INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

Astrocyte–Axon Interactions in Central White Matter Energy Metabolism: The Roles of Glycogen and Lactate

Regina Wender

A dissertation submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

University of Washington

2000

Program Authorized to Offer Degree: Physiology and Biophysics

UMI Number: 9976083

Bell & Howell Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

In presenting this dissertation in partial fulfillment of the requirements for the Doctoral degree at the University of Washington, I agree that the Library shall make its copies freely available for inspection. I further agree that extensive copying of the dissertation is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for copying or reproduction of this dissertation may be referred to Bell and Howell Information and Learning, 300 North Zeeb Road, Ann Arbor, MI 48106-1346, to whom the author has granted "the right to reproduce and sell (a) copies of the manuscript in microform and/or (b) printed copies of the manuscript made from microform."

Signature Regina Walker

Date JUNE 5, 2000

University of Washington

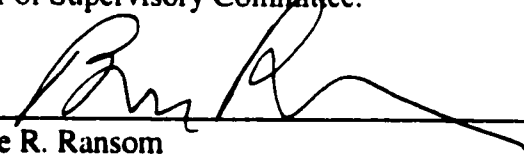
Graduate School

This is to certify that I have examined this copy of a doctoral dissertation by

Regina Wender

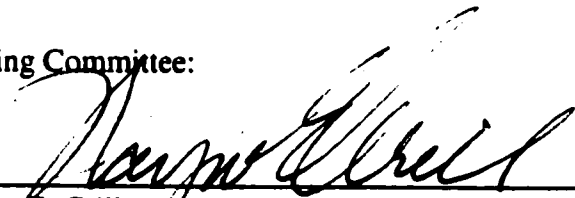
and have found that it is complete and satisfactory in all respects, and that any and all revisions required by the final examining committee have been made.

Chair of Supervisory Committee:



Bruce R. Ransom

Reading Committee:



Wayne E. Crill

Bruce R. Ransom

H. Richard Winn

Date: 5/31/00

University of Washington

Abstract

Astrocyte–Axon Interactions in Central White Matter Energy Metabolism: The Roles of Glycogen and Lactate

Regina Wender

Chairman of Supervisory Committee:

Professor and Chairman Bruce R. Ransom

Department of Neurology

The goal of this research was to investigate the role of astrocytic glycogen in central nervous system (CNS) white matter (WM) metabolism. Experiments were designed to test two hypotheses: (1) Metabolites other than glucose can support function of WM axons. (2) During glucose withdrawal in CNS WM, astrocytes supply energy substrate to axons in the form of lactate, derived from glycogen; and axon function and survival depend on astrocytic glycogen in the absence of exogenous glucose. Experiments were performed on the isolated rat optic nerve, a central WM tract, using electrophysiological and biochemical techniques. Axon function was

determined by quantitatively monitoring the area under the stimulus-evoked compound action potential (CAP). To address the first hypothesis, we tested compounds that were able to enter energy pathways. The CAP was maintained for 2 hr in physiological saline containing 10 mM glucose. 20 mM lactate, 20 mM pyruvate, 10 mM fructose, or 10 mM mannose supported axon function as effectively as did glucose; 10 mM glutamine provided partial support. In the next phase of the study we examined whether astrocytic glycogen sustains axon function during, and enhances axon survival after, 60 min of glucose deprivation. Exposure of nerves to glucose-free perfusate had no effect on CAP area for 30 min, after which the CAP rapidly failed. Irreversible injury, measured as incomplete recovery of the CAP compared with control, was sustained. Glycogen content of the tissue fell 30 min after glucose withdrawal, compatible with rapid turnover in the absence of glucose. Up-regulation of glycogen increased latency to CAP failure and improved CAP recovery; down-regulation of glycogen decreased latency to CAP failure and reduced recovery. Lactate transport blockers were used to determine whether lactate represented the fuel derived from glycogen and shuttled to axons. The blockers, when applied during glucose withdrawal, decreased latency to CAP failure and decreased CAP recovery. These results indicated that in the absence of glucose, astrocytic glycogen was metabolized to lactate, which was subsequently transferred to axons for fuel. This study represents the first demonstration of the importance of astrocytic glycogen for axon function and survival under conditions of glucose withdrawal.

