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Cytochalasin inhibition of hexose transport by platelets

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Previously we described a two-transporter model (T_1, T_2) for galactose uptake by platelets (Horne, M.K. and Hart, J.S. (1986) Biochim. Biophys. Acta 856, 448–456). In the current work we have sought corroborative evidence for this model by studying the effects of cytochalasins on this transport system. Of the various cytochalasins tested, cytochalasin B was the most potent inhibitor (I) of galactose transport, whereas cytochalasin A was less inhibitory and dihydrocytochalasin B and cytochalasin E had no inhibitory effect. The same order of potency was observed for the inhibition of L-glucose diffusion into platelets. The mechanism of cytochalasin B inhibition was investigated in detail. Inhibition of T_1 was competitive and required a higher concentration of cytochalasin B ($K_{i_1} \approx 1.7\ \mu M$) than inhibition of T_2, which was of a mixed type ($K_{i2} \approx 0.8\ \mu M$). The effect of cytochalasin B on T_2 could be accounted for by a membrane alteration which enhanced the affinity of the transporter for galactose while simultaneously preventing passage of the TSI complex into the cell. Since a similar effect on membrane permeability would also explain cytochalasin B inhibition of L-glucose diffusion, it is hypothesized that cytochalasin B binds to a membrane structure shared by T_2 and the passage for L-glucose. The differences in cytochalasin B sensitivity and mechanism of inhibition manifested by T_1 and T_2 support our original hypothesis that galactose is indeed transported by kinetically distinct agencies and suggest that these may be physically distinct as well.

Introduction

Galactose enters platelets by a mechanism which is suppressed by phlorizin, a relatively specific inhibitor of facilitated hexose diffusion [1]. The uptake pathway appears to differ from that for glucose, mannose, and fructose, however, since these sugars, but not galactose, compete with 2-deoxyglucose for cellular entry [2]. With zero-*trans* methodology we recently examined radiolabeled galactose uptake by platelets more carefully [3]. After statistically comparing one- and two-transporter models for the data, we concluded

Abbreviations: galactose, D-galactose; DMSO, dimethyl sulfoxide; [^{3}H]galactose, D-[1-^{3}H]galactose; L-[^{14}C]glucose, L-[1-^{14}C]glucose; EDTA, ethylenediaminetetraacetic acid; v, initial uptake velocity; S, extracellular hexose concentration; V, maximal v; K_m, S yielding $V/2$; v_i, initial uptake velocity in the presence of an inhibitor; V_i, maximal v_i; I, inhibitor; K_i, inhibition constant; K_s, dissociation constant; T_1, galactose transporter-1; T_2, galactose transporter-2; D, diffusion constant; D_i, diffusion constant in the presence of an inhibitor; S.D., standard deviation; S.E., standard error.

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that galactose transport into platelets is kinetically biphasic. A similar system for galactose uptake has also been reported for red cells [4], which, however, appear to absorb glucose with single-component kinetics [5]. The biphasic mechanism of galactose entry into platelets and red cells may represent the activity of functionally independent transporters. On the other hand, the kinetics could also be explained by a single transporter with an affinity and capacity for galactose which is modulated by the extracellular concentration of the sugar [6].

In an effort to corroborate the dual nature of the platelet system we have tested potential inhibitors of galactose uptake for evidence of differential effects on the transporters. In preliminary experiments cytochalasin B appeared to display this property. This compound and its congener cytochalasin A are well known inhibitors of hexose transport in many cells [7–14]. Although this has not been documented directly with platelets, cytochalasin B inhibition of hexose transport has been inferred from studies demonstrating that platelet metabolism of exogenous glucose is reduced by cytochalasin B [15]. Furthermore, platelet membranes bind cytochalasin B, and a portion of this binding is prevented by high concentrations of glucose [16]. These observations, therefore, have suggested a direct interaction of cytochalasin B with platelet hexose transporters.

Further work with the cytochalasins in our laboratory has revealed that although both of the hypothetical transport activities are affected by cytochalasin B, they differ in sensitivity to the cytochalasin and in the mechanism by which they are inhibited. The results we are reporting here, therefore, provide further evidence that galactose transport by platelets is mediated by two kinetically distinguishable agencies.

