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Utilization of Mannose by Astroglial Cells

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Uptake and metabolism of mannose were studied in astroglia-rich primary cultures derived from neonatal rat brains. A saturable component of mannose uptake was found with half-maximal uptake at 6.7 ± 1.0 mM mannose. In addition, a non-saturable component dominated the uptake at high concentrations of mannose. Glucose, cytochalasin B, or phloretin in the incubation buffer inhibited the carrier-mediated uptake of mannose. Within the astroglial cells mannose is phosphorylated to mannose-6-phosphate. In cell homogenates, the K_M value of mannose-phosphorylating activity was determined to be 24 ± 7 μM. The V_{max} value of this activity is only 40% that of glucose-phosphorylating activity. Mannose-6-phosphate was converted to fructose-6-phosphate by mannose-6-phosphate isomerase. The specific activity of this enzyme in homogenates of astroglial cultures was higher than that of hexokinase. Two products of mannose utilization in astroglial cells are glycogen and lactate. The amounts of each of these products increased with increasing concentrations of mannose. In contrast to the generation of lactate, that of glycogen from mannose was enhanced in the presence of insulin. In conclusion, we suggest that mannose is taken up into the cells of astroglia-rich primary cultures by the glial glucose transporter and is metabolized to fructose-6-phosphate within the astroglial cells.

KEY WORDS: Astroglia; glycogen; hexokinase; lactate; mannose; mannose-6-phosphate isomerase.

INTRODUCTION

Oxygen and glucose are the most important substrates for the production of energy in the brain (1). Besides glucose, the brain is also able to take up many other compounds, among them mannose (2–4). Only scarce data exist regarding the metabolism of mannose in the brain. Experiments with brain slices (5) or with perfused brain preparations (6) showed that mannose could be used as substrate for the production of lactate and CO_2. However, in these model systems the cell type(s) which utilize(s) mannose were not identified. Therefore, astroglia-rich cultures were used to investigate the possibility of an astroglial localization of mannose utilization.

In order to utilize mannose, the cells must be equipped with an uptake system for mannose and with enzymes that can convert mannose to an intermediate of glucose metabolism. To our knowledge direct studies of mannose transport in brain cells have not been reported so far. The inhibition by mannose of the uptake of glucose or glucose analogues in brain microvessels (7–9), neurons (10,11), and astroglial cells (10) may only indirectly point to mannose transport.

After uptake into the cells mannose might be phosphorylated by hexokinase (HK). Mannose was found to be a substrate for the HK isolated from brain (12). The next step in the metabolism of mannose is the conversion of mannose-6-phosphate (M6P) to fructose-6-phosphate (F6P), an intermediate of glycolysis. For this reaction

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mannose-6-phosphate isomerase (M6PI) is essential, an enzyme present in brain (13–15).

During investigations on the function of glycogen in astroglial cells mannose was found to be utilized in astroglia-rich primary cultures for the synthesis of glycogen, glucose-6-phosphate (G6P), and lactate (16). Since glycogen (17) as well as glycogen phosphorylase (18–21) are predominantly localized in astroglial cells, this cell type must be able to metabolize mannose.

Here we demonstrate the uptake of mannose into astroglial cells and the enyzmatic conversion of mannose to intermediates of glucose metabolism. Parts of these results have been published in abstract form (22).