TABLE OF CONTENTS

List of Figures iii
List of Tables iv

Chapter 1: Introduction 1
Glial–neuronal interactions and brain energy metabolism 1
Carbohydrate metabolism in the CNS 5
Glucose 5
Glycogen 7
Lactate as a fuel for white matter 11
Lactate dehydrogenase (LDH) 11
Monocarboxylate transporters (MCTs) 12
Hypoglycemic brain injury 14
Brain energy metabolism: the role of astrocytic glycogen 16
Rat optic nerve (RON) as a CNS white matter model 18

Chapter 2: Research design and methods 20
Preparation 20
Curve fitting 22
Test solutions 24
Ion-sensitive microelectrodes 25
Transmission electron microscopy 26
Glycogen and protein assays 27
Data analysis 28

Chapter 3: Metabolic substrates other than glucose support axon function and improve recovery from hypoglycemia in central white matter 29
INTRODUCTION 29
RESULTS 31
Ability of glucose and other substrates to support CNS axon function 31
Ability of substrates to improve CAP recovery from glucose deprivation 37
DISCUSSION 43
Glial–axonal interactions in the absence of glucose 43
Alternative energy substrates 45

Chapter 4: Astrocytic glycogen stores influence axon function and survival during glucose deprivation in central white matter 50
INTRODUCTION 50
RESULTS 52
Ultrastructural identification of astrocytic glycogen in the RON 54
Glycogen content of RON 55
Glycogen content and axon function 58
Blockade of lactate transport and axon function during glucose withdrawal 60
DISCUSSION 67

References 75
Vita 85

LIST OF FIGURES

Figure Number — Page

Figure 1.1. Schematic diagrams of how glial cells and neurons might interact with one another in terms of energy metabolism. 2
Figure 1.2. Catabolism of glucose 7
Figure 1.3. Glycogen dynamics 9

Figure 3.1. Biochemical pathways by which tested substrates might be used to generate energy in the RON in the absence of glucose 31
Figure 3.2. Comparison of the ability of monocarboxylates and glucose to support axon function. 33
Figure 3.3. Comparison of the ability of monosaccharides and amino acids to support axon function. 35
Figure 3.4. Efficacy of glucose at different concentrations to prevent functional injury when substituted for 60 min. 38
Figure 3.5. Efficacy of monocarboxylates or amino acids at different concentrations to prevent injury when substituted for glucose for 60 min. 40
Figure 3.6. Comparison of percent CAP recoveries for different substrates at different concentrations. 42

Figure 4.1. Effects of glucose withdrawal for 60 min on the rat optic nerve CAP area. 53
Figure 4.2. Ultrastructural evidence of glycogen deposition in astrocytes in adult rat optic nerve 54
Figure 4.3. Glycogen content of rat optic nerves following glucose deprivation and pharmacological manipulation 57
Figure 4.4. Effect of nerve glycogen content on axon function during, and recovery from, a 60-min period of glucose deprivation. 59
Figure 4.5. Glucose deprivation caused extracellular alkalosis, and lactic acid supported RON function in the absence of glucose 62
Figure 4.6. Effect of the lactate transport inhibitors quercetin, 4-CIN, and *p*CMBS on RONs exposed to 0 mM glucose or 20 mM lactate for 60 min. 64
Figure 4.7. Summary of CAP recoveries following aglycemia with inhibition of lactate transport, with and without lactate supplementation 66
Figure 4.8. Schematic illustration of how astrocytic glycogen appears to fuel axons in the absence of glucose. 71

