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Enzymes of Glucose Metabolism in Cultured Human Gliomas: Neoplasia Is Accompanied by Altered Hexokinase, Phosphofructokinase, and Glucose-6-Phosphate Dehydrogenase Levels

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The enzymes of glycolysis and selected enzymes of the pentose phosphate pathways were measured by fluorometric methods in extracts prepared from cultures of normal cortical human astrocytes and from cultures derived from low-grade (II) or high-grade (IV) gliomas. The hexokinase and phosphofructokinase levels of the low-grade glioma-derived line were not significantly different from those of the normal astrocyte cultures. However, the activities of hexokinase and phosphofructokinase were consistently and significantly increased in the high-grade glioma-derived lines. The activity of glucose-6-phosphate dehydrogenase was significantly decreased in all glioma-derived lines and by more than 90% in the high-grade-derived lines. Other enzymes of the glycolytic pathway were not significantly different from those of normal astrocytes, or they showed a variation inconsistently related to the neoplastic state. Glucose flux is not apparently regulated to a significant degree of hexokinase in glioma-derived lines, since the measured V_{max} values are in substantial excess over the measured flux rates. Reversible binding of hexokinase to the particulate fraction was observed in both the normal astrocytes cultures and the high-grade glioma-derived lines. A twofold displacement of particulate hexokinase by ATP, ADP, 1-*O*-methylglucose, sorbitol-6-phosphate, and dibutyryl cyclic AMP was observed in the high-grade glioma-derived lines. The degree of displacement by various agents and the basal ratio of free/bound was not significantly different between the transformers and the nontransformants. The hexokinase from both the gliomas and the normal astrocytes was noncompetitively inhibited by the glucose analogue 2-deoxy-*d*-glucose. Phosphofructokinase activity is close to the observed glucose flux rates in both the normal astrocyte and the glioma-derived cultures. The phosphofructokinase activity of normal astrocytes is activated twofold or more by ADP, AMP, fructose-2,6-diphosphate, and P_i. However, these same ligands activate

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phosphofructokinase by less than twofold in a typical high-grade glioma-derived line. ATP, dibutyryl cyclic AMP, and citrate inhibit glioma and normal astrocytic phosphofructokinase, but the magnitude of the inhibition is much less than in the glioma-derived lines.

KEY WORDS: glia; gliomas; brain glycolysis; hexokinase; phosphofructokinase; glucose-6-phosphate dehydrogenase.

INTRODUCTION

The metabolism of gliomas has been the subject of a resurgence of interest, driven in part by the advent of positron emission tomography and the application of [^{18}F]-fluorodeoxyglucose to assess *in situ* metabolic rates (DeLaPaz *et al.*, 1981; Di Chiro *et al.*, 1982). We recently demonstrated that human gliomas show a grade-dependent increase in the local cerebral metabolic rate for glucose (LCMRglc).[5] *In situ*, high-grade gliomas (astrocytoma grade IV or glioblastoma) have an LCMRglc 1.6-fold greater than that of normal contralateral brain (DeLaPaz *et al.*, 1981; Di Chiro *et al.*, 1982; Cummins *et al.*, 1984).

In a previous paper (Galarraga *et al.*, 1986) we demonstrated a close correspondence between the maximum LCMRglc measured in gliomas *in situ* and the glucose consumption rates of cultured gliomas derived from these same tumors. Since the rates of glucose consumption *in situ* and *in vitro* were close despite the different milieux, we postulated that gliomas are altered genotypically, resulting phenotypically in an increased glucose uptake. We also asserted that the close correspondence between glucose uptake rates suggested that the tissue culture model was a valuable one, and thus we sought to use cultured gliomas to determine the metabolic lesion which results in elevated glucose uptake.

Analogous to gliomas, hepatoma cell lines can be isolated which express various dedifferentiated characteristics and rapid growth rates (Weber, 1977a, b). Glucose consumption is highest in the less differentiated and more rapidly growing cell lines; this is correlated with increased activities of hexokinase and phosphofructokinase (Weber, 1977b). Alterations in the levels or isozyme patterns of various glycolytic enzymes have been observed in gliomas by others (Perria *et al.*, 1964; Timperley, 1980; Lowry *et al.*, 1983; for a review, consult Wolleman, 1971).

