Glucose Metabolism in the Brain

EDITED BY

DONARD S. DWYER

International Review of Neurobiology, Volume 51

International
REVIEW OF
Neurobiology

Volume 51

Glucose Metabolism

IN THE

Brain

International Review of Neurobiology

Volume 51

Glucose Metabolism IN THE Brain

EDITED BY

DONARD DWYER
Departments of Psychiatry and Pharmacology
LSU Health Sciences Center
Shreveport, Louisiana

ACADEMIC PRESS
An imprint of Elsevier Science
Amsterdam Boston London New York Oxford Paris
San Diego San Francisco Singapore Sydney Tokyo

Pub: Please supply copyright page.

CONTENTS

Insulin-Like Growth Factor-1 Promotes Neuronal Glucose Utilization During Brain Development and Repair Processes

CAROLYN A. BONDY AND CLARA M. CHENG

CNS Sensing and Regulation of Peripheral Glucose Levels

BARRY E. LEVIN, AMBROSE A. DUNN-MEYNELL, AND VANESSA H. ROUTH

Glucose Transporter Protein Syndromes

DARRYL C. DE VIVO, DONG WANG, JUAN M. PASCUAL, AND YUAN YUAN HO

Glucose, Stress, and Hippocampal Neuronal Vulnerability

LAWRENCE P. REAGAN

Glucose/Mitochondria in Neurological Conditions

JOHN P. BLASS

Energy Utilization in the Ischemic/Reperfused Brain

JOHN W. PHILLIS AND MICHAEL H. O'REGAN

Diabetes Mellitus and the Central Nervous System

ANTHONY L. MCCALL

Diabetes, the Brain, and Behavior: Is There a Biological Mechanism Underlying the Association between Diabetes and Depression?

ALAN M. JACOBSON, JACQUELINE A. SAMSON, KATIE WEINGER, AND CHRISTOPHER M. RYAN

Schizophrenia and Diabetes

DAVID C. HENDERSON AND ELISSA R. ETTINGER

Psychoactive Drugs Affect Glucose Transport and the Regulation of Glucose Metabolism

DONARD S. DWYER, TIMOTHY D. ARDIZZONE, AND RONALD J. BRADLEY

This Page Intentionally Left Blank

CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

Timothy D. Ardizzone (503), Departments of Psychiatry and Pharmacology, LSU Health Sciences Center, Shreveport, Louisiana 71130[1]

John P. Blass (325), Dementia Research Service, Burke Medical Research Institute, Weill Medical College of Cornell University, White Plains, New York 10605

Carolyn A. Bondy (189), Developmental Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892

Ronald J. Bradley (503), Departments of Psychiatry, LSU Health Sciences Center, Shreveport, Louisiana 71130

Clara M. Cheng (189), Developmental Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892

Darryl C. De Vivo (259), Department of Pediatric Neurology, Colleen Giblin Research Laboratories for Pediatric Neurology, Columbia Presbyterian Medical Center, New York 10032

Gerald A. Dienel (1), Department of Neurology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205

Ambrose A. Dunn-Meynell (219), Neurology Service, VA Medical Center, East Orange, New Jersey 07018 and Department of Neurosciences, New Jersey Medical School, Newark, New Jersey 07103

Donard S. Dwyer (159, 503), Departments of Psychiatry and Pharmacology, LSU Health Sciences Center, Shreveport, Louisiana 71130

Elissa R. Ettinger (481), Schizophrenia Program, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114

Kurt Heininger (103), Department of Neurology, Heinrich Heine Universität, D-40597 Düsseldorf, Germany

David C. Henderson (481), Schizophrenia Program, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114

Leif Hertz (1), Hong Kong DNA Chips, Ltd., Kowloon, Hong Kong, China

[1]Current affiliation: Department of Neurology, Vontz Center for Molecular Studies, University of Cincinnati Medical Center, Cincinnati, Ohio 45267.

Yuan Yuan Ho (259), Department of Neurology, Colleen Giblin Research Laboratories for Pediatric Neurology, Columbia Presbyterian Medical Center, New York 10032

Alan M. Jacobson (455), Joslin Diabetes Center, Harvard Medical School, Boston, Massachusetts 02215

Barry E. Levin (219), Neurology Service, VA Medical Center, East Orange, New Jersey 07018 and Department of Neurosciences, New Jersey Medical School, Newark, New Jersey 07018

Anthony L. McCall (415), James M. Moss Professor in Diabetes in Internal Medicine, University of Virginia School of Medicine, Division of Endocrinology & Metabolism, Department of Internal Medicine, UVA Health System, Charlottesville, Virginia 22908

Michael H. O'Regan (377), Biomedical Sciences, School of Dentistry, University of Detroit Mercy, Detroit, Michigan 48219

Juan M. Pascual (259), Department of Neurology, Colleen Giblin Research Laboratories for Pediatric Neurology, Columbia Presbyterian Medical Center, New York 10032

John W. Phillis (377), Department of Physiology, Wayne State University School of Medicine, Detroit, Michigan 48201

Lawrence P. Reagan[2] (289), Laboratory of Neuroendocrinology, The Rockefeller University, New York 10021[1]

Vanessa H. Routh (219), Department of Pharmacology and Physiology, New Jersey Medical School, Newark, New Jersey 07103

Christopher M. Ryan (455), Harvard Medical School, Joslin Diabetes Center, Boston, Massachusetts 02215[3]

Jacqueline A. Samson (455), McLean Hospital, Harvard Medical School, Boston, Massachusetts 02215

Ian A. Simpson (159), Department of Neuroscience and Anatomy, Penn State University College of Medicine, Hershey, Pennsylvania 17033

Susan J. Vannucci (159), Departments of Pediatrics and Neuroscience and Anatomy, Penn State University College of Medicine, Hershey, Pennsylvania 17033

Dong Wang (259), Department of Neurology, Colleen Giblin Research Laboratories for Pediatric Neurology, Columbia Presbyterian Medical Center, New York 10032

Katie Weinger (455), Joslin Diabetes Center, Harvard Medical School, Boston, Massachusetts 02215

[2]Current affiliation: Department of Pharmacology, Physiology, and Neuroscience, University of South Carolina School of Medicine, Columbia, South Carolina 29208.

