

Sugar transport across the hepatocyte plasma membrane

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Physiologically D-glucose transport across the hepatocyte plasma membrane differs from that in most other cells. Most cells in the body extract glucose from the blood as a source of energy or carbon. The liver on the other hand responds to its environment and acts as a 'glucostat'. As the level of glucose in the blood rises, i.e. during feeding, the liver must take it up and store it as glycogen and as the blood glucose level falls so the liver helps to restore it. The carbohydrate metabolism in the liver is under hormonal control, especially by insulin and glucagon, and it is important that the transport system should be able to accommodate these changes in the direction of flux.

A number of years ago Cahill *et al.* (1958) investigated the relationship between the free glucose concentration in the liver and that in the plasma. They found the concentrations to be equal whether the net flow was into or out of the liver. They also demonstrated that D-glucose injected intravenously equilibrated rapidly with that in the liver. On the basis of these observations they suggested that transport of glucose occurred by passive diffusion. This interpretation, coupled with the technical problems associated with transport studies with the intact liver or liver slices, led to little further work on the subject.

Reinvestigation of the system provided Goresky (1967) and Williams *et al.* (1968) with evidence that the transport was in fact carrier-mediated. Both groups observed stereospecificity (D-glucose was transported orders of magnitude faster than L-glucose) and Williams *et al.* (1968) were able to show saturation kinetics (see Table 1), and inhibition by phlorrhizin, but no effect of insulin in the perfused rat liver. Other investigators have accumulated data for anaesthetized dogs (Goresky & Nadeau, 1974), conscious sheep (Hooper & Short, 1977) and again the perfused rat liver (Sestoft & Fleron, 1974); a summary of the kinetic data is shown in Table 1.

All the above results obtained with intact liver suffer from enormous technical problems. In particular, transport cannot be measured directly and data are obtained from elimination of sugar, with the added complication that the liver is a heterogeneous tissue with a number of compartments. This has necessitated some fairly complex modelling to obtain kinetic parameters.

The introduction of techniques for preparation of isolated

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hepatocytes (Berry & Friend, 1969) has provided a far more convenient system for the study of hepatic transport systems. The problems of heterogeneity and multiple compartments are removed, although some caution must be used in interpreting results as all the hepatocyte plasma membrane is in contact with the incubation medium. Baur & Heldt (1977) have used isolated hepatocytes to study the specificity of uptake of a variety of metabolites including monosaccharides. They obtained results similar to those of Williams *et al.* (1968), although with a considerably lower maximum velocity of glucose uptake (Table 1).

We have also used isolated hepatocytes to perform a more detailed study of the sugar transport across the plasma membrane (Craik & Elliott, 1979, 1980). Previous studies (Baur & Heldt, 1977) indicated that transport into the cells was very rapid, so we chose to perform the incubations at 20°C rather than 37°C to improve the chance of measuring true initial rates. To obtain accurate measurements within 2.5 s we made use of the fact that phloretin is a more potent inhibitor than phlorrhizin of the transport system, and were able to stop the movement of sugars essentially instantaneously by the rapid addition of a cold solution of phloretin, HgCl₂ and KI (final concentrations of 195, 52 and 33 mM) to the incubation mixture. This mixture proved to be effective in allowing storage for up to 20 min and washing without any significant leakage of sugar into or out of the cells.

To avoid problems of metabolism of the sugar we performed all our initial experiments using 3-O-methyl-D-glucose, which is not metabolized. This allowed transport to be measured under four separate conditions: (i) zero *trans* entry (entry of sugar into depleted cells), (ii) zero *trans* exit (efflux of sugar from preloaded cells into sugar-free medium), (iii) equilibrium exchange entry (movement of radiolabelled sugar into cells with equal internal sugar) and (iv) equilibrium exchange exit (as (iii) but efflux of radiolabelled sugar). The results of those studies are shown in Table 2. To avoid complications due to endogenous glucose or glucose production from glycogen, hepatocytes were prepared from starved rats.

A wide range of sugar concentrations was used (0.1–100 mM 3-O-methyl-D-glucose) and the data were analysed by the weighted method of Bannister *et al.* (1976) and the unweighted method of Wilkinson (1961). The close agreement obtained between the two methods suggested that the experimental methodology produced no significant systematic errors. Plots of initial rate of transport against concentration of substrate on logarithmic scales (Hoare, 1972) yield a line of gradient approaching unity at substrate concentrations below K_m , indi-

Table 1. Summary of kinetic parameters for sugar uptake

System	K_m (mM)	V_{max} (37°C) [mmol·min ⁻¹ ·(l of cell H ₂ O) ⁻¹]
D-Glucose		
Perfused rat liver (Williams <i>et al.</i> , 1968)	17	345
Anaesthetized dog liver (Goresky & Nadeau, 1974)	120	1700
Rat hepatocytes (Baur & Heldt, 1977)	30	70*
D-Fructose		
Perfused rat liver (Sestoft & Fleron, 1974)	67	50
Rat hepatocytes (Baur & Heldt, 1977)	>100	>160*
D-Galactose		
Rat hepatocytes (Baur & Heldt, 1977)	100	160*

* Measured at 20°C but corrected on basis of Arrhenius plot.