EXPERIMENTAL PROCEDURE

Materials. Dulbecco's modified Eagle's medium (DMEM) was obtained from Flow Laboratories (Meckenheim, F.R.G.); ethanol, fetal calf serum (prepared by Gibco), D-glucose, and the scintillation fluid eco plus were purchased from C. Roth (Karlsruhe, F.R.G.); DMEM without glucose was from Biochrom (München, F.R.G.). Cell culture dishes and 96-well microtiter plates were obtained from Nunc (Wiesbaden, F.R.G.). Amyloglucosidase form Aspergillus niger (EC 3.2.1.3; 14 U/mg), yeast glucose-6-phosphate dehydrogenase (EC 1.1.1.49; 350 U/mg, yeast glucose-6-phosphate isomerase (G6PI; EC 5.3.1.9; 350 U/mg), glutamate-pyruvate transaminase from pig heart (EC 2.6.1.2; 80 U/mg), yeast HK (EC 2.7.1.1; 140 U/mg), lactate dehydrogenase from rabbit muscle (EC 1.1.1.27; 550 U/mg), yeast M6PI (EC 5.3.1.8; 60 U/mg), ATP, M6P, and NAD were from Boehringer (Mannheim, F.R.G.). NADP was purchased from Biomol (Hamburg, F.R.G.). Bovine serum albumin, cytochalasin B, D-mannose, and phloretin were from Sigma (Deisenhofen, F.R.G.). Penicillin G and streptomycin sulfate were purchased from Serva (Heidelberg, F.R.G.). D-[U-^{14}C]mannose (10.0 GBq/mmol) was from Amersham Buchler (Braunschweig, F.R.G.). All other chemicals, of the highest purity available, were obtained from E. Merck (Darmstadt, F.R.G.).

Cell Culture. Astroglia-rich primary cultures derived from brains of neonatal Wistar rats were prepared and cultured as described (23). Cells were seeded in plastic culture dishes (60 mm in diameter) and grown in culture medium (90% DMEM, 10% FCS, 20 units/ml penicillin, and 20 μg/ml streptomycin sulfate). The medium was renewed every 7th day. The studies presented here were carried out on 13- to 21-day-old cultures; in this range the results obtained did not depend on the age of the cultures.

Uptake Experiments. The uptake experiments with [^{14}C]mannose were performed in analogy to the uptake studies described by Stahl et al. (24).

Experimental Incubation and Determination of Glycogen Content and Lactate Concentration. After removal of the culture medium, the cells were washed twice with 3 ml of glucose-free DMEM (37°C, pH 7.4) and preincubated (2 h) with 3 ml of this medium in a Heraeus cell incubator containing an atmosphere of 10% CO_2/90% air. For the main incubation (3 h) the cells were kept in DMEM with the concentrations of hexoses indicated for each experiment. Experiments were terminated by aspirating the medium and washing the cells three times with 4 ml of ice-cold phosphate-buffered saline (10 mM potassium phosphate buffer, pH 7.4, containing 150 mM NaCl), sucking off the buffer and freezing the culture dishes at −25°C. The glycogen content was determined as described (25). For assaying the concentration of lactate in the incubation medium, the medium was diluted with H_2O (1:10) and 200 μl of this solution were mixed in a well of a microtiter plate with 200 μl of reaction mixture (500 mM glutamate/NaOH buffer, pH 8.9, 2.8 mM NAD, 0.56 U glutamate-pyruvate transaminase, 5.5 U lactate dehydrogenase). After the incubation (60 min, 37°C) the absorbance was measured at 340 nm against diluted medium without lactate in an ELISA reader (Titertek Multiskan MCC/340, Flow Laboratories, Meckenheim, F.R.G.).

Preparation of the Cell Homogenates for Assaying Enzyme Activities. Astroglia-rich primary cultures were washed 3 times with 4 ml ice-cold phosphate-buffered saline and once with 20 mM HEPES/NaOH buffer pH 7.3. The cells were then incubated on ice for 10 min with 0.4 ml of the 20 mM HEPES/NaOH buffer pH 7.3. After scraping the lysed cells off the dish the suspension was centrifuged (5 min, 12,000 *g*). Aliquot parts of the supernatant were used for the assessment of enzyme activities.

Enzyme Assays. The reaction mixtures for measuring the activities of HK and M6PI contained 100 mM HEPES/NaOH buffer pH 7.3, 2 mM $MgSO_4$, 0.76 mM NADP, and 3.5 U glucose-6-phosphate dehydrogenase in a final volume of 1 ml. In addition, the reaction mixture (37°C) contained 5 mM glucose and 0.99 mM ATP for the HK assay, and 5 mM M6P and 7 U G6PI for the M6PI assay. The time-proportional increase in absorbance at 340 nm (Shimadzu photometer UV-120-01, Shimadzu Europe, Duisburg, F.R.G.) was followed. For determining kinetic parameters for the phosphorylation of glucose and mannose the supernatants obtained from several dishes were combined. Aliquot parts (100 μl) of the combined solutions were assayed for activity (at 22°C) in wells of a microtiter plate (final concentrations in the total volume of 400 μl: various concentrations of the hexose under study, 100 mM HEPES/NaOH buffer pH 7.3, 2 mM $MgSO_4$, 0.99 mM ATP, 0.76 mM NADP, 1.75 U/ml glucose-6-phosphate dehydrogenase, and if mannose was present, 3.5 U/ml G6PI plus 1.5 U/ml M6PI) in an ELISA reader (see above) at room temperature.