[3]Current affiliation: Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, 15213.

PREFACE

Glucose is an essential energy source for the adult human brain. When blood glucose levels fall by half from a normal fasting value (to about 2–3 mM), there is some cognitive impairment and, at glucose levels below 1 mM, mental confusion is evident and coma may result from sustained glucose deprivation. Recent studies have demonstrated that optimum neuronal function and even cell survival are determined by the efficiency of energy metabolism. Thus, cells that use glucose as their primary energy source are less susceptible to stress and harmful conditions than cells that use other energy sources or that exhibit higher rates of glycolysis. Although our knowledge about the critical role of glucose metabolism in the maintenance of high level brain function has grown considerably in recent years, the various factors that regulate glucose uptake and utilization in the CNS are not well understood. Moreover, the brain must regulate the relative use of glucose, glutamine, and ketone bodies for energy under normal circumstances and especially during development and aging.

The regulation of glucose metabolism in humans is a complex process that proceeds via a two-way street connecting the brain and peripheral tissues. The CNS monitors glucose levels and exerts indirect control over both the utilization and production of glucose via hormone secretion and innervation. Peripheral tissues, including muscle, fat, pancreas, and liver, are responsible for direct control over the transport, synthesis, storage, and metabolism of glucose. Specific signals are conveyed back to the brain and nonspecific information, such as the overall metabolic state, can produce a significant impact on brain function. For example, injury to brain tissue in stroke is much more extensive in patients with hyperglycemia or diabetes as compared to patients with normal levels of blood glucose. Similarly, tardive dyskinesia, a serious movement disorder induced by neuroleptic drugs, occurs more frequently in patients with elevated blood glucose levels. Furthermore, a number of neurologic and psychiatric conditions are associated with alternations in energy metabolism in both the brain and peripheral tissues. Research efforts aimed at providing insights into the relationship between glucose metabolism and brain dysfunction should be a high priority.

The purpose of this volume is to bring together the latest research related to both basic and clinical aspects of glucose metabolism in the brain. The first two chapters by Drs. Hertz and Dienel and Dr. Heininger cover

general aspects of energy metabolism in the CNS, including the use of alternative sources of energy and the evolutionary basis for these metabolic processes. Drs. Dwyer, Vannucci, and Simpson provide an update on glucose transporter proteins that are expressed in the brain in relation to the regulation of glucose uptake. Drs. Bondy and Cheng discuss the important role of insulin-like growth factors in neuronal development and neuroprotection. The mechanisms by which the brain senses glucose and integrates other metabolic signals are summarized by Drs. Levin, Dunn-Meynell, and Routh. The clinical significance of glucose transport and glucose transporter proteins is elegantly illustrated in the chapters by Dr. De Vivo and colleagues (on glucose transporter deficiencies) and by Dr. Reagan (on the effects of stress in the CNS). The next three chapters describe neurological aspects of energy metabolism specifically, the role of mitochondria in a variety of disorders (Dr. Blass), the interrelationship between ischemic injury and glucose metabolism (Drs. Phillis and O'Regan), and the clinical implications of diabetes for brain function (Dr. McCall). The final three chapters concern the interface between behavioral disorders and glucose metabolism. Dr. Jacobson and colleagues describe various associations between major depression and diabetes, Drs. Henderson and Ettinger summarize the evidence for biological connections between schizophrenia and abnormal glucose metabolism, and last, Drs. Dwyer, Ardizzone, and Bradley discuss the drugs used to treat various psychiatric disorders in relation to their effects on glucose transport. In each chapter, there is special emphasis on possible future directions for research on the various topics covered here.

I wish to thank Dr. Noelle Gracy and others at Academic Press for their encouragement and support for this project. I also want to thank all of the contributors who ultimately made this volume possible. Tremendous creative energy was harnessed for this effort and the finished product represents a state-of-the-art summary of the field that will serve as an indispensable reference for many years to come. Finally, to the reader, I express our collective appreciation for your interest in this area of research and I hope that this information stimulates further exchange of ideas, challenges to the prevailing dogma, and the design of additional experiments that take the field to new vistas.

Donard S. Dwyer

ENERGY METABOLISM IN THE BRAIN

Leif Hertz

Hong Kong DNA Chips, Ltd., Kowloon, Hong Kong, China

Gerald A. Dienel

Department of Neurology, University of Arkansas for Medical Sciences
Little Rock, Arkansas 72205

0074-7742/02 $35.00

I. Introduction

Studies of glucose metabolism in the brain reflect a dichotomy due to the fact that the complex, integrating functions of the brain can only be studied in the intact, functioning brain in the conscious individual (human or animal), whereas properties of brain cells, cell-cell interactions, and mechanisms are most readily evaluated *in vitro* under controlled conditions using brain slices, subcellular fractions or purified, isolated cells of different types. *In vivo* studies have most commonly been done in studies with labeled 2-deoxy-D-glucose (DG) or 2-fluorodeoxyglucose (FDG). "Imaging" with DG revolutionized investigations of correlations between brain function and brain metabolism (Sokoloff *et al.,* 1977), because this glucose analog enables local functional analysis of hexokinase activity *in vivo,* from which local rates of glucose utilization can be calculated under steady-state conditions. On the other side, it is becoming overwhelmingly clear that such studies represent only one aspect of brain function, i.e., the "big picture," identifying the pathways and magnitude of functional metabolic activities; the underlying contributions of different cells and cell types in the brain are not identified and quantified, and the character of the energy-requiring processes are not determined. Brain cells can behave metabolically in very different manners in response to various stimuli and interact so that one cell type may generate a glucose metabolite (e.g., glutamate or lactate), which then undergoes "metabolic trafficking" to sustain function, to be further metabolized in a different cell type, or even to leave the activated area. These heterogeneous interactions have the consequence that imaging of overall brain metabolism cannot provide a picture of glucose metabolism at the cellular level.