LIST OF TABLES

TABLE I. CAP area after 120 min exposure to test substrates..... 36

Acknowledgments

The work that went into this dissertation project was made possible by the assistance, support, and encouragement of many people. I owe a particular debt of gratitude to Angus Brown, a fine teacher, colleague, and friend. Angus helped me at every stage of the project, from planning experiments through making electrodes, analyzing data, and preparing manuscripts. He has given me the benefit of his seemingly infinite patience and has been unfailingly generous in sharing ideas, lab techniques, and time. Angus has helped immeasurably in bringing this work to fruition. Thomas Möller has been extraordinarily patient and generous with his time. He has helped and guided me through everything from noisy headstages to judicious data analysis to crashed computers. I am most indebted to Angus and Thomas for making my years in the lab so productive and educational. I will miss their good company and their counsel.

I would like to thank the faculty members who kindly agreed to serve on my Supervisory Committee: William Catterall, Wayne Crill, Bruce Ransom, Karen Thomas, H. Richard Winn, and William Zagotta. Among these people, several are due particular recognition:

I am greatly indebted to my thesis adviser, Bruce Ransom, who encouraged me in the early crucial stages of my work. He invited me to work in his lab early in his tenure at UW. He has guided me in the development and shaping of my project, discussed ideas, and helped me to refine my writing and to grow as a researcher. He has provided access to important resources, equipment, and scientific counsel.

I extend especially warm thanks and appreciation to H. Richard Winn, who has patiently (usually!) put up with me for six years, first as my preceptor when I began medical school, then as my lab adviser, and currently as a key member of my

Supervisory Committee. He has provided counsel and encouragement and has supported my research intellectually and financially. His rigorous standards and constructive criticism have expanded my horizons and strengthened this work.

Wayne Crill was a crucial link in my decision to pursue my Ph.D. in Physiology and Biophysics. He is the model of what a mentor should be: He has always challenged me to meet his demanding standards and has been a staunch supporter when it has been most needed. My years and work as a graduate student have been more productive and more rewarding because of him.

My special admiration and appreciation for the members of the Winn Laboratory cannot be overstated. Over the past six years they have become valued friends and colleagues, and I am indebted to them for their support, feedback, professionalism, and good humor. They have advised me, critiqued my work, teased me mercilessly, encouraged me when results were scarce, and cheered me on when experiments worked.

Raymond Swanson and Kevin Farrell (University of California, San Francisco) lent their expertise to help with assaying glycogen, and Joel Black (Yale University) was kind enough to help with the electron microscopy figure.

A special word of thanks is due to Kass Klemz, who has helped me through endless mazes of paperwork and has made the impossible possible.

Finally, I thank my parents and brothers, whose support and constant encouragement made this endeavor possible.

Chapter 1: Introduction

The goal of this research was to investigate the role of astrocytic glycogen in central nervous system (CNS) white matter (WM). Experiments were designed to test two hypotheses: (1) Metabolites other than glucose can support function of WM axons. (2) During glucose withdrawal in the CNS, astrocytes supply energy substrate to axons in the form of lactate, derived from astrocytic glycogen; and axon function and survival depend on astrocytic glycogen in the absence of exogenous glucose. Experiments were performed on the acutely isolated rat optic nerve, a central WM tract, using electrophysiological and biochemical techniques.

Glial–neuronal interactions and brain energy metabolism

Golgi, more than a century ago, suggested that glial cells feed neurons. He based this on his microscopic observation that glial cells are often positioned between blood vessels and neurons, with their end-feet intimately surrounding blood vessels (Kuffler and Nicholls, 1966; Somjen, 1988). This idea gained credibility when it was learned that astrocytes are the only cells in the mammalian brain that contain significant glycogen (Borke and Nau, 1984; Cataldo and Broadwell, 1986), the main energy reserve in the brain (Lowry, 1964). The anatomic arrangement of astrocytes, neurons, and capillaries suggested that blood glucose might be taken up preferentially by astrocytes (**Fig. 1.1.A**).