Table 2. Summary of kinetic parameters for sugar transport across the hepatocyte plasma membrane at 20°C

Data from Craik & Elliott (1979, 1980 and unpublished work), calculated by the method of Wilkinson (1961) (\pm S.E.M. with numbers of initial rates in parentheses).

Sugar and condition	K_m (mM)	V_{max} [mmol·min ⁻¹ ·(l of cell H ₂ O) ⁻¹]
3-O-Methyl-D-glucose		
Zero trans entry (27)	20 \pm 3	82 \pm 5
Zero trans exit (18)	17 \pm 5	84 \pm 8
Equilibrium exchange entry (30)	18 \pm 5	86 \pm 10
Equilibrium exchange exit (18)	18 \pm 4	79 \pm 5
D-Glucose		
Zero trans entry (63)	66 \pm 14	220 \pm 19
D-Fructose		
Zero trans entry (38)	212 \pm 32	291 \pm 26
D-Galactose		
Zero trans entry (24)	174 \pm 48	288 \pm 48
2-Deoxy-D-glucose		
Zero trans entry (21)	167 \pm 45	404 \pm 62

cating that the system is not polyvalent towards 3-O-methyl-D-glucose. The identity of the kinetic constants for 3-O-methyl-D-glucose indicates, that at least for this sugar, the system is symmetrical without any trans acceleration (as seen for human erythrocytes and D-glucose: Levine & Stein, 1966).

The initial use of 3-O-methyl-D-glucose proved fortunate. As can be seen from Table 2 both the K_m and V_{max} for this sugar are considerably lower than for the other, physiologically more interesting, sugars used. These high values tend to make the actual numbers less reliable. There is again no evidence from logarithmic plots of polyvalency for any of these sugars. Studies of competition between the sugars shown in Table 2 are consistent with a single carrier system with wide specificity. The apparent K_i values, consistent with competitive inhibition, are all similar to the appropriate K_m values for the sugars.

Our results indicate that the monosaccharide transport system in the hepatocyte plasma membrane has a high K_m and high V_{max} for all the metabolically important sugars. The system appears to be symmetrical for 3-O-methyl-D-glucose, and preliminary results indicate a similar situation for D-glucose. The K_m and V_{max} values are similar to the higher values obtained by others (the rates at 37°C are approximately three times those at 20°C, thus the V_{max} for D-glucose becomes 660 mmol·min⁻¹·(l of cell H₂O)⁻¹ although somewhat higher than those obtained by Baur & Heldt (1977) with hepatocytes. This discrepancy is at least partly explained by systematic underestimation of the initial rates by these workers (see Craik & Elliott, 1979).

In the physiological context these kinetic parameters mean that the transport system is ideally suited for its function. Transport should not limit metabolism, either for uptake or output. The glucose phosphorylating capacity (hexokinase: 0.7 μ mol·min⁻¹·g wet wt⁻¹; glucokinase: 4.3 μ mol·min⁻¹·g wet wt⁻¹) and glucose 6-phosphate dephosphorylating capacity (glucose 6-phosphatase: 17 μ mol·min⁻¹·g wet wt⁻¹) (Newsholme & Start, 1973) are well below the transport capacity (unidirectional flux of approximately 460 μ mol·min⁻¹·g wet wt⁻¹). Even assuming these enzymes to

be working at their maximum activity, a concentration gradient of less than 1 mM (inside lower) or less than 3 mM (inside higher) would be sufficient to ensure that the net transport flux was not limiting at physiological D-glucose concentrations. It is clear that even under these extreme conditions only relatively small concentration gradients are necessary for the liver to fulfil its function [this being consistent with the early data of Cahill *et al.* (1958) where no significant concentration gradients were found].

In conclusion, the transport of sugars across the hepatocyte plasma membrane appears to be mediated by a facilitative diffusion system of both high K_m and high V_{max} , which is hormone-insensitive. This system allows the liver to fulfil its physiological role without limitations imposed by transport of sugars into and out of the cell.

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