Determination of Protein Content. Protein was determined according to Bradford (26; uptake experiments) with the Bio-Rad Protein Assay (Bio-Rad Laboratories, München, F.R.G.) or according to Lowry et al. (27; all other experiments), using bovine serum albumin as a standard.

Presentation of Data. Each experiment was carried out two or more times with comparable results. In the Tables and Figures the data of one representative experiment are presented as mean values of duplicates (glycogen content, lactate release, mannose uptake) or triplicates (enzyme activities). In the Figures, the vertical bars span the differences between the individual values (duplicates) or, respectively, represent the standard deviations. The bars have been omitted when they were smaller than the symbols representing the mean values. In the Tables the data are given as mean values ± SD or ± deviation from the mean value (duplicates).

RESULTS

The uptake of mannose from a 1 mM solution into the cells of an astroglia-rich primary culture as a function of time is shown in Fig. 1. A phase of rapid uptake lasting about 1 min was followed by a phase of slow intracellular appearance of radioactivity. Within 1 min approximately 80% of the uptake found after 10 min has been accomplished (Fig. 1). Therefore, an incubation

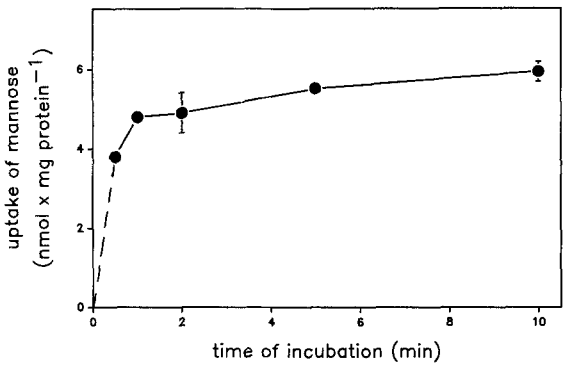

Fig. 1. Time course of the uptake of mannose in an astroglia-rich primary culture (14-day-old; 0.5 mg protein per dish). The incubation buffer contained 1 mM mannose and 14.8 KBq [^{14}C]mannose.

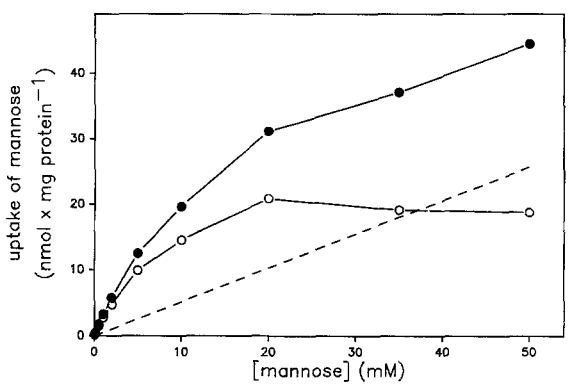

Fig. 2. Uptake of mannose in an astroglia-rich primary culture as a function of the concentration of extracellular mannose. The values for the saturable component of uptake (○) were calculated by subtracting the corresponding data of the dotted line (hypothetical curve for mannose uptake by diffusion alone) from the experimentally determined data (●). The cells were incubated for 1 min with mannose of the indicated concentrations and 14.7 KBq of [^{14}C]mannose. The 19-day-old culture contained 0.7 mg protein per dish.