A variety of *in vitro* methods have been used to assess metabolic activities in different brain cell types and in subcellular structures. Apart from the difficulty that these methods provide no direct information about metabolic activities in the functioning brain *in vivo,* they are almost all encumbered with potential methodological problems. Immunohistochemical studies of enzymes and substrate carriers in intact brain tissue have given much useful information, but with one notable exception (see SectionII.D.1), they *only* provide information about the amount of enzyme or transporter protein, *not* about the dynamic, condition-dependent activity of the enzyme, or the transporter. Information about enzyme and transporter activities in different cell types can be obtained in cellularly homogenous preparations, today most often cultured cells, derived from immature tissues, but differentiating during the culturing. Well-differentiated primary cultures of neurons and astrocytes have similar rates of oxidative metabolism and similar contents of adenine nucleotides as the brain *in vivo* (Hertz and Peng, 1992a; Silver and Erecinska, 1997). However, tissue culture methodology has the potential

source of error that the cultured cells may differ in metabolic characteristics from their *in vivo* counterparts, in part because of the very feature that makes them attractive for metabolic studies, namely their homogeneity and ensuing lack of cellular interactions and exposure to a temporal sequence of trophic factors, known to play critical roles in the development of the central nervous system (CNS). This source of error does not apply to preparations of different cell types or subcellular fractions obtained by dissociation of intact brain tissue followed by gradient centrifugation, but the resulting cellular or subcellular (e.g., synaptosomes, mitochondria) fractions have been rendered ischemic (i.e., exposed to severe energy failure and accompanying autolytic processes from which they may never fully recover), removed from their natural surrounding, and physically damaged, especially in older studies, due to exposure to more or less harsh treatment during their isolation. Accordingly, these preparations as well as brain slices show lower metabolic activities and contents of ATP than intact brain (Hertz and Schousboe, 1986), although the ATP/ADP ratio in carefully prepared synaptosomal preparations approaches that in the brain (Erecinska *et al.,* 1996), suggesting the presence of a functional, metabolically intact component.

Nevertheless, by combining different methodologies and continuously maintaining the *in vivo* situation as the general standard to which results obtained with different cellular and subcellular techniques must be compared, a picture of cellular interactions in glucose metabolism has emerged, and information has been obtained about the identity of energy-requiring and energy-yielding processes. Perhaps even more importantly, these studies have triggered the development of *in vivo* methods, primarily utilizing nuclear magnetic resonance imaging and spectroscopy, which have confirmed and further expanded many observations made *in vitro.* In this review, we will first discuss pathways and regulation of glucose metabolism in the functioning brain in the conscious human or animal during rest and during stimulation; this will be followed by a description of mechanisms which increase glucose metabolism *in vitro.* Combination of these two approaches allows a tentative determination of not only the quantitative contributions to glucose metabolism by some of the major cell types, but also identification of mechanisms creating a demand for metabolically generated energy and their relationships to functional activation and neurotransmission.

II. Pathways and Regulation of Glucose Utilization

A. Oxidative and Nonoxidative Metabolism

Metabolism of glucose is tightly regulated to generate ATP and provide carbon for biosynthetic reactions in conjunction with local functional

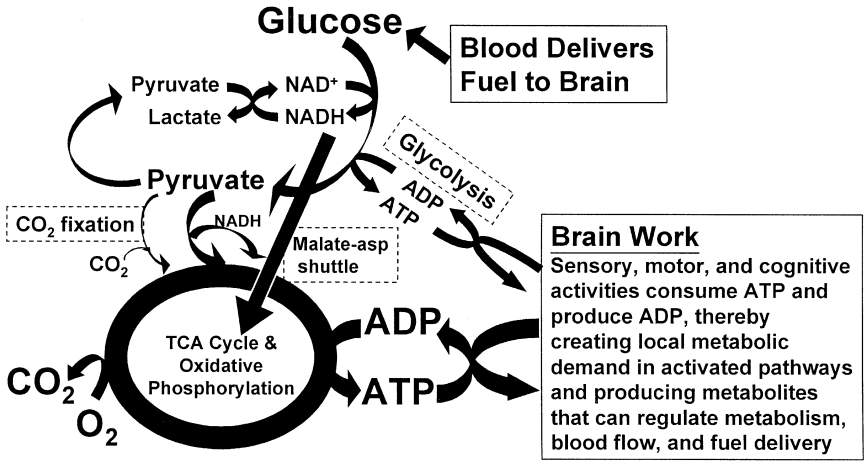

FIG. 1. ATP-ADP cycling links brain function and glucose metabolism. Functional tasks activate neuronal signaling and consumption of neuronal and glial ATP, thereby stimulating glucose utilization (CMR_{glc}) in specific brain structures. By-products of metabolism stimulate local blood flow to increase local delivery of glucose and oxygen. Cytoplasmic NADH is oxidized via lactate dehydrogenase and/or the malate-aspartate (asp) shuttle (see Fig. 4 and text), depending on conditions in the cell. Both the glycolytic pathway (glucose to pyruvate) and pyruvate oxidation in the tricarboxylic acid (TCA) cycle generate ATP for working brain. The glycolytic pathway can be rapidly activated, whereas the TCA cycle has the highest energy yield (see Fig. 2). (Adapted from G. A. Dienel. Energy generation in the central nervous system. *In* "Cerebral Blood Flow and Metabolism, 2nd ed." (L. Edvinsson and D. Krause, eds.), 2002, Lippincott Williams & Wilkins.©)

activities of the brain (Fig. 1). The catabolic process has nonoxidative (glycolytic) and oxidative components, and branch points can divert a portion of the glucose carbon from energy production toward other uses. Oxidative metabolism of pyruvate via the tricarboxylic acid (TCA) cycle produces ATP in high yield via the electron transport system and links bioenergetics to the large amino acid pools. In whole brain at steady state >90% of the glucose is oxidatively degraded as can be concluded from a ratio between rates of utilization of glucose (CMR_{glc}) and of oxygen (CMR_{O_2}) of at least 5.5, which is close to the theoretically expected ratio of 6. In the resting (i.e., not specifically stimulated) human brain, CMR_{glc} is 0.3 μmol/min/g wet wt., compared to 0.7 μmol/min/g wet wt. in the rat brain (Sokoloff, 1986).

