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3-O-Methyl-D-Glucose Uptake in Isolated Rat Hepatocytes

Effects of Dexamethasone

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SUMMARY

We examined the uptake of 3-*O*-methyl-D-glucose, a nonmetabolizable hexose, by isolated rat hepatocytes. The uptake of 3-*O*-methyl-D-glucose was linear for 1 min at 22°, and Lineweaver-Burk analysis demonstrated an apparent K_m of ~6 mM. Cytochalasin B (40 μM) and phloridzin (2 mM) inhibited 3-*O*-methyl-D-glucose uptake by 88% and 63%, respectively. D-Glucose (20 mM) inhibited the initial rate of 3-*O*-methyl-D-glucose uptake by 55% ($p < 0.001$), whereas L-glucose was without any significant effect. The uptake of 3-*O*-methyl-D-glucose remained unchanged in the presence of Na^+ (0–150 mM) in the incubation medium. After 30 min dexamethasone inhibited glucose uptake (the maximal effect being achieved in a time- and concentration-dependent manner) at 2 μM and 0.5 μM concentrations by 50% and 25%, respectively. Dexamethasone produced a decrease in the V_{max} but did not change the K_m. Insulin, glucagon, gastric inhibitory polypeptides, and pancreozymin had no effect on 3-*O*-methyl-D-glucose uptake in isolated hepatocytes. These findings are consistent with the conclusion that 3-*O*-methyl-D-glucose uptake in isolated rat hepatocytes occurs via a stereospecific, carrier-mediated, facilitated diffusion process. Dexamethasone decreases this process of facilitated diffusion in the isolated hepatocyte.

INTRODUCTION

Glucose transport has been studied extensively in a variety of tissues [adipocytes (1), lymphocytes (2), thymocytes (3), and erythrocytes (4)]. However, little information is available regarding hepatic glucose transport (5, 6). It was generally believed that the hepatic plasma membrane was freely permeable to glucose (7). More recently, however, it was shown that perfused rat liver takes up D-glucose more rapidly than L-glucose and that D-glucose uptake is inhibited by phloretin (8). These data suggest that a specific D-glucose transport system exists in hepatocytes. However, the perfused liver preparation does not lend itself to direct examination of the precise kinetics of the putative D-glucose transport system (7, 8). On the other hand, isolated hepatocytes have been employed in extensive kinetic studies of amino acid transport in liver (9). Baur and Heldt (6), employing D-[^{14}C] glucose, suggested that this hexose is transported across the isolated hepatocyte membrane by a carrier-mediated diffusion process. However, since D-glucose is known to be rapidly and extensively metabolized by hepatocytes (10), uptake of D-glucose may not reflect transport per se. More recently, Craik and Elliott (11) employed 3-*O*-methyl-D-glucose, a nonmetabolizable hexose (12), to study the kinetics of sugar transport in hepatocytes. We have studied the uptake of this hexose to determine whether a specific glucose transport system is present in isolated rat hepatocytes. In addition, we have studied the effect of various hormones on the uptake of 3-*O*-methyl-D-glucose by isolated hepatocytes.

MATERIALS AND METHODS

3-*O*-D-[*methyl*-^{3}H]Glucose (80 Ci/nmole), [^{3}H]inulin, and Aquasol scintillation cocktail were obtained from New England Nuclear Corporation (Boston, Mass.). Phloridzin, cytochalasin B, collagenase Type I, 3-*O*-methyl-D-glucose, pancreozymin, dexamethasone, hydrocortisone, and predniosolone were purchased from Sigma Chemical Company (St. Louis, Mo.), and bovine serum albumin (Fraction V) was obtained from Rehis Chemical. Penicillin, streptomycin, and trypan blue were obtained from GIBCO (Grand Island, N. Y.). Crystalline porcine insulin and porcine glucagon were a gift of Eli Lilly Laboratories (Indianapolis, Ind.), and GIP[1] was a gift of Dr. V. Mutt, Stockholm.