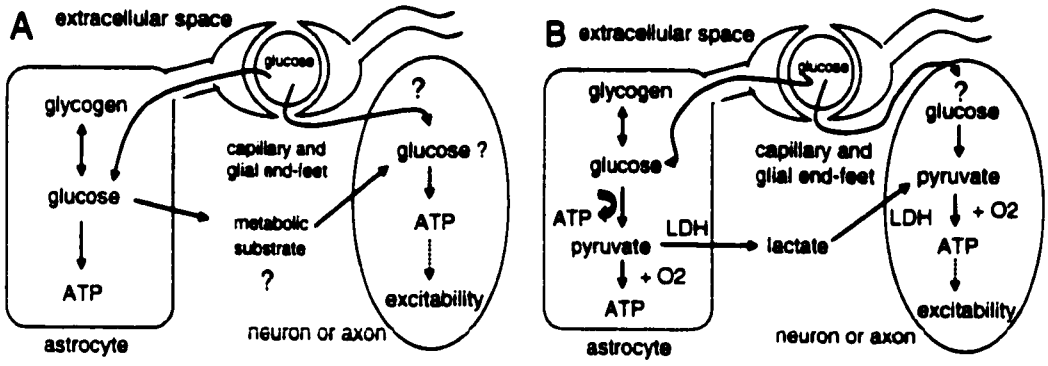


Figure 1.1. Schematic diagrams of how glial cells and neurons might interact with one another in terms of energy metabolism. (A) Golgi proposed that glia feed neurons. For anatomical reasons, glucose was suspected of entering glia first and then being transported to neurons as glucose or some other substrate. (B) Evidence-based theory of glial-neuronal interaction. Glia take up more glucose than do neurons. In the mammalian CNS, lactate is exported from glial cells, and some neurons (perhaps most) use this as a fuel for aerobic energy production. Neurons and glia have distinct transport mechanisms for uptake of lactate (Bröer et al., 1997) and express different isoforms of the enzyme lactate dehydrogenase (LDH) (Tholey et al., 1981; Pellerin et al., 1998); the neuronal form of LDH facilitates the conversion of lactate to pyruvate for subsequent aerobic metabolism. The use of lactate is energy efficient: two moles of lactate produce 28 moles of ATP, nearly the same as for the oxidization of glucose to CO_2 (30 moles of ATP; note that this value supersedes the traditional estimate of 36 ATP) (Hinkle et al., 1991).

Astrocytes would then "share" this fuel with neurons, either as glucose or as another useable substrate. The transfer would occur across brain extracellular space (ECS): the approximate distance between neighboring cells' plasma membranes is 20 nm, which enables substances released from one cell to diffuse almost instantly to adjacent cells (Ransom and Sontheimer, 1992; Nicholson, 1995). It should be emphasized that nearly every neuron in the brain shares common ECS with adjacent astrocytes. Recent observations in the retinas of the honeybee (Tsacopoulos et al.,

1994) and guinea pig (Poitry-Yamate et al., 1995) have moved toward substantiating the theory that glial cells can provide fuel to neurons. The role of glycogen in this glial–neuronal interaction remains unclear. The experiments described herein critically address this important issue using the isolated rat optic nerve, a CNS WM tract.

In the honeybee drone retina, the neural elements, the photoreceptors, contain mitochondria and produce energy mainly by oxidative phosphorylation (Tsacopoulos and Magistretti, 1996). They are surrounded by glial-like cells that are filled with glycogen, contain few if any mitochondria, and produce energy by glycolysis. The glial-like cells alone take up glucose and, consistent with this, they alone have significant hexokinase activity (see below). Energy metabolism in the photoreceptors increases when they are activated by light, and they signal nearby glial-like cells to increase their uptake of glucose. Biochemical experiments indicate that the photoreceptors take up and oxidize alanine released by the glial-like cells (Tsacopoulos et al., 1994). Alanine is taken up by a sodium-dependent transporter and converted to pyruvate, which can enter the citric acid cycle. Consistent with its role as the main energy supply for the photoreceptors, alanine is present in high concentration in the ECS (Cardinaud et al., 1994). Analogous experiments have been carried out in the guinea pig retina (Poitry-Yamate et al., 1995). The major difference is that mammalian retinal glial cells (Müller cells) produce lactate, not alanine, for export to the adjacent photoreceptors. In that study, ^{14}C-labeled lactate