time of 1 min was used for investigating the mechanism of mannose transport in astroglial cells. With increasing concentrations of mannose in the incubation buffer, the uptake of mannose increased without reaching a plateau (Fig. 2). At high concentrations of mannose a non-saturable component dominated total uptake of mannose. Therefore, a hypothetical curve for uptake by the non-saturable component alone was constructed as the parallel to the curve representing the linear component of uptake through the origin. The corresponding data on this curve (Fig. 2, dotted line) were subtracted from the experimentally determined data of total uptake and the resulting graph (Fig. 2, open symbols) represented the saturable component of uptake alone. A similar approach has been used in previous uptake studies with astroglial cells (24,28,29). The saturable component of uptake was analyzed according to Hanes (30). Half-maximal uptake as determined from 3 separate cultures was reached at 6.7 ± 1.0 mM mannose. The uptake of mannose from a 1 mM solution was reduced to 38% of control in the presence of 25 mM glucose and strongly inhibited in the presence of the inhibitors of glucose transport, cytochalasin B (31) or phloretin (32) (Table I). Accordingly, the uptake of 2-deoxyglucose (DG) into astroglia-rich primary cultures was strongly inhibited by the presence of mannose in the incubation buffer (data not shown).

The synthesis of glycogen and the release of lactate into the culture medium served as indicators for the mannose taken up. Both entities increased with increasing concentrations till about 5 mM mannose in the culture medium, at which concentration a plateau of the concentration/response curve was reached (Fig. 3). For both hexoses a half-maximal glycogen content was found at concentrations of 2 mM (Fig. 3A). In the presence of mannose there was slightly less release of lactate into the medium than in the case when glucose was the substrate. (Fig. 3B). Half-maximal release of lactate was found at concentrations of approximately 1 mM hexose (Fig. 3B).

Insulin in the incubation medium enhanced the content of glycogen in both glucose and mannose fed cultures (Table II). In the presence of glucose insulin doubled the content of glycogen, while the increase was somewhat less pronounced if mannose was present (Table II). In contrast, irrespective of the hexose present, insulin had only a negligible effect (110% of control) on the appearance of lactate in the culture medium (Table II).

The first step in the metabolism of mannose is the phosphorylation to M6P. In order to test for the presence of this enzymatic activity, homogenates of astroglia-rich primary cultures were investigated for phosphorylation

Table I. Inhibition of the Uptake of Mannose Into Rat Astroglia-Rich Primary Cultures by Compounds Which Affect Glucose Transport

Substance	Concentration	Uptake of Mannose (% of control)
Glucose	25 mM	38 ± 3.0
Cytochalasin B	50 μM	14 ± 0.6
Phloretin	100 μM	13 ± 0.7

The cells were incubated for 1 min in buffer containing 1 mM unlabeled and 14.8 KBq of labeled mannose, and the compounds indicated. The 21-day-old culture contained 0.6 mg protein per dish.

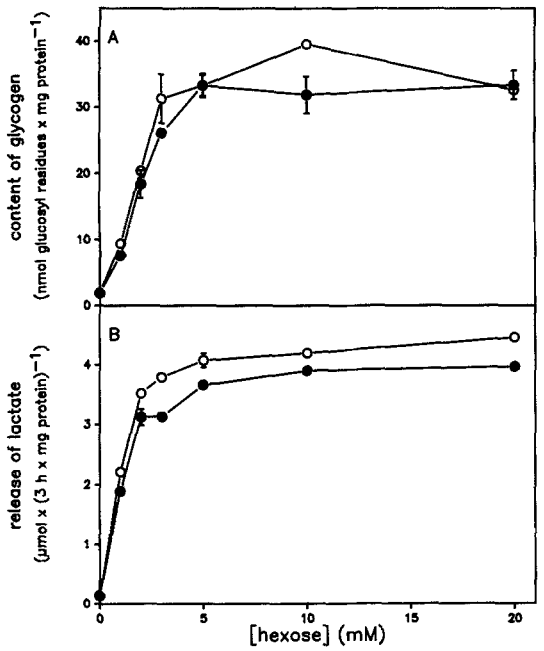

Fig. 3. Content of glycogen in astroglia-rich primary cultures (A) and of lactate in the culture medium (B) after incubation (3 h) in DMEM with the given concentrations of mannose (●) or glucose (○). For depletion of glycogen, the cells were preincubated (2 h) in DMEM without glucose. The 15-day-old cultures contained 1.6 mg protein per dish.