Hepatocyte isolation procedure. Hepatocytes were prepared from male Sprague-Dawley rats weighing 200–250 g. Liver cells were isolated by a modification of the collagenase method of Jeejeebhoy *et al.* (13). The method is described in more detail elsewhere (14).

[1] The abbreviations used are: GIP, gastric inhibitory polypeptide; KRP buffer, Krebs-Ringer phosphate buffer.

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The cells were washed three times with cold KRP buffer (containing 1.20 mM NaCl, 4 mM KCl, 1.2 mM $MgSO_4$, and 2.4 mM KH_2PO_4, pH 7.4) to remove the collagenase which may remain in the cell suspension. After the final wash, the cells were suspended in KRP buffer to a cell concentration of 4–5 × 10^6 cells/ml, as estimated by hemocytometer count (Clay Adams, Parsippany, N. J.). This procedure yielded 400–700 × 10^6 viable cells/liver. Cell viability was determined by trypan blue exclusion (15) and found to be between 85% and 95%.

3-O-Methyl-D-glucose uptake studies. In most experiments, isolated hepatocytes (5 × 10^5 cells/tube) were incubated in quadruplicate in tubes (12 × 75 mm) containing KRP buffer and 2% bovine serum albumin at 22° in a shaking water bath. Uptake experiments were initiated by the addition of a mixture of 3-*O*-D-[*methyl*-^{3}H] glucose (3–4 μCi/tube) and unlabeled 3-*O*-methyl-D-glucose (2 mM). The final incubation volume was 250 μl. The incubation was terminated by quickly transferring 200-μl aliquots of the cell suspension to plastic Microfuge tubes (Eppendorf, 1.5 ml) containing 1 ml of ice-cold KRP buffer containing 2 mM $HgCl_2$. The tubes were immediately centrifuged for 20 sec at 18,000× *g* in a Beckman Microfuge. The supernatant was aspirated and the pellet was washed twice with 1 ml of ice-cold KRP buffer. The centrifuge tube was then cut just above the pellet and the portion containing the cells was placed in a scintillation vial. The ^{3}H content was determined after the addition of 0.5 ml of formic acid (88%) and 10 ml of Aquasol. The amount of 3-*O*-methyl-D-glucose trapped in the extracellular space of the sedimented cell pellet was determined using [^{3}H]inulin, and all data were corrected for this factor (16). The intracellular water was calculated according to the method of Kletzien *et al.* (17). All data are expressed as means ± standard deviation; their statistical significance was evaluated using Student's *t*-test.

RESULTS

Characterization of 3-O-methyl-D-Glucose Uptake in Isolated Rat Hepatocytes

As shown in Fig. 1, the uptake of 3-*O*-methyl-D-glucose by isolated rat hepatocytes was a linear function of the number of hepatocytes, over a range of 1–13 × 10^5 cells/tube. Within this range, the coefficient of variation of 3-*O*-methyl-D-glucose uptake was 2.5%. The amount of trapped 3-*O*-methyl-D-glucose in the extracellular space ranged from 4% to 8% of the total uptake. Values for intracellular water varied between 0.32 and 0.35 μl/5 × 10^5 cells without differences between control and hormone-treated cells.

Figure 2 shows the time course of 3-*O*-methyl-D-glucose uptake at 22°. The uptake by isolated hepatocytes was rapid, and the initial velocities were approximated from 60-sec time points. After this initial period, the uptake of 3-*O*-methyl-D-glucose tended to level off, reaching a steady-state level after 4 min. Lineweaver-Burk analysis (Fig. 3) yielded a K_m of 5.88 mM and a V_{max} of 420 nmoles/min/5 × 10^5 cells.