We present data which suggest that tissue cultures derived from various gliomas lines show phenotypic changes in enzymes regulating glycolysis, which are consistent with the observed increased glycolytic rate.

MATERIALS AND METHODS

Cell Culture. Procedures for deriving cultured glioma cells from surgical materials and maintaining stock cultures are described elsewhere (Kornblith and Szypko, 1978; Galarraga *et al.*, 1986).

[5]Abbreviations used: LCMRglc, local cerebral metabolic rate for glucose: dbcAMP, dibutyryl cyclic AMP; F2,6 diP, fructose 2,6-diphosphate; G1,6 diP, glucose 1,6-diphosphate; G6PDH, glucose-6-phosphate dehydrogenase; HK, hexokinase; PFK, phosphofructokinase; PEP, phosphoenolpyruvate; DTT, dithiothreitol; BSA, bovine serum albumin; DHAP, dihydroxyacetone phosphate; GA3P, glyceraldehyde-3-phosphate.

Assay of Glycolytic and Pentose Phosphate Pathway Enzymes. Confluent cultures from one or more 75-cm^2 flasks were washed 2 × in SB buffer (Loreck *et al.*, 1987) cooled to 4°C. The monolayer was scraped with a policeman into an aliquot (usually 2 ml) of 50 m*M* sodium potassium phosphate buffer, pH 7.2. The cell suspension was transferred to an ice-cold glass Potter–Elvehjem type homogenizer with a tight-fitting Teflon pestle, and the cells were homogenized on ice with 20 strokes.

Enzyme assays were conducted directly in a Farrand Ratio-2 fluorometer under conditions where the product was lead by a fivefold excess of auxiliary enzymes to the oxidation or reduction of pyridine nucleotides. All assays are based on the efforts of Lowry and Passonneau (1972; Lowry *et al.*, 1964) modified slightly to accommodate the relatively low yields of tissue cultured cells. To begin each reaction, 10–100 μl of homogenates was added to 1.0 ml of reagent in 10 × 75-cm^2 borosilicate tubes. Details of buffers, auxiliary enzymes, substrate concentrations, and added protein content [as determined on homogenates by the methods of Lowry *et al.* (1951)] are described in Table I. Under the conditions described, enzymatic activities were linear with respect to time for at least 15 min and linear with respect to added protein content. Apparent activity in the absence of substrate (usually 10% or less of the observed rate) was taken to be the blank and was subtracted.

Hexokinase Binding. Confluent flasks of normal astrocytes or glioma-derived cultures were washed and scraped as above, and the cells resuspended in 2.0 ml of 50 m*M* Tris buffer, pH 8.1. Cells were homogenized by 50 strokes. To assess the degree of binding to the particulate fraction, 100- to 150-μl aliquots were incubated on ice in Beckman Airfuge tubes for 30 min. The tubes were then centrifuged for a further 30 min in a Beckman Airfuge at 4°C and 80,000*g*. Aliquots of the upper phase were carefully withdrawn and assayed for hexokinase activity as described in Table I. The pellet was resuspended in 50 μl of homogenization buffer by brief sonication (10 pulses, 20% duty cycle, power output 1 on a Bransonic sonicator fitted with a microtip). The particulate fraction was independently assayed for hexokinase as described in Table II. For the studies reported here, the sum of the hexokinase activities in the pellet and supernatant fraction always exceeded 85% of the activity of the homogenates.

Materials. Enzymes and substrates were purchased from Boehringer–Mannheim Co. or Sigma Chemical Co. Tissue cultures reagents were purchased from either Grand Island Biological Supply or MA Bioproducts. Tissue culture ware was manufactured by Costar Plastics or Falcon Plastics.