Table II. Influence of Insulin on the Content of Glycogen in Astroglia-Rich Primary Cultures and on the Release of Lactate Into the Culture Medium in the Presence of Mannose or Glucose

Hexose	Presence of Insulin	Glycogen Content (nmol/mg)	Lactate Release (μmol/(3 h × mg))
Glucose	−	32.6 ± 0.6	4.46 ± 0.05
Mannose	−	33.4 ± 2.2	3.97 ± 0.11
Glucose	+	69.9 ± 3.3	4.95 ± 0.17
Mannose	+	55.1 ± 11.6	4.46 ± 0.01

For depletion of glycogen the astroglia-rich primary cultures were preincubated (2 h) in DMEM without glucose. After the main incubation (3 h) in DMEM with 20 mM glucose or mannose in the presence or absence of insulin (1 μM), the medium was saved for the assessment of lactate released from the cells, and the cells were washed and frozen for determination of glycogen. The content of glycogen is presented as nmol glucosyl residues per milligram of protein. The 15-day-old cultures contained 1.6 mg protein per dish.

of mannose. No matter whether mannose or glucose was used as a substrate the concentration-activity plot showed saturation kinetics of the phosphorylating activities (Fig. 4). The K_M value for mannose determined in 3 separate experiments was found to be 24 ± 7 μM. The V_{max}

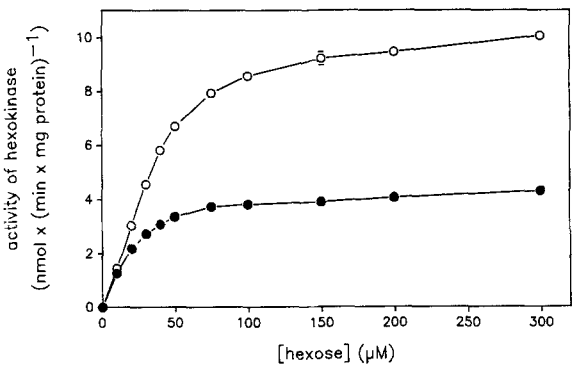

Fig. 4. Relationship, at 22°C, between the specific activities of hexokinase in a homogenate (soluble fraction) of an astroglia-rich primary culture (18-day-old culture) and the concentrations of glucose (○) and mannose (●).

Table III. Specific Activities of HK and M6PI in the Soluble Fraction of Homogenates of Astroglia-Rich Primary Cultures

Enzyme	Specific Activity (nmol × (min × mg protein)$^{-1}$)
Hexokinase	28.9 ± 4.5
Mannose-6-phosphate Isomerase	39.8 ± 0.8

The enzyme activities were measured in HEPES/NaOH buffer, pH 7.3, at 37°C, the culture was 20 days old. For further details see Experimental Procedure.

value for the phosphorylation of mannose varied with the preparation of the homogenate, although it was not dependent on culture age. In three experiments with 13-, 14- and 21-day-old cultures it was determined as 2.9, 1.7, and 4.3 nmol × (min × mg protein)$^{-1}$. In the respective experiments the V_{max} value of HK for glucose was 7.9, 3.2, and 10.1 nmol × (min × mg protein)$^{-1}$, i.e., in each of the experiments the mannose phosphorylating activity amounted to approximately 44% of that for glucose. For generation of glycogen or lactate M6P has to be converted to F6P, an intermediate of glycolysis. Therefore, the presence of M6PI is essential. Indeed, this enzyme was found in homogenates of astroglia-rich primary cultures with a specific activity that was nearly 50% higher than that of HK (Table III).

DISCUSSION

Although the uptake of mannose into the brain through the blood brain barrier has been known for many

years (2–4), the cell type(s) in brain that utilize(s) mannose (5,6) remained unknown. Primary cultures derived from the brains of newborn rats are dominated by astroglial cells (21,33) and contain only minor populations of oligodendroglial, phagocytic, and ependymal cells, but no neurons. Therefore, these cultures are well suited to address the question whether mannose can be utilized by astroglial cells.