B. GLYCOLYSIS

1. *Glycolytic Pathway*

Glucose enters the cytoplasmic compartment of brain cells from a capillary or the extracellular space via an equilibrative glucose transporter.

Glucose breakdown takes place in "stages," beginning with its phosphorylation at the C6 position by hexokinase, metabolically "primed" by hydrolysis of one molecule of ATP. Most glucose-6-phosphate (glucose-6-P) is converted to pyruvate (Table I), but glucose-6-P can be diverted from the glycolytic pathway by entry into the pentose-P shunt pathway to produce NADPH and five-carbon compounds, or it can be converted to glucose-1-P and utilized for synthesis of glycogen, galactose, glycoprotein, and glycolipids (Fig. 2; see color insert). Myo-inositol is also synthesized from glucose-6-P and serves as the precursor for the phosphatidylinositide signaling molecules. The second glycolytic step, formation of fructose-6-P also produces a branch point product for biosynthetic pathways; small quantities are converted to mannose-6-P (for synthesis of fucose, and complex carbohydrates via GDP-mannose) or glucosamine-6-P (a precursor for sialic acid). Thus, the initial phase of glucose metabolism requires ATP to "prime" each glucose molecule, and the first two metabolic steps yield "branch point" metabolites that are precursors for important but quantitatively minor metabolic pathways.

The controlling and most highly regulated reaction of the glycolytic pathway is the second ATP-dependent phosphorylation to fructose-1,6-bisphosphate (fructose-1,6-P_2) carried out by 6-phosphofructo-1-kinase (Passonneau and Lowry, 1964), the activity of which is governed by many downstream metabolites (see the next section). Formation of fructose-1,6-P_2 is followed by splitting of the 6-carbon compound into two triose phosphates (triose-P), dihydroxyacetone-phosphate (dihydroxyacetone-P), and glyceraldehyde-phosphate (glyceraldehyde-P). This sets the stage for a series of oxidation-reduction reactions that generate cytoplasmic NADH and ATP. Of the two triose phosphates, only glyceraldehyde-P is oxidized, but new glyceraldehyde-P is generated from dihydroxyacetone-P, catalyzed by triose-P isomerase. Glyceraldehyde 3-P dehydrogenase produces 1,3-bisphosphoglycerate plus NADH, which must be reoxidized to NAD^+, either via the malate-aspartate shuttle (MAS) and associated with generation of ATP (Section II.E.1), or by conversion of equimolar amounts of pyruvate to lactate, without any ATP synthesis (Section II.E.3). Two molecules of ATP per molecule of glucose are generated by the next step which is carried out by phosphoglycerate kinase. The 3-P-glycerate undergoes a mutase reaction to shift the phosphate group to the two position, followed by dehydration by enolase to form phosphoenolpyruvate (PEP); conversion of PEP to pyruvate by pyruvate kinase produces two more molecules of ATP per molecule glucose. Pyruvate is also a branch point metabolite (Fig. 2); it can either (1) enter mitochondria for conversion to acetyl-CoA and serve as substrate for oxidative metabolism or biosynthesis of fatty acids or acetylcholine; (2) be reduced to lactate in the cytosol for later oxidation and/or export from the cell; (3) be converted to alanine by transamination; or (4) be converted to oxaloacetate in the

TABLE I
ENZYMATIC STEPS OF THE GLYCOLYTIC PATHWAY

Sequential enzymatic step	Reaction[a]	Maximal velocity[b] (μmol min^{-1} g wet wt^{-1})	
		Mouse	Human
1. Hexokinase	Glucose + ATP → glucose-6-P + ADP	11	4
2. Phosphohexose isomerase	Glucose-6-P ↔ fructose-6-P	55	58
3. 6-Phosphofructo-1-kinase	Fructose-6-P + ATP → fructose-1,6-P_2 + ADP	9	1
4. Aldolase	Fructose-1,6-P_2 ↔ glyceraldehyde-3-P + dihydroxyacetone-P	5	4
5. Triose phosphate isomerase	Dihydroxyacetone-P ↔ glyceraldehyde-3-P		747
6. Phosphoglyceraldehyde dehydrogenase	2Glyceraldehyde-3-P + 2P_i + 2NAD^+ ↔ 2glycerate-1,3-P_2 + 2NADH	52	2
7. 3-Phosphoglycerate kinase	2Glycerate-1,3-P_2 + 2ADP + 2P_i → 2glycerate-3-P + 2ATP	167	
8. Phosphoglyceromutase	2Glycerate-3-P ↔ 2glycerate-2-P	39	41
9. Enolase	2Glycerate-2-P ↔ 2phosphoenolpyruvate + 2H_2O	30	
10. Pyruvate kinase	2Phosphoenolpyruvate + 2ADP + 2P_i → 2pyruvate + 2ATP	118	70
Net reaction	Glucose + 2ADP + 2NAD^+ + 2P_i → 2pyruvate + 2ATP + 2NADH		
Cytoplasmic oxidation of NADH by lactate dehydrogenase	2NADH + 2pyruvate ↔ 2NAD^+ + 2lactate	59	66
Mitochondrial oxidation of NADH[c]	2NADH + O_2 + 4-6ADP + 4-6P_i → 2NAD^+ + 4-6ATP + 2H_2O		

[a] Reactions do not include hydrogen ions.