RESULTS

The activities of glycolytic enzymes and the regulatory enzymes of the pentose phosphate pathway in cultured normal human cortical astrocytes and one low-grade (II) and two high-grade (IV) glioma-derived lines are shown in Table II. Consistent and statistically significant differences between normal human cortical astrocytes and high-grade glioma-derived cell lines are seen for only three enzymes: hexokinase (HK), phosphofructokinase (PFK), and glucose-6-phosphate dehydrogenase (G6PDH). HK and PFK are elevated in grade IV glioma-derived lines by 1.5 to 1.9-fold and 4.5 to 7.5-fold, respectively. G6PDH is decreased in both high-grade

Table I. Method of Fluorometric Assay for Glycolytic Enzymes

Enzyme	Buffer	Substrate	Auxiliary enzyme	Protein (μg)
Hexokinase	50 m*M* Tris, pH 8.10 1 m*M* $MgCl_2$ 300 μ*M* ATP 3 μ*M* $NADP^+$	Glucose, 1 m*M*, or 2-deoxy-*d*-glucose	Glucose-6-phosphate dehydrogenase, 0.2 U/ml	3–100
Phosphoglucoisomerase	As above 0.5 m*M* DTT	Fructose-6-phosphate, 1 m*M*	As above	2–20
Phosphofructokinase	50 m*M* imidazole, pH 7.0 150 m*M* K acetate 15 μ*M* NADH 0.01% BSA	As above ATP, 1 m*M*	Glycerol-3-phosphate dehydrogenase, 0.3 U/ml Aldolase, 0.1 U/ml Triose phosphate isomerase, 10 U/ml	3–25
Aldolase	130 m*M* imidazole 1 m*M* $MgCl_2$ 5 m*M* mercaptoethanol 1 m*M* EDTA 1 m*M* Na_2HASO_4 30 μ*M* NADH	Fructose-1,6-diphosphate, 1 m*M*	Triose phosphate isomerase, 1.25 U/ml Glyceraldehyde-3-phosphate dehydrogenase, 0.4 U/ml	2–10
Triose phosphate isomerase	50 m*M* imidazole, pH 7.6 10 μ*M* NADH	Glyceraldehyde-3-phosphate, 1 m*M*, 0.84 U/ml	Glycerol-3-phosphate dehydrogenase	0.3–2.0
Triose phosphate isomerase	150 m*M* imidazole, pH 7.6 1 m*M* $MgCl_2$ 5 m*M* mercaptoethanol 1 m*M* EDTA 1 m*M* Na_2HASO_4 30 μ*M* NADH	Dihydroxyacetone phosphate dehydrogenase, 1 m*M*	Glyceraldehyde-3-phosphate	As above
Glyceraldehyde-3-phosphate dehydrogenase	As above	Glyceraldehyde-3-phosphate, 1 m*M*	None	3–70

Phosphoglyceromutase	50 m*M* imidazole, pH 7.0 75 m*M* KCl 2 m*M* $MgCl_2$ 1 m*M* ADP 10 μ*M* NADH	3-Phosphoglycerate, 1 m*M*	Enolase, 0.01 U/ml Pyruvate kinase, 0.2 U/ml LDH, 0.6 U/ml	0.2–5
Enolase	As above	2-Phosphoglycerate, 1 m*M* LDH, 0.6 U/ml	Pyruvate kinase, 0.2 U/ml	1–9
Pyruvate kinase	As above	Phosphoenol pyruvate, 1 m*M*	LDH, 0.6 U/ml	0.4–35
Lactate dehydrogenase	50 m*M* imidazole, pH 7 1 m*M* $MgCl_2$ 10 μ*M* NADH	Pyruvate, 1 m*M*	None	0.02–0.3
Glucose-6-phosphate dehydrogenase	50 m*M* Tris, pH 8 1 m*M* $MgCl_2$ 0.5 m*M* DTT 50 μ*M* $NADP^+$	Glucose-6-phosphate, 1 m*M*	None	0.1–5
6-Phosphogluconate dehydrogenase	40 m*M* Tris, pH 8 0.1 m*M* EDTA 30 m*M* NH_4 acetate 5 m*M* $MgCl_2$ 30 μ*M* $NADP^+$	6-Phosphogluconate, 1 m*M*	None	1.5–27

[a] Enzymes were measured directly in 1.0 ml (final volume) directly in the fluorometer. Protein (whole homogenates) was measured as described under Materials and Methods. Under these conditions, the activity was linear with respect to time and volume of added homogenate. Blanks consisted of substrate-free reaction mixtures; the observed activity in the absence of substrate was usually less than 10% of the total and was subtracted.