Effect of cytochalisin B and phloridzin on 3-O-methyl-D-glucose uptake. As shown in Fig. 4, the rate of 3-*O*-methyl-D-glucose uptake was markedly inhibited when

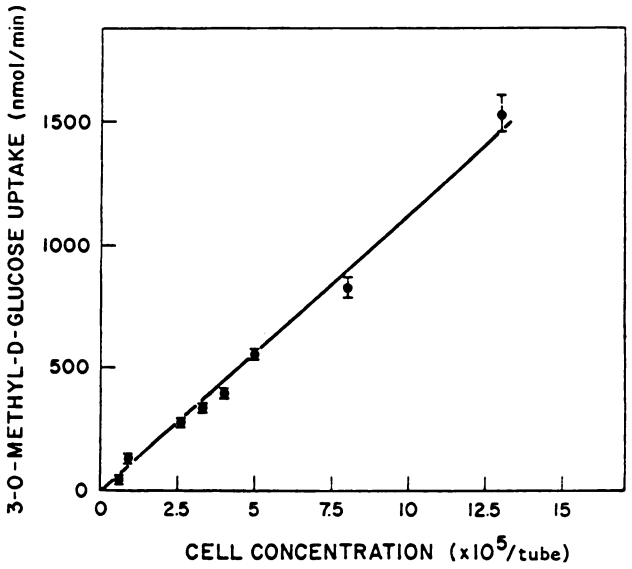

FIG. 1. *Relationship between 3-O-methyl-D-glucose uptake and cell concentration*

The values represent means ± standard deviation of four experiments

hepatocytes were preincubated with 40 μM cytochalasin B for 20 min. Under these condition, uptake of 3-*O*-methyl-D-glucose was linear for 1–2 min and equilibrium had not been reached after 20 min. The effect of cytochalasin B on the time course of 3-*O*-methyl-D-glucose uptake revealed 85% inhibition at 1 min as compared with 47% inhibition at 10 min and 15% inhibition at 20 min (Fig. 4).

Preincubation of hepatocytes with 2 mM phloridzin resulted in a 63% inhibition of 3-*O*-methyl-D-glucose uptake at 1 min (data not shown), 420 ± 7 nmoles/min/5 × 10^5 cells with control and 155 ± 11 nmoles with phloridzin. At 0.2 mM, the inhibition was 33%.

Effect of hexoses on 3-O-methyl-D-glucose uptake. The uptake of 3-*O*-methyl-D-glucose was inhibited by D-glucose 55% and 85% inhibition at 20 mM and 40 mM, respectively (data not shown). In contrast, L-glucose (20 mM) had no significant effect on 3-*O*-methyl-D-glucose uptake. At 40 mM, L-glucose produced a statistically significant (albeit small) inhibition of 10% uptake (data not shown).

3-*O*-Methyl-D-glucose uptake was examined as a function of the Na^+ concentration in the range of 0–150 mM by replacing NaCl with equimolar amounts of choline chloride. The rate of uptake remained unchanged.

Effect of Hormones on 3-O-methyl-D-glucose Uptake in Isolated Hepatocytes

Incubation of isolated hepatocytes with insulin (10^{-3}–10^{-11} M), glucagon (10^{-3}–10^{-11} M), GIP (2 × 10^{-3}–10^{-11} M), and pancreozymin (2–25 units/ml) for up to 2 hr did not significantly affect 3-*O*-methyl-D-glucose uptake (data not shown).

However, dexamethasone was found to have an inhibitory effect on the rate of uptake of 3-*O*-methyl-D-glucose (Fig. 4). Therefore, we examined in detail the kinetics of this effect. The inhibition of 3-*O*-methyl-D-glucose by dexamethasone depended on the time of incubation and on the concentration of inhibitor. As shown in Fig. 5, the