Materials and Methods

Materials. Cytochalasin B, cytochalasin A, dihydrocytochalasin B, cytochalasin E, D-galactose (containing < 0.01% glucose), and dimethyl sulfoxide (DMSO) were obtained from the Sigma Chemical Corporation (St. Louis, MO). Concentrated solutions of the cytochalasins were prepared in either 95% ethanol or DMSO and kept at −20°C until used. [^{3}H]Galactose (385 GBq/mmol, 98% pure by paper chromatography) and L-[^{14}C]glucose (2.15 GBq/mmol, 98% pure by paper chromatography) were products of the Amersham Corporation (Arlington Heights, IL).

A stock solution of 270 mM galactose was prepared in 15 mM Tris (pH 7.4), to give a final, measured osmolality of 290 mosM. A trace amount of [^{3}H]galactose was added to this stock and used to check the accuracy of subsequent dilutions, which were made with phosphate-buffered saline, ethylenediaminetetraacetic acid (EDTA) (90 mM NaCl, 20 mM sodium phosphate, 10 mM Na_2EDTA (pH 7.4), 260 mosM). The specific radioactivity of the final galactose dilutions were adjusted to 0.37–37 GBq/mmol by adding more [^{3}H]galactose.

Platelet preparation. Human platelets were separated from fresh blood as previously described (17) and suspended at room temperature in phosphate-buffered saline with 10 mM EDTA. The final platelet concentration was measured with a Coulter S-Plus (Coulter Electronics, Inc., Hialeah, FL). Contaminating red or white cells were less than one per 10^6 platelets.

Galactose transport measurements. Transport was measured at room temperature by previously described methods with the platelets suspended in phosphate-buffered saline with 10 mM EDTA [3]. This procedure involves rapid mixing of a platelet aliquot with an equal volume of a specific [^{3}H]galactose concentration for 10 s, followed by a 200-fold dilution with 0–2°C isotonic saline. The final osmolality of the platelet-galactose mixtures is estimated to be 260–265 mosM. The diluted samples are immediately filtered through 0.4 μm filters. The radioactivity retained by the filters is quantitated by liquid scintillation spectrometry and is a measurement of the galactose associating with the cells over the 10-s incubation at room temperature. Under these conditions uptake of galactose by the cells is linear for approx. 30 s, and galactose metabolites are not detectable by thin-layer chromatography [3]. Points at time zero (nonspecific, background trapping of [^{3}H]galactose by the filtered platelets) are obtained by adding the platelet aliquots and the [^{3}H]galactose separately to the cold diluting buffer and then immediately filtering.

When the effect of cytochalasins on transport was studied, equal concentrations of a cytochalasin were produced in the platelet suspension and in the [^{3}H]galactose solutions by adding small amounts of the cytochalasin stocks in a volume of 95% ethanol or DMSO always less than 2% of the volume of the platelet suspension. The platelets were then mixed with the [^{3}H]galactose for 10 s and processed as outlined above. Controls were run with identical concentrations of ethanol or DMSO without cytochalasin. There were no appreciable differences in transport in the presence of these solvents compared with transport measurements in phosphate-saline-EDTA alone. Background [^{3}H]galactose retained by the filters (time zero) was not affected by the presence of cytochalasin.

Measurement of L-glucose diffusion. Platelets suspended in phosphate-saline-EDTA were mixed with an equal volume of L-[^{14}C]glucose in the same buffer for periods of up to two hours. At intervals 50-μl aliquots were diluted 200-fold with cold saline as in the galactose transport experiments and filtered through 0.4 μm filters. When cytochalasins were included in the suspensions, an equal volume of ethanol or DMSO was added to a control.