Table II. Activities of Key Enzymes of Glucose Metabolism in Normal Astrocytic Cultures and Low-Grade and High-Grade Cultures[a]

Enzyme	nmol/min mg protein ± SE			
	Normal astrocytes	SCH-II	SAN-IV	MILL-IV
Hexokinase	9.8 ± 1.1	6.6 ± 0.6	18.6 ± 2.5[b]	15.1 ± 1.8[b]
Phosphoglucosiomerase	122.5 ± 17.6	28.2 ± 3.0[b]	875.9 ± 47[b]	60.7 ± 11.5
Phosphofructokinase	0.8 ± 0.4	0.7 ± 0.2	3.6 ± 0.4[b]	6.0 ± 0.8[b]
Aldolase	11.3 ± 1.5	7.8 ± 0.7	9.2 ± 0.3	5.2 ± 0.7[b]
Triose phosphate isomerase (DHAP)	142.2 ± 19.9	84.2 ± 6.4	67.1 ± 12.8	—[c]
Triose phosphate isomerase (GA3P)	1267.9 ± 99	1037.2 ± 45	802.6 ± 67	95.4 ± 11.6[b]
Glycerol-3-phosphate dehydrogenase	0.5	0.5	0.6	0.5
Glyceraldehyde-3-phosphate dehydrogenase	4.7 ± 0.7	5.5 ± 1.1	8.6 ± 0.9	4.2 ± 0.9
Phosphoglyceromutase	95.8 ± 7.8	169.9 ± 14.6[b]	76.8 ± 3.5	128.5 ± 8.2
Enolase	92.5 ± 5.0	201.4 ± 8.1	125.4 ± 5.6[b]	73.4 ± 3.7[b]
Pyruvate kinase	107.3 ± 3.4	45.5	57.0 ± 3.8[b]	310.3 ± 17.7[b]
Lactate dehydrogenase	964.5 ± 80.0	2002.4 ± 59.2[b]	134.4 ± 48.5	231.4 ± 7.9[b]
Glucose-6-phosphate dehydrogenase	195.0 ± 13.1	115.5 ± 6.0[b]	18.1 ± 2.2[b]	17.0 ± 1.4[b]
6-Phosphogluconate dehydrogenase	4.7 ± 0.3	4.7 ± 0.2	9.0 ± 0.1[b]	3.3 ± 0.1[b]

[a]Enzymes were measured in duplicate on three or more confluent flasks of normal astrocytes or low-grade (II)- or high-grade (IV)-derived glioma lines. Triose phosphate isomerase was measured in both directions, using dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GA3P) as substrates.
[b]Statistical evaluation was done by the Student t test. Significant at $P < 0.05$ compared to the normal astrocytes.
[c]Not done.

glioma-derived lines by more than 90% compared to normal human cortical astrocyte cultures. For all other enzymes, no consistent or significant grade-dependent changes were observed. In some cases (e.g., phosphoglucoisomerase, pyruvate kinase, and 6-phosphogluconate dehydrogenase), statistically significant differences were seen when each line was compared to the normal human cortical astrocyte cultures, but the levels seemed to reflect individual differences between specific cell lines and were not apparently related to the pathological grade.

In the low-grade glioma-derived lines, G6PDH was altered in the same direction as in the high-grade glioma-derived lines, but to a lesser extent. HK activity in the low-grade glioma-derived line was somewhat less than that of the normal human cortical astrocyte homogenates, and the phosphoglyceromutase and enolase were increased 1.7- and 2.2-fold, respectively.

In the normal human cortical astrocyte cultures and in the glioma-derived cultures, PFK had the lowest specific activity of all the glycolytic enzymes, followed by glyceraldehyde-3-phosphate dehydrogenase and HK. In all cases, the specific activity of HK was greater than that of PFK.