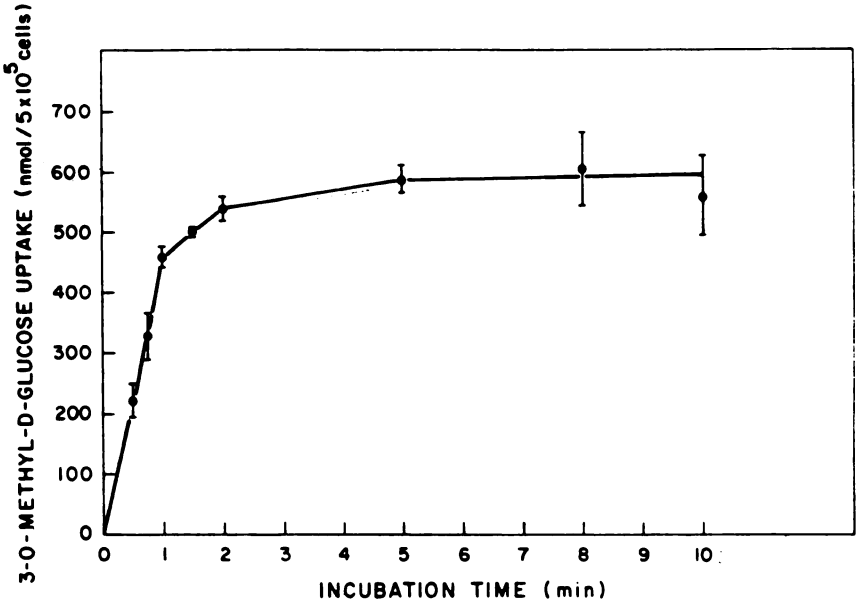

FIG. 2. *Time course of 3-O-methyl-D-glucose uptake*

Hepatocytes (5 × 10^5 cells/tube) were incubated at 22° and 3-*O*-D-[*methyl*-^{3}H]glucose (2 mM) was added. At the indicated times, the radioactivity retained by the cells was determined after centrifugation. The data are the means from four different experiments ± standard deviation.

minimal concentration of dexamethasone which produced a significant ($p < 0.01$) decrease in 3-*O*-methyl-D-glucose uptake was 0.5 μM. At a higher concentration (2 μM), a significant ($p < 0.01$) inhibition was seen after only 3 min of incubation whereas at the lower concentration of 0.5 μM, 3-*O*-methyl-D-glucose uptake did not decrease significantly until after 10 min of incubation. At 0.1 μM, incubation of up to 2 hr caused no significant inhibition of 3-*O*-methyl-D-glucose uptake. The time required for maximal inhibition of the uptake of 3-*O*-methyl-D-glucose at 2 μM and 0.5 μM dexamethasone was 30 min.

We studied further the delayed inhibitory effect of 3-*O*-methyl-D-glucose uptake observed at low concentrations of dexamethasone. Dexamethasone (0.5 μM, 60-min incubation) decreased the initial velocity by 35%. In equilibrium, inhibition of 3-*O*-methyl-D-glucose uptake was insignificant (Fig. 4).

As depicted in Fig. 3, the decrease in 3-*O*-methyl-D-glucose uptake by dexamethasone (0.5 μM) was due to a significant decrease in the V_{max} (420 versus 230 nmoles/min/5 × 10^5 cells; $p < 0.01$), whereas the K_m (5.88 versus 5.75 mM) remained unchanged. When hepatocytes were exposed to dexamethasone in the presence of insulin (10^{-6}–10^{-11} M) the effect of dexamethasone on 3-*O*-methyl-D-glucose remained unchanged (data not shown).

In addition to dexamethasone, other glucocorticoids (prednisolone and hydrocortisone) were also found to decrease the initial rate of uptake of 3-*O*-methyl-D-glucose in isolated hepatocytes (data not shown). A 50% inhibition of the 3-*O*-methyl-D-glucose uptake was observed at 1.5 μM dexamethasone, 10 μM prednisolone, and 25 μM hydrocortisone.