Data analysis. Weighted least-squares regression curves were fitted by computer to plots of initial uptake velocity, v, versus extracellular galactose concentration, S, by adjusting the parameters V (maximal initial uptake velocity) and K_m (S yielding $V/2$) [3,18]. With the F-test fits of single rectangular hyperbolas (one-transporter models) were compared with fits of curves representing the sum of two rectangular hyperbolas (two-transporter models). Because the errors in the estimates of K_m and V are skewed, approximate standard errors were calculated from logarithmic transformations of the parameters [18]. All of the models included a minor component of a diffusional transport which was estimated experimentally with L-glucose and treated as a constant in fitting curves to the galactose transport data.

Results

Effect of various cytochalasins on galactose transport

The effect of 10 μM cytochalasin B, cytochalasin A, dihydrocytochalasin B, and cytochalasin E on V was measured with 0.01 mM and 25 mM galactose. As shown in Table I, cytochalasin B produced the greatest reduction in v_i/v, while cytochalasin A was less inhibitory and dihydrocytochalasin B and cytochalasin E had no measurable effect. When cells exposed to 10 μM cytochalasin B were washed extensively, their ability to transport 0.01 mM galactose was completely restored.

Effect of various cytochalasins on the platelet uptake of L-glucose

Diffusion coefficients (D) describing the uptake of L-glucose by platelets were calculated as v/S, 1/min per 10^{15} cells. The effect of 10 μM cytochalasin B, cytochalasin A, dihydrocytochalasin B, and cytochalasin E on these coefficients is shown in Table II. Only the effect of 10 μM cytochalasin B was statistically significant ($P < 0.001$), although the mean D in the presence of 10 μM cytochalasin A was moderately reduced ($P = 0.13$). The effect of cytochalasin B on D was dependent on the cytochalasin B concentration, as shown in Fig. 1, but independent of L-glucose concentration (data not shown).

Cytochalasin B inhibition of galactose transport

The effect of cytochalasin B on galactose trans-

TABLE I

EFFECT OF CYTOCHALASINS ON GALACTOSE TRANSPORT RATE

The data are shown as means ± 1S.D., based on the number (N) of replicates indicated in parentheses. CA, CB and CE, cytochalasin A, B and E, respectively.

Cytochalasin	v_i/v	
	0.01 mM galactose	25 mM galactose
10 μM CB	0.12 ± 0.017 ($N = 4$)	0.079 ± 0.066 ($N = 4$)
10 μM CA	0.37 ± 0.12 ($N = 3$)	0.32 ± 0.075 ($N = 3$)
10 μM dihydroCB	1.1 ± 0.25 ($N = 3$)	0.92 ± 0.072 ($N = 3$)
10 μM CE	0.94 ± 0.012 ($N = 3$)	1.1 ± 0.26 ($N = 3$)

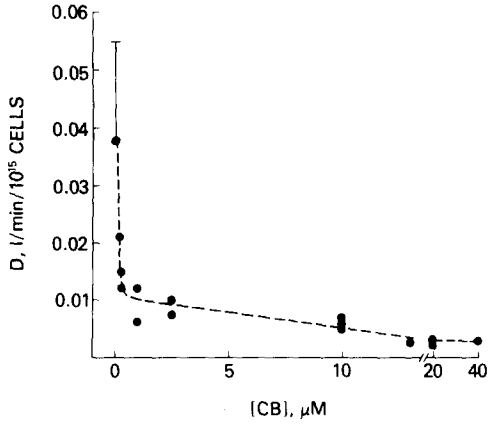

Fig. 1. The effect of cytochalasin B (CB) concentration on the coefficient of L-glucose diffusion into platelets. The data for [CB] = 0 are expressed as the mean ± 1 S.D. based upon 18 separate measurements. Note the break in the horizontal axis above 13 μM cytochalasin B. The line was fitted visually.

port was first quantitated by comparing v in the presence of the inhibitor (v_i) with v determined in a control experiment using the same platelet preparation and solvent (ethanol) concentration. Two concentrations of galactose were studied, 0.01 mM and 25 mM. With 0.01 mM galactose $I_{0.5}$ approximated 1 μM, while with 25 mM $I_{0.5}$ approximated 0.4 μM (Fig. 2).