HK and PFK are thought to be the regulatory enzymes of brain glycolysis (Lowry *et al.*, 1964) and the glucose flux rates in brain are substantially less than the observed V_{max} values for these enzymes (Lowry and Passonneau, 1964). One possible

Table III. Kinetic Parameters of Hexokinase from Normal Astrocyte Cultures and Low- and High-Grade-Derived Cultures[a]

Line	Glucose			2-Deoxyglucose		
	K_m[b]	V_{max}[c]	V_{max}/K_m	K_m[b]	V_{max}[c]	V_{max}/K_m
Normal astrocytes	0.07	7.7	109.8	0.6	1.37	2.28
SCH-II	0.08	8.0	107.1	0.5	3.0	5.5
SAN-IV	0.08	17.6	228.5	—	—	—
BER-IV	—	—	—	0.3	5.2	17.4
MIL-IV	0.10	18.3	182.5	0.3	3.74	12.5

[a]The kinetic constants for hexokinase in crude homogenates were determined as described under Materials and Methods. ATP concentration was 1 mM. Glucose and 2-deoxyglucose were varied from 0.1 to 10 × K_m. Each progress curve was fit by linear regression; the r value was 0.95 for each curve.
[b]Units, mM.
[c]Units, nmol/min/mg protein.

interpretation is that HK and PFK operate below their respective V_{max} values either due to substrate availability (e.g., glucose transport) or due to inhibition by allosteric means. Since HK and PFK have low specific activities in normal human cortical astrocyte cultures and in glioma-derived lines, it seems likely that these enzymes regulate glycolytic flux as well. Thus, we chose to examine the properties of HK and PFK in greater detail.

The regulation of the flux through the pentose phosphate pathway and its control by G6PDH are treated in the following paper (Loreck *et al.*, 1987).

Table III shows the kinetic characteristics of HK measured in crude homogenates with glucose and 2-deoxy-*d*-glucose as substrates. The K_m values for glucose are very similar for the normal human cortical astrocyte cultures, the low-grade glioma-derived lines, and the high-grade glioma-derived line. The V_{max} values exhibit the same relative activities for the various lines as shown in Table II. The K_m values for 2-deoxy-*d*-glucose are increased seven fold in both the normal human cortical astrocyte cultures and the low-grade glioma-derived lines but by no more than threefold in the grade (IV) glioma-derived lines. Furthermore, the V_{max} values of the HK from the high-grade glioma-derived lines indicate that 2-deoxy-*d*-glucose is not a simple competitive inhibitor of glucose. This complicated kinetic picture is not likely the result of feedback inhibition of 2-deoxy-*d*-glucose-6-phosphate, since under the conditions of the assay the steady-state concentration of 2-deoxy-*d*-glucose-6-phosphate can be calculated (Lowry and Passonneau, 1972) to be less than 20 μM.

The differences in the kinetic properties imply that the HK of high-grade glioma-derived cultures is a different form from that found in either the low-grade glioma-derived line or the normal human cortical astrocyte cultures.

HK in brain is readily and reversibly bound to the outer membrane of the mitochondria (Long, 1952; Crane and Sols, 1953). In rodent brain, the bound form has a lower K_m and a higher apparent V_{max} for glucose than the cytosolic form (Bachelard, 1967). Transition between compartments is promoted by nucleotide phosphates, hexoses, hexose phosphates (Wilson, 1978), and salts (Felgner and Wilson, 1977). The particulate binding of HK in high-grade glioma-derived lines is shown in Table IV. The percentage bound in the absence of agent (90%) is close to

Table IV. Mitochondrial Binding of Hexokinase in MIL-IV[a]

Addition	Supernatant (nmol/min/mg protein)	% in supernatant
None	2.08	10.9
ATP	2.66	20.0
ADP	3.16	24.0
AMP	0.07	0.5
Dibutyryl cAMP	3.29	25.0
1-*O*-Methylglucose	3.3	25.0
Sorbitol-6-P	3.04	23.0

[a]Binding of hexokinase was done as described under Materials and Methods. The final concentration for each agent was 1.0 mM. HK activity and protein content were measured in the supernatant and particulate (after resuspension) fractions. Recovery was always greater than 85%. Each value represents the mean of two or more separate cultures.

