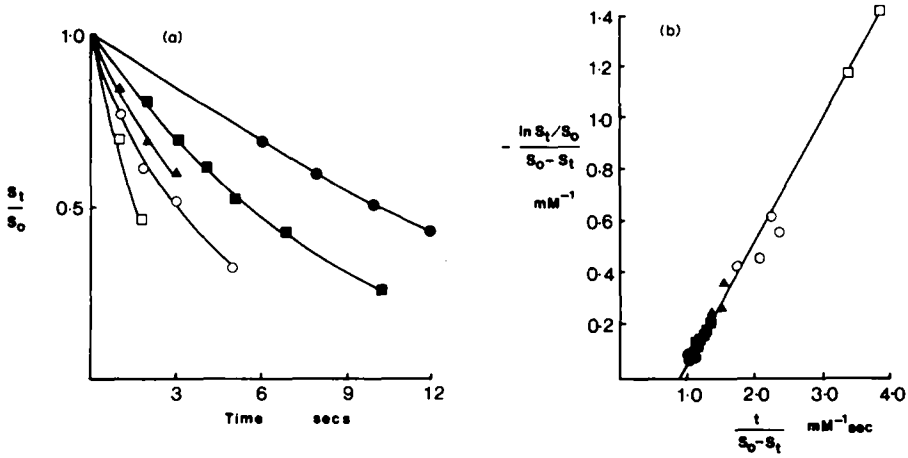

Fig. 3. (a) Zero-*trans* efflux of 3-*O*-methyl-D-glucose at 37°C in cells pretreated with 10 nM insulin. (□) 1 mM, (○) 3 mM, (▲) 5 mM, (■) 10 mM, (●) 20 mM (130 observations). (b) An integrated rate equation replot of the zero-*trans* efflux of 3-*O*-methyl-D-glucose in insulin-treated cells (K_{zt}^{io} = 2.66 ± 0.26 mM, V_{zt}^{io} = 1.19 ± 0.07 mM/s).

in the absence of insulin is higher than that for influx but this is probably due to the long loading times employed to give internal 3-*O*-[^{14}C]methyl-D-glucose. Prolonged incubations or rough handling of basal cells gives an increase in the basal transport.

In view of the difficulties involved in measuring zero-*trans* exit, we decided to confirm the indications of an overall symmetry by carrying out infinite-*cis* experiments. We found technically the easiest and most accurate way to determine the infinite-*cis* entry kinetic parameters was to follow the time course of entry (Fig. 4) of a high substrate concentration and to use the integrated rate equation (Eqn. 1). When the *cis*(external) concentration is much greater than the K_m value, the intercept on the abscissa of the infinite-*cis* entry plot (Fig. 5) gives the internal K_m value [13], while the intercept on the ordinate gives the infinite-*cis* entry V. Eqn. 1 assumes the carrier model for transport but the parameters obtained by the use of this equation can readily be re-interpreted in terms of other models for transport. In the presence of insulin, K_{ic}^{oi} = 6.51 ± 0.83 mM and V_{ic}^{oi} = 0.98 ± 0.09 mM/s. In the basal state, K_{ic}^{oi} = 9.03 ± 3.28 mM and V_{ic}^{oi} = 0.066 ± 0.013 mM/s. Hence, the infinite-*cis* entry parameters confirm values for the entry V and the internal K_m obtained in the zero-*trans* entry and zero-*trans* exit experiments, respectively.

Whitesell and Gliemann [1] have also studied a time course for net uptake of a high 3-*O*-methyl-D-glucose concentration, but their analysis assumed rather than demonstrated symmetrical affinity constants.

There are several advantages of the infinite-*cis* entry experiments as a means of measuring the transport parameters. One is that errors due to the variation in the applied concentration are minimal because a single substrate concentration is used. Also, timing errors are small because the high substrate concentration gives a low net rate of uptake. Long time intervals can be studied even in the presence of insulin. We find that the use of the integrated rate