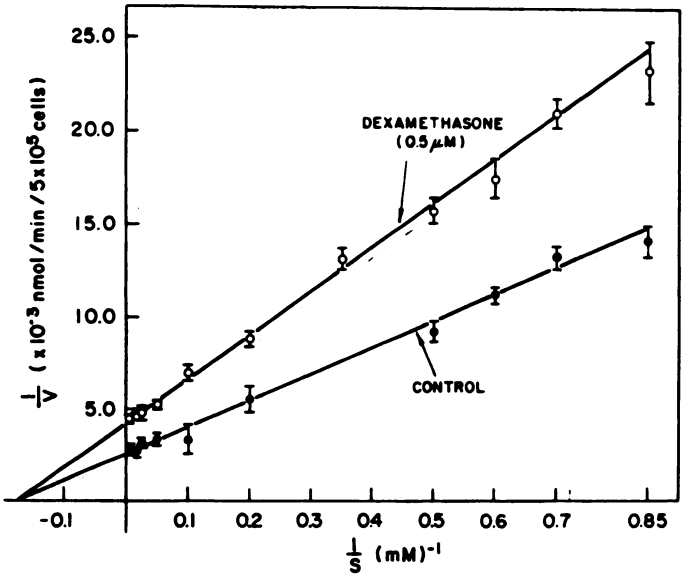

FIG. 3. *Lineweaver-Burk plot of initial velocity of 3-O-methyl-D-glucose uptake in hepatocytes*

Hepatocytes (5 × 10^5 cells/tube) were incubated for 60 min with various concentrations of 3-*O*-methyl-D-glucose and a fixed concentration of 3-*O*-D-[*methyl*-^{3}H]glucose in the presence (○) and absence (●) of dexamethasone. The uptake was assayed after 1 min of incubation. Values represent means ± standard deviation of five experiments.

DISCUSSION

Current findings demonstrate that 3-*O*-methyl-D-glucose uptake in isolated rat hepatocytes occurs via a specific, carrier-mediated, facilitated diffusion process. It is recognized that the uptake of hexoses by various cells is a rapid process, making kinetic analysis of glucose transport rather difficult (1). However, since in the present study 3-*O*-methyl-D-glucose uptake was linear over the initial 1-min period (Fig. 2), the technique employed provides a reasonable means for determining the kinetics of 3-*O*-methyl-D-glucose uptake. The K_m value (6 mM)

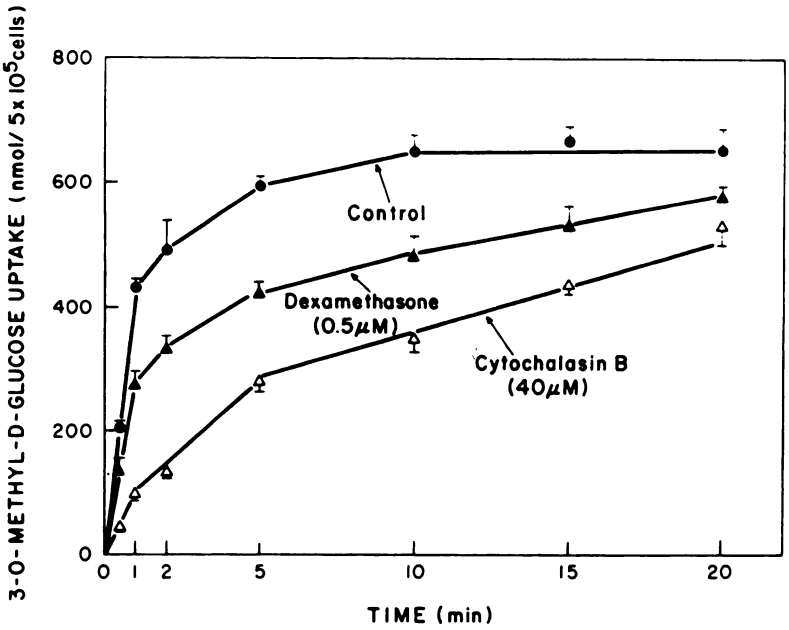

FIG. 4. *Inhibition of 3-O-methyl-D-glucose uptake by cytochalasin B and dexamethasone in isolated hepatocytes*