Table V. Kinetic Constants of Cytosolic and Particulate Hexokinase[a]

Condition	K_m (mM)	V_{max} (nmol/min/mg protein)
Homogenate	0.02	20.8
Supernatant	0.11	2.9
Particulate	0.03	25.9

[a]SAN-IV cells were homogenized and centrifuged as described under Materials and Methods. The supernatant and particulate fractions were separated, and the kinetic constants were determined on each as described under Materials and Methods and in Table III. The kinetic constants were determined by linear regression analysis of progress curves ($r = 0.98$). The results are from a typical experiment, consisting of duplicate determinations on 10 glucose concentrations.

that reported for rodent brain (Crane and Sols, 1953). Hexokinase is displaced from the particulate fraction by treatment with, in order of increasing potency, dibutyryl cyclic AMP, 1-*O*-methylgucose, ADP, sorbitol-6-phosphate, and ATP. The kinetic constants for the free and bound and forms are shown in Table V. In the particulate form the K_m is decreased by 73% and the V_{max} is increased by 125%, as compared to the crude homogenate. Assuming that in the resting state (1) 90% of the hexokinase is bound, (2) the intracellular concentration of glucose approaches the extracellular concentration, and (3) the enzyme obeys Michaelis–Menten kinetics in the cell, the calculated maximum flux through HK could approach 25 nmol/min/mg protein, a value substantially in excess of the observed value of 1.38 nmol/min/mg protein for the SAN-IV cell line (Galarraga *et al.*, 1987). These calculations imply that under the conditions that we presume operate in the cultured glioma cell, the HK is inhibited $100 - [(1.38/25) \times 100]$ or approximately 95%, which is close to the predicted value of HK inhibition observed in mouse brain (Lowry and Passonneau, 1964). In order for the degree of HK binding to regulate glucose flux, one can calculate that the bound form cannot exceed a few percent of the total, substantially less than the observed value of 90%.

Although the availability of normal human cortical astrocyte cultures has prevented us from examining the HK binding in comparable detail, HK

compartmentation does not appear to differ substantially from that of the glioma-derived lines. HK appears to be 80–90% bound under control conditions, and it is displaced an additional 10% after treatment with 3.3 m*M* CTP. Thus mitochondrial binding may not play a direct role in regarding glycolytic flux in either normal astrocytes or glioma-derived lines. We conclude that the measured HK activity in the high-grade glioma-derived lines and in normal human cortical astrocyte cultures is far in excess of the flux rates, and the disparity cannot be accounted for by reversible compartmentation. Hexokinase activity either is inhibited allosterically or is flux-limited at some "downstream" step.

PFK is a highly regulated allosteric enzyme. At present, its major known activators include AMP, dibutyryl cyclic AMP (dbcAMP) fructose 2,6-diphosphate (F2,6 diP), glucose 1,6-diphosphate (Gl,6 diP), and P_i; inhibitors include ATP, ADP, phosphoenolypyruvate (PEP), and citrate (Clark and Patten, 1980; Newsholme *et al.*, 1977; Foe and Kemp, 1982; Bosca *et al.*, 1982; Foe and Trujillo, 1980; Claus *et al.*, 1980; Pinella and Luque, 1981). To determine the effects of these physiological regulators on the activity of PFK, ligands were coincubated with normal astrocytic or SAN-IV homogenates for up to 30 min. The results are shown in Table VI.

The PFK from normal astrocytes is inhibited 71% by a fivefold increase in ATP. PFK is activated at both 1.0 and 5.0 m*M* ATP by ADP, AMP, PEP, F2,6 diP, and P_i. At both ATP concentrations, normal astrocytic PFK is inhibited by dbcAMP and by citrate. Gl,6 diP has a mixed effect, slightly stimulating PFK activity at 1.0 m*M* ATP but inhibiting at 5.0 m*M* ATP.

The PFK of the SAN-IV glioma-derived cell line is only slightly (28%) inhibited by a fivefold increase in ATP. No ligand significantly activates PFK at low ATP levels. At high ATP levels, slight (28%) activation is observed after treatment with ADP and AMP. The most effective stimulator at higher ATP levels is F2,6 diP (1.7-fold).

SAN-IV PFK is substantially inhibited by dbcAMP and PEP and moderately inhibited by Gl,6 diP at both ATP levels. Significant citrate inhibition is seen only at 5.0 m*M* ATP levels. AMP, F2,6 diP, and P_i showed no effect which was not in the range of experimental error.

The relative sensitivities of normal astrocytic or SAN-IV PFK toward each ligand can be evaluated by comparing the activity ratio (activity at 1.0 divided by activity at 5.0 m*M* ATP). A value close to 1.0 would indicate a relatively unregulated PFK. The normal astrocytic PFK responds to the various ligands in a manner which closely resembles that of skeletal muscle, liver, hepatocytes, heart, and brain (see Discussion). The activity ratios are greater than 1.0 for all ligands except PEP. For the SAN-IV cell line, the activity ratio is close to 1 for all ligands except citrate, indicating a PFK that is substantially dampened in its response to various allosteric regulators.