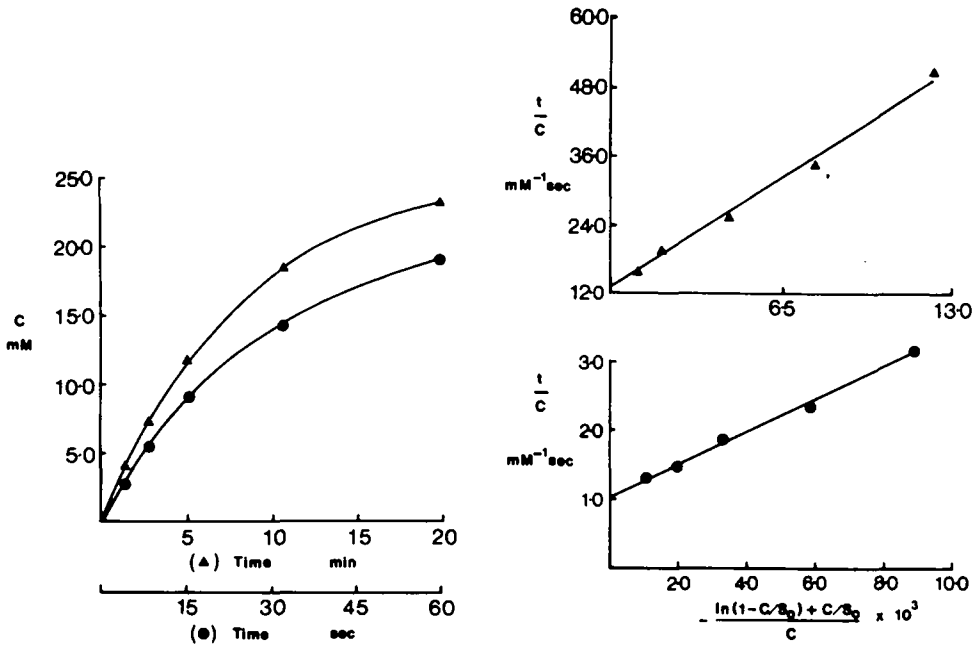

Fig. 4. (●) A time course for net influx of 40 mM 3-*O*-methyl-D-glucose (infinite-*cis* entry) at 37°C in cells pretreated with 10 nM insulin (51 observations). (▲) A time course for net influx of 40 mM 3-*O*-methyl-D-glucose (infinite-*cis* entry) at 37°C in the absence of insulin (49 observations).

Fig. 5. (●) An integrated rate equation analysis of the infinite-*cis* entry data shown in Fig. 4. K_{ic}^{pl} = 6.51 ± 0.83 mM, V_{ic}^{pl} = 0.98 ± 0.09 mM/s. (▲) An integrated rate equation analysis of the infinite-*cis* entry data shown in Fig. 4. K_{ic}^{pl} = 9.03 ± 3.28 mM, V_{ic}^{pl} = 0.066 ± 0.13 mM/s.

equation confers no particular disadvantage in spite of the formidable appearance of the log term.

Likewise, infinite-*cis* exit has technical advantages when compared with zero-*trans* exit. In this experiment, a single high internal concentration of 3-*O*-methyl-D-glucose is used and the apparent permeability is low even in the presence of insulin. The efflux is linear with respect to time until the internal concentration falls below the saturating concentration for the internal site. A time point of 20 s in the presence of insulin and 4 min in the absence of insulin has been used to measure the efflux rate in the presence of varying external concentrations of 3-*O*-methyl-D-glucose (Fig. 6). $1/v$ is plotted against the external concentration and the external K_m is given by the intercept on the abscissa. The calculated kinetic parameters in insulin-treated cells are K_{ic}^{io} = 3.60 ± 1.33 mM; V_{ic}^{io} = 1.76 ± 0.63 mM/s. In the absence of insulin K_{ic}^{io} = 4.54 ± 1.32 mM; V_{ic}^{io} = 0.106 ± 0.026 mM/s. These values therefore confirm the estimates of the efflux V and the influx K_m found in the zero-*trans* experiments and show that infinite-*cis* transport is also symmetric.

Taken collectively (Table I), the results indicate that the transport system handles 3-*O*-methyl-D-glucose symmetrically and that insulin does not change either the internal or the external affinity constant for this glucose analogue. It would be interesting to determine whether other sugars are also handled symmetrically by this transport system. Foley et al. [9] have shown that

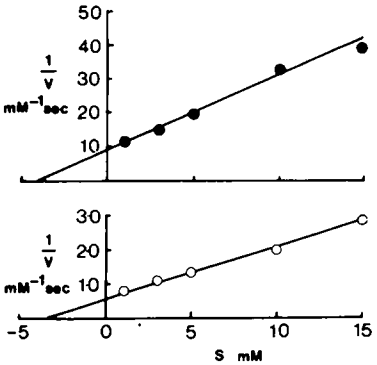

Fig. 6. Infinite-*cis* efflux of 40 mM 3-*O*-methyl-D-glucose at 37°C in cells pretreated with insulin (○) and basal cells (●). In insulin-treated cells, K_{1c}^{io} = 3.60 ± 1.33 mM, V_{1c}^{io} = 1.76 ± 0.63 mM/s (47 observations). In basal cells, K_{1c}^{io} = 4.54 ± 1.32 mM, V_{1c}^{io} = 0.106 ± 0.026 mM/s (60 observations).

2-deoxy-D-glucose is dissimilar to 3-*O*-methyl-D-glucose in its interaction with the adipocyte sugar transporter and thus a conclusion that the transporter is a simple symmetrical carrier is probably erroneous. Foley et al. [9] have described a pore model for transport in adipocytes. The new kinetic parameters reported here may be useful in the assignment of rate constants and constraints for such a model.

The present results do not directly pertain to mechanisms by which insulin activates sugar transport, but the lack of a large insulin-dependent change in K_{zt}^{io}, K_{1c}^{oi} and K_{1c}^{io} does eliminate the possibility that carriers could be redistributed between inner and outer plasma membrane surfaces in response to insulin. It is therefore of interest to note that a redistribution of transporter components between the plasma membrane and an internal storage site have recently been suggested by Cushman and Wardzala [15] and Suzuki and Kono [17]. Such an effect would give an overall increase in the number of functional transporters at the plasma membrane and would be consistent with the observed insulin-dependent changes in V for zero-*trans* and infinite-*cis* entry and exit.

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