Hepatocytes (5×10^5 cells/tube) were preincubated with 40 μM cytochalasin B (△) or 0.5 μM dexamethasone (▲) at 22° for 20 min and 60 min, respectively, before the addition of 2 mM 3-*O*-D-[*methyl*-^{3}H]glucose. At the indicated time, the radioactivity retained in the cells was determined. The values represent means ± standard deviation of five experiments.

observed in the present study (Fig. 3) is in the physiological range and compares favorably with the values reported in studies with rat adipocyte plasma membranes (18) and human adipocytes. However, this finding is not in agreement with the results of Craik and Elliott (11). On the other hand, this K_m value is substantially lower than the values reported for D-glucose uptake by hepatocytes (30 mM) (7) and in perfused liver (17 mM) (8). The higher K_m values reported for D-glucose in other studies probably reflect the fact that the latter hexose is metabolized after entry into the cell.

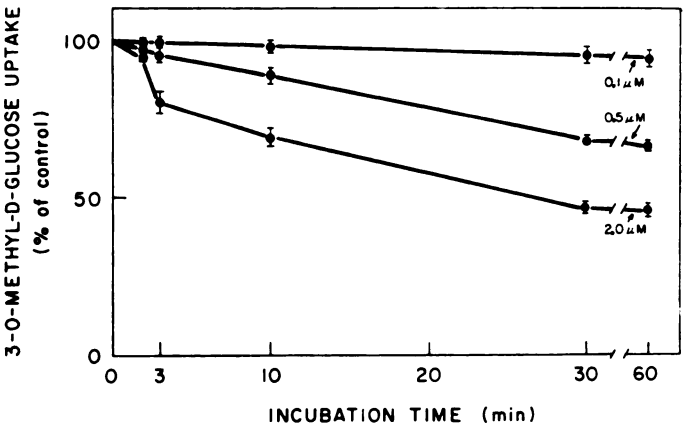

FIG. 5. *Time course of inhibitory effects of different concentrations of dexamethasone on 3-O-methyl-D-glucose uptake*

Hepatocytes were preincubated with different concentrations of dexamethasone for varying times at 22° before the addition of 3-*O*-D-[*methyl*-^{3}H]glucose. The uptake was determined after 1 min of incubation. The results are expressed as a percentage of the 3-*O*-methyl-D-glucose uptake of the control. Each value is the mean ± standard deviation of four experiments

The data showing an inhibitory effect of cytochalasin B (Fig. 4) and phloridzin are in agreement with previous findings in other cells (1, 3). The failure of even high concentrations of cytochalasin B to inhibit completely the uptake of 3-*O*-methyl-D-glucose without equilibrium being reached indicates that simple diffusion across the hepatocyte membrane does contribute by some 10–15% to the total uptake of 3-*O*-methyl-D-glucose. Similar results have been reported for brown and white fat cells (1). Nevertheless, the inhibitory effects of cytochalasin B support the conclusion that uptake of 3-*O*-methyl-D-glucose across the plasma membrane of isolated hepatocytes is not governed solely by simple diffusion, but is largely a carrier-mediated transport process. The possible existence of a cytochalasin B binding site in the hepatocyte membrane which is involved in sugar transport has been suggested in a previous study (19).

The data on the effect of other hexoses at 3-*O*-methyl-D-glucose uptake by hepatocytes indicate that 3-*O*-methyl-D-glucose uptake is stereospecific and that D-glucose and 3-*O*-methyl-D-glucose share a common transport system. Another possibility is that of the inhibitory effect of L-glucose, which might be due to competition for diffusion into the cell. The uptake of L-glucose may occur in part via the D-glucose transport system, but with a very low affinity.

The results (data not shown) concerning the effect of sodium concentration on 3-*O*-methyl-D-glucose indicate that transport of this hexose in isolated hepatocytes is not an active sodium-dependent process. This finding is in agreement with data on D-glucose uptake by hepatocytes (6).