DISCUSSION

Rapidly dividing high-grade gliomas *in situ* have a LCMRglc 1.6-fold greater than that of normal contralateral brain (Cummins *et al.*, 1984). When these tumors are explanted into culture and cell lines are established, the characteristic absolute

Table VI. Effects of Allosteric Modulators on PFK Activity in Normal Astrocytes and Glioma-Derived Lines[a]

	Normal astrocytes					SAN-IV				
Ligand	[ATP]/ (1 m*M*)	%	[ATP] (5 m*M*)	%	[1 m*M* ATP]/ [5 m*M* ATP]	[ATP] (1 m*M*)	%	[ATP] (1 m*M*)	%	[1 m*M* ATP]/ [5 m*M* ATP]
Control	1.34	100	0.39	100	3.4	1.55	100	1.12	100	1.38
ADP, 1 m*M*	3.69	275	0.67	172	5.5	1.50	97	1.43	128	1.05
AMP, 1 m*M*	6.37	475	0.57	146	11.2	1.55	100	1.40	125	1.11
dbcAMP, 1 m*M*	0.97	72	0.01	1	(97)	0.40	26	0.93	83	0.43
PEP, 1 m*M*	1.66	124	1.70	436	1.0	0.01	1	0.01	1	(1)
F2,6 diP, 10 μM	6.97	507	2.0	513	3.5	1.70	110	1.90	170	0.89
G1,6 diP, 10 μM	1.52	113	0.01	1	(152)	1.33	86	1.02	91	1.30
Citrate, 1 m*M*	0.18	13	0.01	1	(18)	1.53	99	0.10	9	15.30
P_i, 1 m*M*	5.86	437	1.47	376	4.0	1.69	109	1.27	133	1.33

[a]The activity of PFK was determined as described in Table I and under Materials and Methods. Activity units, nmol/min/mg protein. These results are from a typical experiment: each value consists of triplicate measurements on two or more culture flasks. The final concentration of fructose-6-phosphate was 0.5 m*M* and the concentration of each allosteric modifier is shown. Rtios in parentheses are subject to uncertainty, since either the numerator or the denominator was low enough to be ambiguous.

rates of glucose uptake for each tumor appear to persist, even though a substantial variation in LCMRglc is observed from tumor to tumor, both *in situ* (DeLaPaz *et al.*, 1981; Di Chiro *et al.*, 1982) and *in vitro* (Galarraga *et al.*, 1987).

That glucose uptake rates can be correlated *in situ* and *in vitro* despite the very different milieux suggests that the process of neoplastic transition from glia to glioma is associated with a genotypic change, reflected phenotypically by alterations in the glycolytic pathway. This is certainly the case for other solid tumors, such as hepatomas (Weber, 1977a, b), which have increased hexokinase and phosphofructokinase activities. The maximal catalytic capacity of these enzymes is elevated in high-grade glioma-derived cell lines; the activities of other glycolytic enzymes are also altered, but to a smaller degree and with an inconsistency that implies no direct relationship to the neoplastic state.

Recently Lowry *et al.* (1983) compared the activities of several key enzymes of energy metabolism in glioblastomas to those in normal cortical samples, and they were able to show that the glioblastoma HK and PFK were decreased and G6PDH was increased. The activities reported by Lowry *et al.* (1983) for the glioblastoma series were within twofold of the activities reported here, but the levels of HK and PFK in the cortical samples were higher than our normal astrocytes by threefold or more. G6PDH activity in cortical samples, however, appears to be much lower than in our normal astrocytic cultures. Taken together, these results imply that astrocytes have, on a per mass basis, less HK and PFK activity but relatively more G6PDH activity than neuronal and nonglial cortical elements.