[b] Rate data from mouse brain were compiled by McIlwain and Bachelard (1985); values from human brain were calculated from data summarized by Sheu and Blass (1999), assuming 100 mg protein (g brain tissue)$^{-1}$. Note that maximal velocities of all steps in the glycolytic pathway greatly exceed the average rate of glucose utilization, i.e., about 0.7 μmol g^{-1} min^{-1} in rat brain and about 0.3 μmol g^{-1} min^{-1} in human brain (Sokoloff, 1986, 1996), demonstrating very high capacity to increase fuel consumption with an abrupt rise in energy demand.

[c] Either 2 or 3 ATP can be formed from each cytoplasmic NADH, depending on the shuttle system that brings the reducing equivalents into the mitochondria. The glycerol-3-P shuttle activity is low in brain, and provides electrons at the level of $FADH_2$, with a total yield of 4 ATP. The malate shuttle is predominant, and transfers electrons to mitochondrial complex 1, yielding a total of 6 ATP (see text). (Modified from G. A. Dienel. Energy generation in the central nervous system. *In* "Cerebral Blood Flow and Metabolism, 2nd ed." (L. Edvinsson and D. Krause, eds.), 2002, Lippincott Williams & Wilkins.©)

mitochondria by pyruvate carboxylase. The moment-to-moment energy status of the cell, tissue oxygen level, relative fluxes of the glycolytic pathway and tricarboxylic acid cycle, and the cell type determine the fate of pyruvate.

To summarize, the glycolytic pathway of glucose metabolism uses two ATP to prime one molecule of glucose and produces two molecules of NADH and four ATP via substrate-level phosphorylation reactions, for a net gain of two ATP per molecule glucose. Oxidation of NADH to NAD^+ by MAS, is under oxygenated conditions, followed by oxidation of NADH in the mitochondria, creating another six molecules of ATP, whereas lactate formation is not associated with ATP formation or utilization.

2. *Metabolic Control by Energy Demand and Levels of Intermediates*

ATP production is closely coupled to brain work, due, in part, to the requirement for ADP as a substrate for the energy-producing reactions (Figs. 1 and 2). If glycolysis were not regulated, metabolism of all available glucose that entered the brain would simply consume glucose and ATP, trap phosphate as triose-P, and produce lactate. Major sites for control of glycolytic flux are hexokinase, phosphofructokinase (PFK), and pyruvate kinase; PFK is the key enzyme.

Type I hexokinase, the predominant isozyme in brain, is normally saturated with substrate and strongly inhibited by its product (glucose-6-P); its kinetic properties are altered by reversible binding to mitochondria. In rat brain, the apparent K_m of hexokinase for glucose is ~0.05 m*M*, which is well below the intracellular glucose concentration in brain, i.e., 2–3 m*M* (Siesjö, 1978; Pfeuffer *et al.*, 2000). The K_m for ATP-Mg^{2+} is about 0.4 m*M*, and brain ATP level is 2–3 m*M*. Brain hexokinase is inhibited by ADP as well as by glucose-6-P ($K_i \cong 10\ \mu M$) and fructose-1,6-P_2, an inhibition which is antagonized by phosphate (P_i). Comparison of glycolytic flux in normal rat brain to maximal hexokinase activity assayed *in vitro* (Table I) indicates that the enzyme is normally inhibited by more than 95%, so brain has high capacity to increase glycolytic flux as needed. Hexokinase I binds to the outer mitochondrial membrane via a pore-forming protein (porin) through which ATP and ADP cross the mitochondrial membrane, thereby giving hexokinase preferential access to mitochondrially generated ATP (Cesar and Wilson, 1998). Binding to mitochondria alters specific epitopic regions in the hexokinase molecule (Hashimoto and Wilson, 2000). The phosphorylation of glucose by brain hexokinase bound to mitochondria is not only *able to* use mitochondrially generated ATP but *selectively uses* ATP formed in mitochondria, which may help coordinate glycolysis and TCA cycle activity (BeltrandelRio and Wilson, 1992a,b; Cesar and Wilson, 1998). When bound to mitochondria, the K_i of hexokinase for glucose-6-P is increased and the K_m for ATP is reduced, suggesting that the bound form is more active.

In smooth muscle preparations, it has been shown that hexokinase association with mitochondria is reduced from 70 to 40% of total hexokinase activity by treatment with DG to increase glucose-6-P content fourfold, suggesting that hexokinase binding to mitochondria is regulated by the metabolic state of the cells (Lynch *et al.,* 1991, 1996). Besides moment-to-moment regulation of activity by metabolic effectors (Wilson, 1995), functional activity modulates hexokinase amount; thus, hexokinase activity increases over several days in structures involved in body fluid regulation in response to water deprivation, diabetes, and aortic baroreceptor denervation (Turton *et al.,* 1986; Krukoff *et al.,* 1986).