TABLE II

COEFFICIENTS OF DIFFUSION OF L-GLUCOSE INTO PLATELETS

The data are shown as means ± 1S.D., based upon the number (N) of replicates indicated in parentheses. CA, CB and CE, cytochalasin A, B and E, respectively.

Inhibitor	D (l/min per 10^{15} cells)
None	0.038 ±0.017 (N = 18)
10 μM CB	0.0054 ± 0.0014 (N = 3)
10 μM CA	0.024 ±0.0067 (N = 4)
10 μM dihydroCB	0.043 (N = 1)
10 μM CE	0.039 ±0.0042 (N = 2)

The effect of 1.0 and 2.5 μM cytochalasin B was then studied over a broad range of galactose concentration. Simultaneous controls with solvent only (DMSO or ethanol) were also run. The data (v versus S) were fitted with regression curves based upon models of one or two transporters. Statistical comparison of the fits indicated that the more complex model was always favored ($P < 0.01$). The diffusional component of transport with each cytochalasin B concentration was estimated with L-glucose (Fig. 1). The mathematical formulation describing uninhibited transport by two saturable transporting systems (T_1, T_2) plus a dif-

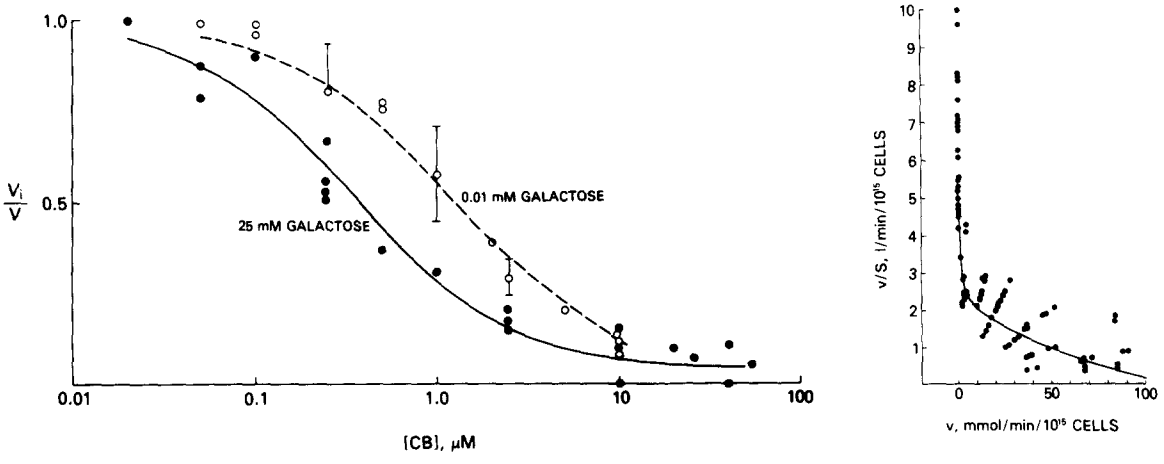

Fig. 2. The effect of cytochalasin B (CB) concentration on the relative transport rate (v_i/v) of 0.01 mM and 25 mM galactose by platelets. The data represent individual measurements or the means ± 1S.D. of at least six replicates. The points have been fitted with curves based upon competitive inhibition of T_1 ($K_{i_1} \approx 1.7$ μM) and mixed inhibition of T_2 ($K_{i_2} \approx 0.79$ μM, $\alpha \approx 0.24$).

Fig. 3. Eadie-Scatchard plot of uninhibited galactose transport data. The line has been computed for a two-transporter model based upon the parameters shown in Table III.