Our data also demonstrate that 3-*O*-methyl-D-glucose uptake in isolated hepatocytes may be under hormonal regulation. Thus, the addition of glucocorticoids de-

creased 3-*O*-methyl-D-glucose uptake mainly because of a lowered V_{max} value (Fig. 3). This is in agreement with findings in adipocytes (16, 20). In terms of the carrier model, a decrease in V_{max} such as that produced by dexamethasone is consistent with a decrease either in the mobility of the carrier or in the effective number of carriers.

At high doses (0.5–2.0 μM), dexamethasone inhibited 3-*O*-methyl-D-glucose uptake within 3–30 min. These rapid effects are unlikely to be due to a decrease in the rate of carrier synthesis, or to be mediated via protein synthesis (21). A rapid effect of dexamethasone was demonstrated in fat cells and in Novikoff hepatoma cells (20, 22) and with cortisol in rat thymocytes (3). On the other hand, at dexamethasone concentrations of 0.1–1 μM, an effect in adipocytes was not observed until after 2 hr (16). Thus, it appears that dexamethasone affects glucose transport differently in hepatocytes (without a lag period) and in fat cells (with a lag period).

It should be noted that hepatocyte viability determined by trypan blue exclusion was not affected by exposure to high concentrations of hormones. The intracellular water space also remained unchanged. Since 3-*O*-methyl-D-glucose is not a glucose metabolite, the current findings support the hypothesis that glucocorticoids exert their effect at the level of glucose transport and decreased generation of glucose-6-phosphate (23).

However, the present data do not explain the mechanism of the inhibition. From the results given in Figs. 3 and 5 it seems that the process is saturable and time-dependent, thus hinting at a possibility of intracellular binding. On the other hand, the apparent inhibition constant is 0.61 μM, 30- to 120-fold higher than the dissociation constant of the glucocorticoid-intracellular receptor complex (3). Therefore, it is questionable whether the inhibition is mediated by steroid receptors. Some specificity exists, since dexamethasone is 6.7 times more potent than prednisolone and 16.7 times more potent than hydrocortisone.

In view of the relatively high concentrations of dexamethasone required to achieve inhibition of glucose transport in hepatocytes, further experiments are needed to clarify whether this effect has any physiological significance *in vivo*. In our previous work (14) with hepatocytes, in contrast to the effect of dexamethasone and other glucocorticoids, 17β-estradiol increased 3-*O*-methyl-D-glucose uptake.

We failed to observe any effect of insulin, glucagon, GIP, and phloridizin on 3-*O*-methyl-D-glucose uptake in hepatocytes. The findings with insulin are in agreement with previous observations with isolated hepatocytes (6) and with cultured hepatocytes (16). On the other hand, insulin has been shown to increase glucose uptake in fat cells (11).