Metabolic control points are best identified by appropriate crossover studies, but until a complete crossover study is done on gliomas, one can only presume that, by analogy to brain (Lowry *et al.*, 1964), the major control points are hexokinase, phosphofructokinase, and perhaps pyruvate kinase. The studies by Kirsch (1965; Kirsch and Leitner, 1967) on the energy metabolism of human gliomas, while incomplete as crossover studies, are consistent with the control point hypothesized by Lowry *et al.* (1964). However, it is somewhat simplistic to infer that the increased glycolytic capacity of hexokinase and phosphofructokinase necessarily means increased flux rates. In fact, several glioma-derived lines exhibited a glucose metabolic rate which was less than that of the normal astrocytic cultures, implying that instantaneous flux rates are still controlled by allosteric means.

Hexokinase activity appears not to limit flux, since (1) the extent of mitochondrial binding does not appear to contribute to the regulation of flux rates; and (2) kinetic properties suggest that in normal astrocytes and low-grade and high-grade glioma-derived cell lines, the measured hexokinase activity is greater than the sum of the fluxes through glycolysis (Galarraga *et al.*, 1987), through the pentose phosphate pathway (Loreck *et al.*, 1987), or into glycogen (Galarraga *et al.*, 1987).

We have used the glucose analogue 2-deoxy-*d*-glucose to probe the kinetic properties of the hexokinases of the glioma-derived cell lines. The kinetic behavior of the enzyme toward this analogue is consistently different toward glucose, hinting at either a possible tumor-specific isozyme or an alteration in the structure of the tumor enzyme.

A much closer agreement of flux rate and enzymatic activity is seen if phosphofructokinase activity is measured in the presence of physiological concentrations of its

various allosteric modifiers. It is known that phosphofructokinase is tightly regulated in brain *in situ* (Lowry *et al.*, 1964a); in addition, the enzyme is subject to phosphorylation (Brand and Soling, 1975). Various isozymic forms have been reported for liver (Dunaway *et al.*, 1981) and in gliomas (Wollemann, 1971). Taken together, this evidence suggests that one critical step in neoplastic transition may involve modification or replacement of "differentiated" forms of phosphofructokinase with forms which can be less tightly regulated.

The PFK of most mammalian tissues (including brain) is inhibited by citrate, ATP, PEP, and ADP and activated by AMP, F2,6 diP, G1,6 diP, P_i, and cyclic AMP. The PFK of normal astrocytes corresponds to this picture, except for ADP and, to a slight extent, PEP, which are stimulatory. The degree of ATP inhibition is profound. Only the copresence of P_i, PEP, or F1,6 diP is adequate to reverse the ATP inhibition.

The PFK of SAN-IV cells exhibits an allosteric profile which differs extensively from that of normal astrocytes. ATP inhibition is slight, and the magnitude of activation is small, even in the presence of agents such as F2,6 diP, AMP, and P_i, which activate the normal astrocytic enzyme as much as fivefold. It is striking, however, that the measured PFK activity in the presence of the various allosteric modifiers is close to the observed glucose flux rates of normal astrocytes and glioma-derived lines. This suggests that the glycolytic rate is closely controlled by PFK activity and that PFK is operating under near-maximum conditions.

A consideration of the behavior of PFK aside, in virtually all aspects the normal astrocytes and the low-grade glioma-derived lines shared very similar properties. The high-grade glioma-derived lines were, on the whole, very different, exhibiting altered properties of glucose uptake (Galarraga *et al.*, 1987) and utilization of alternative carbon sources (J.G., D.J.L., and C.J.C., personal observations). The highly neoplastic character of high-grade glioma-derived lines is also demonstrated by the altered maximal catalytic capacities and kinetic properties of hexokinase and phosphofructokinase.

Our results imply that in high-grade glioma-derived lines (1) increased glycolytic capacity is observed at the hexokinase and phosphofructokinase steps; (2) the instantaneous flux appears to be closely regulated by allosteric means, primarily at the PFK step; and (3) the allosteric properties of hexokinase and phosphofructokinase differ from those of normal astrocytes, which in turn permit the observed increased glycolytic rate. While the mechanisms that permit altered regulation are not known, it is clear that, compared with normal astrocytes, the altered allosteric properties of gliomas make them less sensitive to certain physiological stresses, and thus they are capable of vastly increasing their anaerobic glycolytic rates. Taken together, these results suggest that glioma metabolism differs from that of brain in its flexibility, reliance on many energy sources, and resistance to metabolic insult.

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