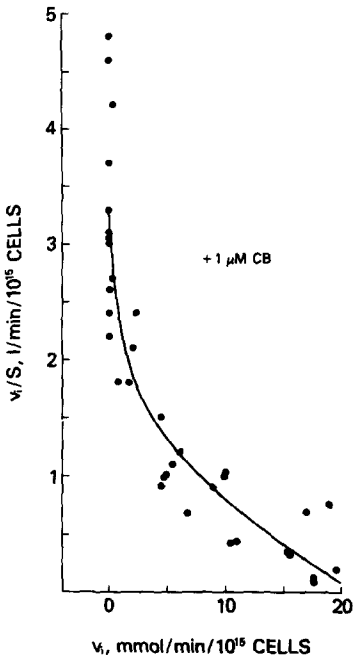

Fig. 4. Eadie-Scatchard plot of galactose transport data in the presence of 1.0 μM cytochalasin B (CB). The line has been computed for a two-transporter model based upon the parameters shown in Table III.

fusional element it as follows

$$v = \frac{V_1 S}{K_{m_1} + S} + \frac{V_2 S}{K_{m_2} + S} + DS$$

The data from the inhibited transport experiments and from the controls are graphed as Eadie-Scatchard plots in Figs. 3–5 [19]. They have been fitted with lines describing two-transporter models based upon the above formula. The parameters V_1, V_2, K_{m_1}, and K_{m_2} derived from these curves are shown in Table III. The apparent

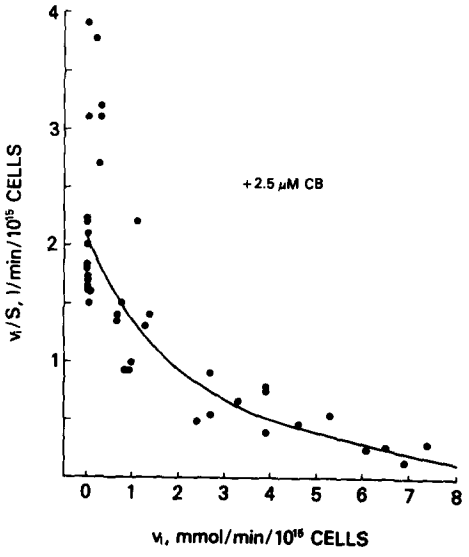

Fig. 5. Eadie-Scatchard plot of galactose transport data in the presence of 2.5 μM cytochalasin B (CB). The line has been computed for a two-transporter model based upon the parameters shown in Table III.

K_{m_1} increased progressively in the presence of 1.0 and 2.5 μM cytochalasin B, whereas the apparent V_1 remained unchanged, consistent with competitive inhibition of T_1. In contrast, the apparent K_{m_2} and V_2 were both reduced by cytochalasin B.

Discussion

Previously we described biphasic kinetics for the uptake of galactose by platelets [3]. In the current study we repeated our original work as a control and derived very similar results (Fig. 3, Table III). In addition, we sought further evidence for the dual kinetic system by analyzing the effect of various cytochalasins on galactose transport.

TABLE III

EFFECT OF CYTOCHALASIN B ON TRANSPORT PARAMETERS

The data in parentheses represent the means ± 1S.E. (approximate), based upon a long-normal distribution of the experimental errors [18].

Cytochalasin B (μM)	0	1.0	2.5
K_{m_1} (mM)	0.32 (0.15–0.68)	0.50 (0.17–1.4)	0.79 (0.43–1.5)
V_1 (mmol/min per 10^{15} cells)	1.1 (0.59–2,2)	1.0 (0.32–3.1)	1.2 (0.60–2.5)
K_{m_2} (mM)	42 (35–50)	14 (11–19)	13 (7.6–23)
V_2 (mmol/min per 10^{15} cells)	87 (81–93)	19 (18–20)	7.7 (6.7–8.9)