used to trace CO_2 production indicated that guinea pig photoreceptors preferentially metabolize lactate even in the presence of glucose.

The evidence from these experiments on the retina, and from a study designed to examine possible physiological stimuli for metabolic coupling between astrocytes and neurons in mammalian gray matter (Pellerin and Magistretti, 1994), support the scheme shown in **Fig. 1.1.B.** It is premature to conclude that glial cells and neurons throughout the brain behave in this manner; more experiments will be necessary to establish this. Nevertheless, *in vitro* studies on mammalian glia and neurons are consistent with the idea that glial cells produce lactate for neuronal consumption. Glucose utilization appears to be higher in astrocytes than neurons (Lopes-Cardozo et al., 1986); astrocytes, but not neurons, export large amounts of lactate (Walz and Mukerji, 1988); and, when glycogen breakdown is induced in astrocytes, they release large amounts of lactate (Dringen et al., 1993). Lactate can serve as an effective energy substrate for neural tissue in the absence of glucose (Schurr et al., 1988; Izumi et al., 1994; Larrabee, 1995; Izumi et al., 1997). Finally, lactate transporters are located in both neurons and glial cells (Bröer et al., 1997), and neurons express the form of lactate dehydrogenase (isoform LDH1) that is appropriate for cells that oxidize lactate for energy production (Tholey et al., 1981; Bittar et al., 1996). One important caveat is worth mentioning: While lactate appears to be a good substrate for brain metabolism, it does not readily cross the blood–brain barrier (Clarke and Sokoloff, 1999). The system under discussion applies strictly to lactate produced within brain parenchyma.

Carbohydrate metabolism in the CNS

Glucose

Transport The entry of glucose into cells is mediated by a family of facilitative glucose transporter proteins (GLUTs). This family comprises isoforms GLUT1–GLUT7, all of which are integral membrane proteins (Vannucci et al., 1997). Although all isoforms have been detected in brain, many at only very marginal levels, only GLUT1 and GLUT3 have robust expression. GLUT1 is very highly expressed in the endothelial cells of the blood–brain barrier and in astrocytes, and GLUT3 is very highly expressed in neurons and in axons (Vannucci et al., 1997), with predominant expression in the neuropil (McCall et al., 1994). Both GLUT1 and GLUT3 are known to operate asymmetrically, and the kinetic characteristics of transport differ depending on the direction of transport (Vannucci et al., 1997).

GLUT2, the hepatic isoform that functions in bidirectional glucose transport, has been identified in some astrocyte populations (Leloup et al., 1994). Furthermore, the other hepatic glucose transporter, GLUT7, which exists as a complex with glucose-6-phosphatase (see below), is also found in some astrocytes (Bell et al., 1993). The presence of the GLUT2 and GLUT7 isoforms, the combined expression of which is necessary in the periphery, e.g., in the liver and kidney, for glucose export, may imply that astrocytes can extrude glucose, as well as lactate, to the ECS. Whether this is possible is yet to be determined.

Metabolism The fundamentals of glucose metabolism are common throughout organ systems. In brief, glucose is taken up into cells, where it is phosphorylated by the cytoplasmic enzyme hexokinase to generate glucose-6-phosphate. In this form, ionized under physiological conditions, the glucose is trapped in the cell, unable to diffuse across the plasma membrane.