The uptake of mannose from a 1 mM solution into the astroglial cells was not linear with time even at incubation periods as short as 30 s to 1 min. However, with the method used it was not reliable to measure uptake of radioactively labeled compounds with incubation times below 30 s. Therefore, it could not be demonstrated unambiguously if initial velocities of uptake were measured, although the linearity of the concentration dependence below 2 mM may suggest that this experiment is based on initial velocities. As a consequence of this ambiguity, an exact kinetic analysis including K_M and V_{max} values was not attempted. Nevertheless, the graph representing total uptake within 1 min as a function of the concentration of extracellular mannose demonstrated clearly the presence of a saturable process in addition to a non-saturable one which dominated total uptake at concentrations higher than 40 mM. A similar course of uptake has been reported for glucose (24) and DG (34) in astroglial cultures. Since with increasing concentrations of mannose the specific radioactivity of the incubation medium decreased, the measured 'cellular' radioactivity did not represent non-specific association of radioactively labeled mannose with the cells. The non-saturable component therefore most probably represents diffusion, although a carrier-mediated process with a K_M value higher than 50 mM cannot be excluded with certainty. Nevertheless, such a K_M value would be of no physiological significance.

If one considers the saturable component of total uptake alone, half-maximal uptake was reached at about 7 mM. This value was derived from an evaluation of the data according to the formal framework given by Hanes (30), although, as discussed in the preceding paragraph, initial velocities could not be established unambiguously. The high value of 7 mM suggests that mannose might not be the physiological substrate of a 'mannose transporter' of its own. A candidate for transporting mannose across the plasma membrane of astroglial cells is the glucose carrier. This hypothesis is supported by the reports that mannose inhibits the uptake of DG into astroglial cultures (10) and of glucose through the blood brain barrier (2–4). In addition, it inhibits the transport of glucose or its analogues DG and 3-O-methylglucose in brain microvessels (8,9), in cultures of neurons (10,11), in oocytes expressing the human brain glucose transporter (35), and in several non-neural cell types (36–38). In turn, the reduction by glucose of mannose uptake into astroglia-rich primary cultures reported here is reminiscent of a similar observation made in erythroleukemic cells (39), although at present we cannot explain the relatively low degree of inhibition. However, stronger support for the above mentioned hypothesis is lent by the notion that cytochalasin B (31) and phloretin (32), inhibitors of glucose transporters blocked the uptake of mannose into astroglia-rich primary cultures almost completely. Cytochalasin B had also been reported to inhibit the uptake of glucose into astroglia-rich primary cultures (24).

These facts lead to the conclusion that mannose is taken up into astroglia-rich primary cultures by the glucose transporter. The fact that the concentration at which half-maximal velocity of transport into astroglial cells is reached is higher for mannose (6.7 mM) than for glucose (1 mM, (24)) might be explained by steric hindrance of the binding of mannose to the transporter caused by the hydroxyl group at position 2 (40).

To our knowledge, a specific 'mannose kinase' has not been reported so far. Since M6PI is necessary in the enzymatic assay for mannose-phosphorylating activity (see Experimental Procedure), phosphorylation of mannose to M6P was demonstrated. Isolated brain HK accepts mannose as substrate (12). Therefore, we suggest that it is HK which phosphorylates mannose in the astroglial cells. The K_M values of brain HK for the substrates mannose and glucose (12) are lower than the K_M values found for hexose phosphorylation in astroglial homogenates (present work). From this one might conclude that some cellular component contained in the homogenate influences the kinetic properties of HK. It is worth pointing out that the K_M value of 40 μM reported here for glucose phosphorylation in astroglial homogenates is identical to previously published data (41). Lower K_M values for mannose than for glucose, as found for the phosphorylation in astroglial cell homogenates (present work), were also reported for HK isolated from brain (12), HK in homogenates from Novikoff ascites-hepatoma cells (42), and HK from pancreatic β-cells (43). The relative maximal rate of phosphorylation of mannose by brain HK was reported to be only 40% that for glucose (12), which agrees well with the data presented here for hexose phosphorylation in the astroglial homogenates. It should be noted that the variations in the V_{max} values of the two phosphorylating activities did not correspond to the different culture ages. Care was also taken to perform lysis and extraction of the cells with high accuracy. Finally, it is highly improbable that var-

iations in the hexokinase activities are due to variations in the extent of contaminating cell populations. Particularly oligodendrocytes contain only low amounts of hexokinase (44). On the other hand, similar variations have also been found concerning other cellular parameters of astroglial cells, e.g., the glycogen content varied about twofold when 19 separate batches of astrocytes were compared (45).