All three isoenzymes (muscle [M], liver [L], and brain [C]) of PFK occur in brain, and quantitative differences in allosteric properties are found between the different isozymes (Zeitschel *et al.,* 1996). Regulation of PFK activity is a major control point for glycolysis (Passonneau and Lowry, 1964). For example, activation of metabolism by ischemia increases the rate of glycolysis greater than four-fold, and causes the concentrations of metabolites upstream of fructose-6,1-P_2 (glucose, glucose-6-P, and fructose-6-P) to fall, whereas the levels of those downstream (between fructose-1,6-P_2 and lactate) rise, indicating rapid PFK activation. PFK is inhibited by compounds that accumulate when the energy charge is high, and it is activated by products of functional metabolic activity (Fig. 2). Energy charge is the relative ATP level of the adenine nucleotide pool, calculated as $[ATP + 0.5ADP]/[ATP + ADP + AMP]$. The cellular concentration of ATP (2–3 m*M*) greatly exceeds those of ADP (about 0.2–0.6 m*M*) and AMP (about 0.05 m*M*), and the normal energy charge is slightly less than one. AMP is produced by adenylate kinase (myokinase) when ADP is used to regenerate ATP; due to the low concentration of AMP, small changes in ATP level are amplified and reflected by larger fractional changes in the amount of AMP. PFK activators include NH_4^+, K^+, P_i, AMP, cAMP, ADP, and fructose-2,6-P_2; inhibitors include ATP, phosphocreatine, 3-P-glycerate, 2-P-glycerate, 2,3-P_2-glycerate, phosphoenolpyruvate, citrate, hydrogen ion (low pH), and Mg^{2+}. Fructose-6-P, fructose-1,6-P_2, ADP, AMP, P_i, and NH_4^+ are all increased in ischemia; these compounds overcome the inhibition of PFK by ATP. Inhibition of PFK by citrate helps to coordinate TCA cycle activity with glycolytic flux. Inhibition by ATP of both PFK and pyruvate kinase slows glycolysis when energy supplies are high. Many regulatory mechanisms act concertedly to fine-tune glucose metabolism to meet local energy demand. Typical metabolite levels obtained in rat brain (Table II) show that brain reserves of energy metabolites are low relative to flux through catabolic pathways (0.3 μmol glucose/min/g wet wt in man; 0.7 μmol glucose/min/g wet wt in the rat). A continuous supply of glucose is, therefore, required to sustain brain function.

TABLE II
REPRESENTATIVE LEVELS OF ENERGY METABOLITES IN FREEZE-BLOWN RAT BRAIN

Compound	Concentration (μmol/g wet wt)
Glycogen	2.8
Glucose	1.6
Glucose-6-P	0.2
Fructose 1,6-P_2	0.01
Dihydroxyacetone-P	0.02
α-Glycerol-P	0.11
Pyruvate	0.09
Lactate	1.4
Citrate	0.28
α-Ketoglutarate	0.22
Malate	0.32
Glutamate	12
Aspartate	3
Glutamine	6
ATP	2.5
ADP	0.6
AMP	0.07
Creatine-P	4

Data are from Veech, R. L., 1980.

3. *Immunohistochemistry of Glycolytic Enzymes and Transporters*

a. Glucose Transporter. Transit of glucose from blood across the capillary endothelium and ultimately into brain cells requires the action of several isoforms of the glucose transporter family. Endothelial cells constituting the blood-brain barrier express the glucose transporter GLUT1, whereas neurons express GLUT3. Neuronal perikarya and proximal dendrites have little immunochemically visualized glucose transporter, but the adjacent neuropil is intensely stained for the glucose transporter (Bagley *et al.,* 1989; Mantych *et al.,* 1993; McCall *et al.,* 1994; Gerhart *et al.,* 1995; Fattoretti *et al.,* 2001), which is densely expressed both pre- and postsynaptically (Leino *et al.,* 1997). GLUT3 is not expressed by astrocytes, oligodendrocytes, or endothelial cells (Nagamutsi *et al.,* 1993; Morro and Yamada, 1994). These cells instead express the glucose transporter GLUT1, which is concentrated in astrocytic end feet and astrocytic processes surrounding synapses (Morgello

et al., 1995; McCall *et al.,* 1996; Yu and Ding, 1998), although it is also present in astrocytic cell bodies (Leino *et al.,* 1997). GLUT1 is expressed in the choroid plexus and ependymal cells (Hacker *et al.,* 1991; Cornford *et al.,* 1998; de los A Garcia *et al.,* 2001), but not in microglia, which express GLUT5 (Payne *et al.,* 1997; Yu and Ding, 1998). There is relatively good regional correlation between staining for glucose transporter and local CMR_{glc} (Wree *et al.,* 1988; McCall *et al.,* 1994; Gronlund *et al.,* 1996). Both GLUT1 and GLUT3 immunostaining increase in abundance in a region-specific manner following chronic seizures (Gronlund *et al.,* 1996).

b. Hexokinase. Some, although not perfect, correlation is found between density of glucose transporter sites and expression of hexokinase, which can be observed in the cytoplasm of neuronal, astrocytic, and choroid plexus cells as well as in the neuropil and purified synaptosomes (Wilkin and Wilson, 1977; Fields *et al.,* 1999). The distribution of hexokinase has been especially well examined in the cerebellar cortex (Kao-Jen and Wilson, 1980). Extensive staining of cytoplasmic regions, with some increased density at mitochondrial profiles was found in most types of neurons and their processes and in astrocytes, whereas oligodendrocytes showed no staining. The expression of dense staining for hexokinase in both neurons and astrocytes is consistent with the finding of almost identical values for hexokinase activities in cultured neurons and astrocytes (Lai *et al.,* 1999); the deficient staining in oligodendrocytes is mirrored by very low activity of hexokinase in cultured oligodendrocytes (Rust *et al.,* 1991), and a much lower CMR_{glc} in white than in gray matter (Sokoloff *et al.,* 1977). An exception to intense neuronal staining was Purkinje cells and part of their dendrites, which showed only little hexokinase expression. Granule cell dendrites were well stained in their proximal parts but void of stain in their terminal digits, which form part of the cerebellar glomeruli; in contrast, the mossy fiber terminals of brain stem neurons, with which the granule cells synapse, exhibited intense staining, as did synaptic vesicles adjacent to the mitochondria. Endothelial cells in brain microvessels express hexokinase activity (Djuricic and Mrsulja, 1979; de Cerqueira Cesar and Wilson, 1995).