Of the many cytochalasins, cytochalasin B, cytochalasin A, dihydrocytochalasin B, and cytochalasin E were selected for study because of the published evidence that their inhibitory potency against hexose transport differs [20]. The comparative studies were performed with a low galactose concentration (0.01 mM) in order to test for inhibition when both transporting agencies (T_1 and T_2) would normally be making relatively large contributions to the observed transport. (When $S = 0.01$ mM, v_1 accounts for approx. 60% of transport, while v_2 accounts for approximately 40%.) Under these conditions residual transport activity in the presence of 10 μM cytochalasin B was 0.12 ± 0.017 (S.D.) of the uninhibited rate and in the presence of 10 μM cytochalasin A, 0.37 ± 0.12 (S.D.) of the uninhibited rate (Table I). Neither 10 μM dihydrocytochalasin B nor 10 μM cytochalasin E showed appreciable inhibition. Therefore, the rank of inhibitory potency observed in other hexose transport systems (cytochalasin B > cytochalasin A > dihydrocytochalasin B, cytochalasin E) appears to hold true in the sensitivity of platelet galactose transport to cytochalasins [20]. From these data it is impossible to predict whether higher concentrations of the inactive cytochalasins would be inhibitory or to know whether cytochalasin A inhibits both or only one of the transporters. On the other hand, in order to account for the level of inhibition observed with 10 μM cytochalasin B (Fig. 2) we must hypothesize that this compound affects both T_1 and T_2.

We also studied the effect of these cytochalasins on the uptake of L-glucose by platelets. Previously we have shown that the rate of L-glucose uptake is much slower than galactose transport and that the process is consistent with simple diffusion [3]. Nevertheless, the current work demonstrates that cytochalasin B markedly inhibits L-glucose uptake, while cytochalasin A has a marginal effect and dihydrocytochalasin B and cytochalasin E have no effect (Table II). Cytochalasin B has been reported to inhibit L-glucose diffusion into cultured hepatoma cells as well [21]. In platelets the relationship of D_i to cytochalasin B concentration appears to be multiphasic (Fig. 1). Approximately 80% of the reduction in D_i occurs with concentrations of cytochalasin B ≤ 1.0 μM. Raising the cytochalasin B concentration to 10 μM decreases D_i by an additional 10%, but above 13 μM cytochalasin B has no further effect. This pattern suggests that L-glucose may diffuse into platelets by at least three routes: one very sensitive to cytochalasin B, one less sensitive to cytochalasin B, and one insensitive to cytochalasin B.

The differences in inhibitory potency among cytochalasin B, cytochalasin A, and dihydrocytochalasin B are noteworthy because these compounds are structurally very similar [22]. The lesser potency of cytochalasin A compared with cytochalasin B correlates with the presence of a hydroxyl group on carbon-20 of cytochalasin B and a keto group at the same position of cytochalasin A. The lack of inhibition by dihydrocytochalasin B, on the other hand, is apparently due to the reduction of the double bond found at carbon-21 of cytochalasin B. Both the hydroxyl at carbon-20 of cytochalasin B and the double bond at carbon-21 would be expected to reduce the hydrophobicity of this region of the molecule. The relatively low hydrophobicity of this locus must be critical in mediating the effects of cytochalasin B on both facilitated and simple diffusion of hydrophilic hexoses through the lipid bilayer of the platelet plasma membrane.

The nature of the inhibition of galactose transport by cytochalasin B was analyzed in more detail. It was shown that the inhibition was completely reversed by washing the cells free of cytochalasin. This has been true in other systems as well [7,9]. The inhibitory effect of cytochalasin B on the transport of 0.01 mM and 25 mM galactose was dose-dependent. However, the $I_{0.5}$ observed with 0.01 mM galactose was higher (approx. 1 μM) than that observed with 25 mM galactose (approx. 0.4 μM) (Fig. 2). This pattern is inconsistent with simple competitive inhibition, in which the $I_{0.5}$ increases with S, and with simple noncompetitive inhibition, in which the $I_{0.5}$ does not vary with S. The pattern, however, is characteristic of uncompetitive inhibition and certain forms of mixed-type inhibition [19]. On the other hand, because the relative contributions of T_1 and T_2 are quite different in transporting 0.01 mM galactose (T_1, approx. 60%) and 25 mM galactose (T_1, approx. 3%), we reasoned that the difference in $I_{0.5}$ might reflect a difference in the sensitivity of the

two systems to cytochalasin B.