For the next step in the metabolism of mannose, the conversion of M6P to F6P, M6PI is required. This enzymatic activity is present in brain (13–15). Astroglia-rich primary cultures contained M6PI at a specific activity higher than that of HK. Knowledge of the activity ratio of HK to M6PI is important for assessing the toxic effects of mannose. Abnormal high concentrations of mannose cause teratogenic effects in rat embryos (46,47). Absence or reduction of the activity of M6PI was the cause of mannose toxicity in Drosophila melanogaster, Ceratitis capitata (48), and in Ehrlich ascites tumor cells (15), probably by accumulation of M6P in the cells (15). However, we can exclude toxic effects in the astroglial cultures, since morphological changes, detachment of cells or cell necrosis were never observed even during incubation with mannose at high concentrations for several days (K. Bergbauer, R. Dringen, R. Schmid, and B. Hamprecht, unpublished results).

Lactate as the product of anaerobic glycolysis was found to be generated from mannose and excreted into the culture medium. The content of lactate in the medium in the presence of mannose was at all concentrations about 90% of that in the presence of glucose. The higher K_M value of the glucose transporter for mannose and the lower V_{max} value of HK for mannose, in comparison to those values for glucose, might be responsible for this small reduction in the excretion of lactate. Accordingly, it would most likely have been the astrocytes in the brain slices and the perfused brain, which generated lactate from mannose in amounts equal to those found in the presence of glucose (5,6).

In liver (49) and Novikoff ascites-hepatoma cells (42), carbon atoms derived from mannose were incorporated into glycogen. For the biosynthesis of glycogen from mannose, the F6P generated by the action of M6PI had to be converted to G6P, the physiological intermediate for the synthesis of glycogen from glucose. This conversion was performed by the activity of the G6PI, a glycolytic enzyme that was found in astroglial cells with a specific activity several fold higher than that of HK (R. Dringen and B. Hamprecht, unpublished results). Thus, all enzymes required for the synthesis from mannose of glucosyl residues of glycogen exist in astroglial cells. Accordingly, astroglia-rich primary cultures synthesized glycogen if exposed to mannose at a concentration of 20 mM (22; R. Dringen, R. Gebhardt, and B. Hamprecht, in press). However, also at lower concentrations glycogen was synthesized from mannose to a similar extent as from glucose.

Insulin was reported to double the content of glycogen synthesized from glucose in cultured astroglial cells (25,50). This enhancement is less, if mannose is the carbon source. In contrast, in the case of either hexose insulin only slightly affected the release of lactate into the culture medium. These data on the action of insulin on astroglial cultures agree well with the enhancement by insulin of glucose incorporation in brain glycogen (51) and the just slight enhancement by insulin of glucose consumption in the perfused brain (52). Obviously, insulin does not generally influence the metabolism of hexoses, but rather specifically regulates the level of glycogen. These results contrast with the situation in hepatocytes, where both the release of lactate (53) and the synthesis of glycogen (54,55) are enhanced in the presence of insulin.

Our studies provide a further example of the metabolic versatility of astrocytes. Besides glucose, ketone bodies (56,57), sorbitol (58) and octanoate (57,59) astroglial cells in culture can also utilize mannose, and, therefore, are candidates for mannose utilization in the brain. Of course, the present study does not exclude the possibility that other cell types in the brain, particularly neurons, are also able to utilize mannose for energy production. What could become the physiological function of mannose in brain or particularly in astrocytes? Mannose taken up by astroglial cells can be metabolized to lactate, which, after release, may be a substrate for energy production in other brain cells. This could especially be the case in the early neonatal brain, the cells of which utilize lactate as the main metabolic substrate (60).

Since mannose does not normally occur in the blood circulation in significant concentrations (61,62), the endowment of astroglial cells with M6PI might be an enigma. The main function of this enzyme in brain might not be in the production of energy but rather in the production of M6P from G6P. This generation of M6P is essential for the synthesis of UDP-mannose, the substrate for the synthesis of mannosyl and fucosyl residues in glycoproteins and glycolipids (63). Therefore, M6PI might be necessary for the proper functioning of membranes in the brain.

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