c. PFK. All three isotypes of PFK have been found by immunohistochemistry in both neurons and astrocytes. M-type PFK is preferentially found perinuclearly, L-type PFK shows a characteristic staining in the cytoplasm and the processes of cells, whereas the C-type antibodies almost homogeneously stain whole cell bodies as well as large dendrites; because the PFK isoenzymes differ with respect to their allosteric properties, their differential distribution in different cell constituents might be of importance for the regulation of brain glycolysis in the different cellular compartments of the brain (Zeitschel *et al.,* 1996).

d. Pyruvate Kinase. Pyruvate kinase is expressed in both neurons and astrocytes, but appears to be especially prominent in large neurons and in nerve terminals (Gali *et al.,* 1981); pyruvate kinase staining may be absent in oligodendrocytes and microglia (van Erp *et al.,* 1988).

e. Lactate Dehydrogenase and Lactate Transporters. There are different isoforms of lactate dehydrogenase. The H4 tetramer, which shows much greater inhibition by a pyruvate/NAD^+ complex at the active site, is predominant in aerobic heart tissue, raising the possibility that the efflux of pyruvate as lactate is minimized and lactate is mainly converted to pyruvate in these tissues. On the other hand, muscle, which has mainly the M4 isoform, can operate anaerobically and needs to produce lactate from pyruvate.

High levels of lactate dehydrogenase reactivity are found in the neuropil in certain, specific afferent terminal fields (Borowsky and Collins, 1989a,b). It has been reported that M4, i.e., the isoenzyme favoring conversion of pyruvate to lactate is enriched in astrocytes compared to neurons (Bittar *et al.,* 1996; Pellerin *et al.,* 1998); however, this observation is not consistent with early work using *in vivo* immunofluorescense, which demonstrated approximately equal distribution of the H and M forms of lactate dehydrogenase in astrocytes and neurons in the CNS (Brumberg and Pevzner, 1975; Pevzner, 1979).

Pyruvate and lactate are transported across cell membranes via an equilibrative monocarboxylic acid transporter (MCT), which exists as different isotypes. It has been suggested that the MCT isotypes expressed by brain cells favor lactate formation and release from astrocytes and lactate uptake into neurons (Broer *et al.,* 1997; Pellerin *et al.,* 1998; Pierre *et al.,* 2000), but this proposal is controversial, since different MCT isoform distributions have been demonstrated by Gerhart *et al.* (1997, 1998) and Hanu *et al.* (2000). Moreover, it should be kept in mind that MCT mediates facilitated diffusion, and that sustained flux of lactate across a cell membrane in a given direction is determined by the transmembrane lactate concentration gradient (together with the H^+ gradient). Maintained net uptake of lactate from extracellular fluid therefore will be governed by rate of metabolism of lactate, which is much slower than the equilibrative transport process (Dienel and Hertz, 2001).

f. Synopsis of Immunochemistry. With the exception of oligodendrocytes, hexokinase is readily detectable in brain parenchymal cells, in brain endothelial cells, and in choroid plexus; at least in some pathways the density is greater pre- than postsynaptically. There is intense staining in the neuropil. PFK and pyruvate kinase are also expressed in both neurons and glial cells, but the level of pyruvate kinase appears to be low in oligodendrocytes. LDH is expressed in the neuropil, especially in specific afferent fields,

and differences exist within both neurons and astrocytes according to the pathways with which they are associated.

C. Formation of Acetyl Coenzyme A (Acetyl-CoA)

1. *Acetyl-CoA Formation from Pyruvate*

Pyruvate oxidation is initiated by pyruvate entry into the mitochondrion, mediated by an MCT. The participation of MCTs both in transmembrane transport of lactate and pyruvate and in the entry of pyruvate from the cytosol into the mitochondria renders it difficult to utilize an MCT inhibitor in order to draw any conclusions about the importance of lactate (or pyruvate) as a metabolic fuel.

Inside the mitochondria, the pyruvate dehydrogenase (PDH) complex (PDHC) catalyzes the first step of pyruvate utilization to produce acetyl-CoA plus CO_2 and NADH from pyruvate, coenzyme A (CoASH) and NAD^+; in this thiamine-dependent step, carbons three and four of glucose (carbon one of pyruvate) are converted to CO_2, whereas the remaining carbon atoms are introduced into the TCA cycle (Fig. 2). Pyruvate dehydrogenase has a K_m for pyruvate of ~0.05 m*M* (Ksiezak-Reding *et al.,* 1982), which is approximately equal to the pyruvate concentration in brain (Siesjö, 1978). The PDH multienzyme complex is composed of pyruvate dehydrogenase tetramers (each with two decarboxylase and two dehydrogenase sites), transacetylase, and lipoamide dehydrogenase. Activity of the PDH complex is regulated by phosphorylation at a serine residue on the pyruvate decarboxylase polypeptide to make PDH *inactive*. A Mg^{2+}- and Ca^{2+}-dependent phosphatase dephosphorylates and activates the PDH complex. Acetyl-CoA and NADH inhibit the active dephosphorylated form of PDH and are also positive effectors of the kinase, which will inactivate the enzyme. CoASH, NAD^+, and pyruvate are all PDH substrates that inhibit the PDH kinase and thereby activate PDH, as does ADP. Thus, metabolic demand regulates pyruvate utilization: increased precursor supply reduces inactivation of the PDH complex by the kinase, and products of the reaction both inhibit the active PDH complex and activate the kinase. Overload of the TCA cycle will cause acetyl-CoA and NADH to rise, thereby turning off pyruvate utilization, whereas increased energy demand raises the ADP level and activates the flux of pyruvate into the TCA cycle. Another stimulus for activation of PDH is an increase in intramitochondrial Ca^{2+}, resulting from transmitter-induced increase in free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) (McCormack and Denton, 1990), as will be discussed in Section IV.D.8. Because PDH is a regulated enzyme and its Km for pyruvate is similar to that of the brain pyruvate

concentration, this metabolic step can be a transient "bottle-neck," in which flux of pyruvate into the oxidative pathway is limited compared to the rate of glycolysis, causing an increase in lactate formation in order to regenerate NAD^+ and maintain glycolytic flux, especially when brain work is suddenly increased.