REFERENCES

1. Czech, M. P. Regulation of the D-glucose transport system in isolated fat cells. *Mol. Cell. Biochem.* **11**:51–63 (1976).
2. Yasmeen, D., A. J. Laird, D. A. Hume, and J. M. Weidemann. Activation of 3-*O*-methyl-D-glucose transport in rat thymus lymphocytes by concanavalin A: temperature and calcium ion dependence and sensitivity to puromycin but not to cycloheximide. *Biochim. Biophys. Acta* **500**:89–102 (1977).
3. Zyskwski, L., and A. Munck. Kinetic studies on the mechanism of glucocorticoid inhibition of hexose transport in rat thymocytes. *J. Steroid Biochem.* **10**:573–579 (1979).
4. Carter, J. R., J. Avruch, and D. B. Martin. Glucose transport by trypsin-treated red-blood cell ghosts. *Biochim. Biophys. Acta* **291**:506–518 (1973).
5. Bachmann, W., and D. Challoner. D-Glucose uptake by a rat liver plasma membrane preparation. *Biochim. Biophys. Acta* **443**:254–266 (1976).
6. Baur, H., and W. H. Heldt. Transport of hexoses across the liver-cell membrane. *Eur. J. Biochem.* **74**:397–403 (1977).
7. Cahill, G. F., J. Ashmore, A. S. Earle, and S. Zottu. Glucose penetration into liver. *Am. J. Physiol.* **192**:491–496 (1958).
8. William, T. F., J. H. Exton, C. R. Park, and D. M. Regen. Stereospecific transport of glucose in the perfused rat liver. *Am. J. Physiol.* **215**:1200–1209 (1968).
9. LeCam, A., and P. Freychet. Neutral amino acid transport: characterization of the A and L systems in isolated rat hepatocytes. *J. Biol. Chem.* **252**:148–156 (1977).
10. Katz, J., P. A. Wals, S. Golden, and R. Rognstad. Recycling of glucose by rat hepatocytes. *Eur. J. Biochem.* **60**:91–101 (1975).
11. Craik, D., and K. F. Elliott. Kinetics of 3-*O*-methyl-D-glucose transport in isolated rat hepatocytes. *Biochem. J.* **182**:503–508 (1979).
12. Czaky, T. Z., and J. E. Wilson. The fate of 3-*O*-$^{14}CH_3$-glucose in the rat. *Biochim. Biophys. Acta* **22**:185–256 (1956).
13. Jeejeebhoy, K. N., J. Ho, A. R. Greenberg, M. J. Phillips, A. Bruce-Robertson, and U. Sodtke. Albumin, fibrinogen and transferrin synthesis in isolated rat hepatocyte suspensions: a model for the study of plasmaprotein synthesis. *Biochem. J.* **146**:141–155 (1975).
14. Madar, Z., N. J. MacLusky, and F. Naftolin. Estrogen stimulation of 3-*O*-methyl-D-glucose uptake in isolated rat hepatocytes. *Endocrinology* **110**:330–335 (1982).
15. Moore, G. E., E. Ito, K. Ulrich, and A. A. Sandberg. Culture of human leukemia cells. *Cancer* **19**:713–732 (1966).
16. Olefsky, J. M. Effect of dexamethasone on insulin binding, glucose transport, and glucose oxidation of isolated rat adipocytes. *J. Clin. Invest.* **56**:1499–1508 (1975).
17. Kletzien, R. F., W. M. Pariza, J. E. Becker, and V. R. Potter. A method using 3-*O*-methyl-D-glucose and phloretin for the determination of intracellular water space of cells in monolayer culture. *Anal. Biochem.* **68**:537–544 (1975).
18. Ludvigsen, C., and L. Jarett. A kinetic analysis of D-glucose transport by adipocyte plasma membranes. *J. Biol. Chem.* **254**:1444–1466 (1979).
19. Biordan, J. R., and N. Alon. Binding of [^{3}H]cytochalsin B and [^{3}H]colchicine to isolated liver plasma membranes. *Biochim. Biophys. Acta* **464**:547–56 (1977).
20. Livingston, N. J., and H. D. Lockwood. Effect of glucocorticoids on the glucose transport system of isolated fat cells. *J. Biol. Chem.* **250**:8353–8360 (1975).
21. Freychet, P., and A. LeCam. Amino acid transport in isolated hepatocytes: effect of glucagon. *Proc. CIBA Found.* **55**:247–268 (1978).
22. Plagemann, P. G. W., and E. D. Renner. Glucocorticoids: competititve inhibition of glucose transport. *Biochem. Biophys. Res. Commun.* **46**:816–823 (1972).
23. O'Neill, I. E., and D. R. Langslow. The action of hydrocortisone, insulin and glucagon on chicken liver hexokinase and glucose-6-phosphatase and on the plasma glucose and free fatty acid concentrations. *Gen. Comp. Endocrinol.* **34**:428–437 (1978).

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