The data to test this hypothesis were gathered by measuring galactose transport over a broad range of hexose concentration in the presence of 1.0 and 2.5 μM cytochalasin B. As in the studies without inhibitor, the biphasic nature of the transport kinetics was obvious in the presence of cytochalasin B (Figs. 4 and 5). However, cytochalasin B caused an asymmetric change in the Eadie-Scatchard curves. The effect of cytochalasin B on the steep limb of the curves, where T_1 activity is apparent, was less pronounced than on the shallow limb of the curves, where T_2 activity dominates. This indicates that T_1 is less sensitive than T_2 to inhibition by cytochalasin B and offers an explanation for the greater $I_{0.5}$ observed with 0.01 mM galactose as compared with 25 mM galactose (Fig. 2).

The increase in apparent K_{m_1} observed in the presence of cytochalasin B without a changes in V_1 indicates that the inhibition of the T_1 transporter is competitive. Calculation of K_{i_1} for a competitive mechanism gives 1.70 μM from the studies with 1.0 μM cytochalasin B and 1.78 μM from the data obtained with 2.5 μM cytochalasin B. This Ki is approximately three to ten times greater than that measured for cytochalasin B inhibition in other hexose transport systems, making the T_1 transporter remarkably insensitive to cytochalasin B [8,9,12,13]. Competitive inhibition is consistent with unpublished data from our laboratory indicating that some of the cytochalasin B which binds to platelet membranes can be displaced by galactose. On the other hand, glucose entry into red cells is non-competitively inhibited by cytochalasin B, apparently due to interaction of the cytochalasin with the endofacial terminus of the transporter, whereas glucose exit is competitively inhibited [13,14]. If these relationships of cytochalasin B binding site to resulting mechanisms of inhibition can be extrapolated to the platelet galactose transport system, the implication is that cytochalasin B reacts with T_1 at its exofacial exposure. However, it is also possible that the competition is mediated by an allosteric interaction between the exofacial galactose binding site of T_1 and a cytochalasin B binding site elsewhere.

The inhibition of T_2 by cytochalasin B is quite different. Clear reductions in both K_{m_2} and V_2 were demonstrated (Table III). Because such a pattern is characteristic of uncompetitive inhibition, an attempt was made to describe the inhibition of T_2 by this mechanism. Since with 25 mM galactose approx. 97% of the transport is mediated by T_2, the K_i for the proposed uncompetitive inhibition of T_2 was estimated from the $I_{0.5}$ observed with 25 mM galactose (Fig. 2)

$$K_i = \frac{I_{0.5}}{1 + K_{m_2}/S}$$

The K_i (approx. 0.2 μM) in the equation for uncompetitive inhibition

$$v_i = \frac{V_2 S}{K_{m_2} + S(1 + I/K_i)}$$

generates values leading to a line very similar to that shown in Fig. 2 for 25 mM galactose. However, the formula for uncompetitive inhibition of T_2 ($K_i = 0.2$ μM) plus competitive inhibition of T_1 ($K_i = 1.7$ μM) produces values for v_i which fit the observations with 0.01 mM galactose very poorly, particularly at cytochalasin B concentrations greater than 1 μM (not shown).

We therefore investigated the possibility of a mixed-type inhibitory mechanism, which in certain cases can also produce a decrease in both K_m and V [19]. The general equilibria for this mechanism are shown in Fig. 6. According to this scheme the inhibitor alters both the dissociation constant (K_s) for the reaction $T + S \leftrightharpoons TS$, as well as the rate constant (k) for the movement of the TS complex through the membrane. Since with sufficient concentrations of cytochalasin B transport of galactose approaches zero (Fig. 2), the hypothetical equilibrium scheme can be simplified by assuming $\beta = 0$. The parameters K_{i_2} and α can be estimated from the horizontal intercepts of K_{m_2}/V_2 versus I and $1/V_2$ versus I [19]. This requires the assumption that K_m approximates K_s, which is supported by studies of red cell glucose transport [23,24]. By these methods $K_{i_2} = 0.79$ μM and $\alpha = 0.24$.