Pyruvate dehydrogenase immunoreactivity has been observed in neuronal cell bodies (with pronounced differences between different neurons), proximal cell processes, and at several locations in the neuropil (Milner *et al.,* 1987; Bagley *et al.,* 1989; Calingasan *et al.,* 1994). No information is available about the cellular distribution of pyruvate dehydrogenase within the neuropil, but cortical mouse astrocytes in primary cultures show higher rates of pyruvate dehydrogenase-mediated flux from [1-^{14}C]pyruvate than cultures of cortical neurons (Hertz *et al.,* 1987).

2. *Acetyl-CoA Formation from Other Sources*

Although brain acetyl-CoA is mainly derived from pyruvate, it can also be formed from fatty acids, ketone bodies, and monocarboxylic acids like acetate. Lipid is not a major energy source in brain, and astrocytes are the only cell type to oxidize fatty acids as primary fuel, whereas neurons, astrocytes, and oligodendrocytes can metabolize ketone bodies (Edmond *et al.,* 1987; Auestad *et al.,* 1991). Formation of acetyl-CoA from acetate is of little physiological importance (although ethanol and the neurotransmitter acetylcholine are metabolized to acetate), but it is of considerable experimental interest, because acetate is preferentially transported into glial cells (Fig. 3A). Autoradiographic studies have localized acetate uptake to neuropil and astrocytes, whereas it is not accumulated into perikarya (Muir *et al.,* 1986). This finding is corroborated by the demonstration that [^{14}C]acetate is taken up much more rapidly in primary cultures of astrocytes than in primary cultures of neurons, as shown in Fig. 3B (O'Dowd, 1995; Waniewski and Martin, 1998). Therefore, acetate can be utilized as a "reporter molecule" of astrocyte metabolism. The first step in acetate metabolism in mammalian brain is conversion to acetyl-CoA by acetate thiokinase (acetyl-CoA synthase) in the presence of CoASH and ATP; this enzyme is present in both cultured astrocytes and synaptosomes (see in the next section). Label from the astrocytically accumulated [^{14}C]acetate can, after formation of acetyl-CoA, become incorporated into TCA-cycle-derived amino acids (Fig. 3A), and may eventually be transported to neurons due to cycling of glutamate, glutamine, and GABA between neurons and astrocytes (Section II.H.2–4). Since acetyl-CoA formation from pyruvate does not proceed in the opposite direction, acetate is not a precursor for pyruvate or for oxaloacetate, which is formed by pyruvate carboxylation (Section II.F.2).

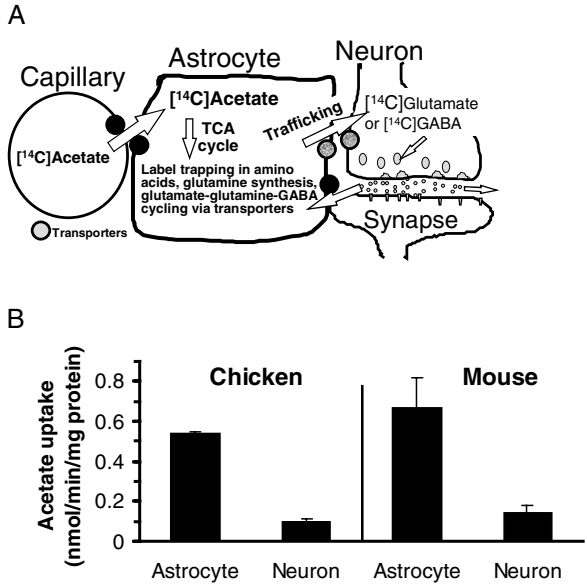

FIG. 3. Acetate is a "glial reporter molecule." (A) Preferential entry into the astrocyte and metabolic trapping in the amino acid pools provides a means for autoradiographic detection of a local increase in astrocytic activity and for NMR assays of astrocyte TCA cycle activity. This schematic drawing illustrates preferential uptake of blood-borne [^{14}C]acetate into astrocytes via a monocarboxylic acid transporter, incorporation into TCA-cycle-derived amino acids in astrocytes, and local trafficking of labeled compounds due to cycling of glutamate, glutamine, and GABA between neurons and astrocytes (see text). (Adapted from G. A. Dienel. Energy generation in the central nervous system. *In* "Cerebral Blood Flow and Metabolism, 2nd ed." (L. Edvinsson and D. Krause, eds.), 2002, Lippincott Williams & Wilkins.©) (B) Rates of [^{14}C]acetate uptake in primary cultures of chick and mouse astrocytes. Acetate uptake was measured during a 10-min period of incubation with 50 μM [2- ^{14}C]acetate in tissue culture medium containing 6 m*M* glucose. The uptake was rectilinear and was calculated from accumulated radioactivity per mg protein and the specific activity of the incubation medium. SEM values are shown by vertical bars. In both chick and mouse cultures acetate uptake is significantly higher ($p < 0.05$ or better) in astrocytes than in neurons. (From O'Dowd, 1995, with the permission of O'Dowd.)

3. *Acetate and "Metabolic Compartmentation"*

a. Metabolic Compartmentation. The preferential uptake of acetate (and some other monocarboxylic acids) into astrocytes is consistent with pioneering tracer labeling experiments carried out between the late 1950s and the 1970s (reviewed in Berl *et al.,* 1975). The patterns of labeling of glutamate and glutamine in whole brain tissue by different radioactively labeled precursors were studied during an experimental period when the specific activities (μCi/mmol) of the compounds of interest were increasing *in vivo.*