When these constants are entered into the formula describing competitive inhibition of T_1, mixed-type inhibition of T_2, and the inhibition of diffusion measured experimentally with L-glucose

(Fig. 1)

$$v_i = \frac{V_1 S}{K_{m_1}(1 + I/K_{i_1}) + S} + \frac{V_2 S}{K_{m_2}(1 + I/K_{i_2}) + S(1 + I/\alpha K_{i_2})} + D_i S$$

the resulting values closely approximate the values observed both with 0.01 and 25 mM galactose. This is reflected in the curves shown in Fig. 2 (v_i/v versus cytochalasin B concentration).

In this scheme for the inhibition of T_2, $0 < \alpha < 1$. Because of this the affinity of the complexes TS and TI for the other reactants (i.e., I and S) is greater than the affinity of the uncomplexed T for I or S. This shifts the equilibrium toward the ternary complex TSI and lowers the apparent K_m. By itself this phenomenon would result in activation rather than inhibition of transport. However, the TSI complex, which is favored by the decreased K_m, cannot pass through the membrane ($\beta = 0$). Therefore, the lower apparent K_m paradoxically promotes inhibition of transport. The ultimate basis for the inhibition of T_2 is actually a reduction in the rate of transmembrane diffusion. Nevertheless, K_{i_2} (approx. 0.8 μM) is as much as 8-fold higher than that reported for other hexose transport systems, making T_2, like T_1, comparatively insensitive to cytochalasin B inhibition [8,9,12,13].

The effect of cytochalasin B on transmembrane diffusion was also observed in the experiments with L-glucose, which is presumed to enter platelets by simple diffusion [3]. Despite the apparent difference in the mechanisms by which galactose and L-glucose are taken up by platelets, the effect of cytochalasins on the two systems is similar in several respects: (1) the relative potency of the

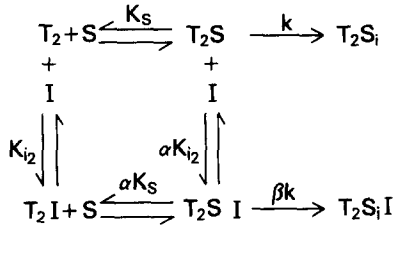

Fig. 6. Equilibria describing mixed-type inhibition of T_2 by cytochalasin B (I). See text for discussion [19].

cytochalasins in inhibiting L-glucose diffusion parallels their relative inhibitory potency against galactose transport (cytochalasin B > cytochalasin A > dihydrocytochalasin B, cytochalasin E); (2) the effect of cytochalasin B on the coefficient of L-glucose diffusion occurs at cytochalasin B concentrations which also affect galactose transport; and (3) the inhibition of L-glucose diffusion by cytochalasin B is not reduced by high concentrations of the hexose, just as the inhibition of T_2 cannot be overcome by high concentrations of galactose.

These observations suggest that inhibition of L-glucose diffusion and inhibition of galactose entry mediated by T_2 are related to the presence of inhibitor at sites with a similar stereospecificity for cytochalasins and a similar lack of affinity for hexose. Both inhibitions, therefore, may be due to cytochalasin B binding to the same plasma membrane structure, which permits nonspecific diffusion of hexoses but which also has stereospecificity for physiologic sugars such as galactose. Such a structure could be a transmembrane gated pore or an intramembranous carrier, since these models are kinetically indistinguishable [25]. We cannot rule out the possibility, however, that inhibition of L-glucose uptake reflects cytochalasin B interaction at a site entirely separate from T_2. Also, since a small portion of L-glucose diffusion appears not to be inhibited by cytochalasin B at all (Fig. 1), multiple routes for L-glucose diffusion may exist.

In conclusion, exposure of platelets to cytochalasins reveals additional differences between the hypothetical transporters for galactose and raises questions about the nature of L-glucose movement across the platelet plasma membrane. The simplest explanation for the dissimilarities between T_1 and T_2 is that they represent the effects of cytochalasin B on physically separate membrane structures, which may or may not also contain a pathway for L-glucose diffusion. Alternatively, the observations could reflect the activity of a single transporter subject to complex steric rearrangements which affect not only its affinity and capacity for galactose, but also its interaction with cytochalasin B. Although we favor the former hypothesis, much work obviously remains to elucidate the true nature of these transporting activities.

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