Glucose-6-phosphate is then passed into the (anaerobic) glycolytic pathway, from which a net two ATPs and two molecules of three-carbon pyruvate are generated. In an aerobic environment, pyruvate is converted by the enzyme pyruvate dehydrogenase to acetyl CoA (and CO_2) for passage into the citric acid cycle. Conversely, in an environment in which oxygen tension is limited, pyruvate is reduced to lactate through the action of the enzyme lactate dehydrogenase (LDH). Lactate can subsequently be reoxidized to pyruvate, which provides on a molar basis 14 ATPs per pyruvate molecule through oxidative phosphorylation (Hinkle et al., 1991; Stryer, 1995). It is estimated that 15% of brain glucose is converted to lactate and does not enter the citric acid cycle (Clarke and Sokoloff, 1999). Of particular importance to this study is the cellular expression of different isoforms of LDH, on which further discussion follows. **Figure 1.2** illustrates the most immediately relevant steps in these pathways.

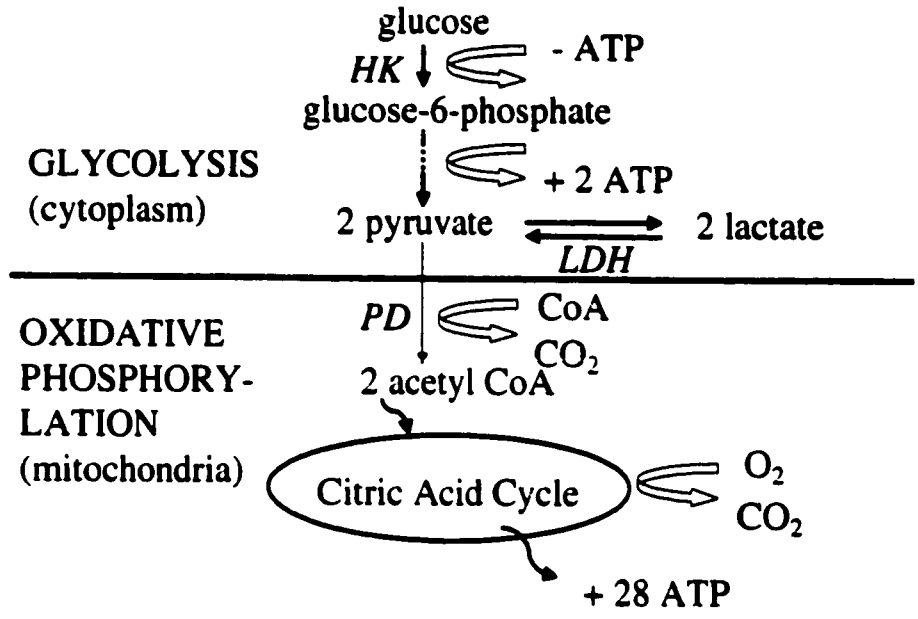


Figure 1.2. Catabolism of glucose (intermediate steps have been omitted for clarity). On entering the cytoplasm, glucose is phosphorylated by the enzyme hexokinase (HK), consuming one ATP, thereby trapping the resultant glucose-6-phosphate in the cell. One molecule of glucose is ultimately converted to two molecules of pyruvate by the glycolytic pathway, from which a net two ATP molecules are generated. In the mitochondria, pyruvate is oxidized by pyruvate dehydrogenase (PD) to generate two molecules of acetyl CoA, which feed into the citric acid cycle. A net 28 ATP molecules are produced through the combined activity of citric acid cycle and oxidative phosphorylation for every glucose molecule metabolized. Alternatively, pyruvate can be reduced in the cytoplasm to lactate through the action of lactate dehydrogenase (LDH).

Glycogen

Glycogen as an energy reserve Glycogen, the single largest energy reserve of the brain (Ibrahim, 1975), is a highly branched polymer of glucose, existing as roughly circular granules (Revel, 1964), which serves as a storage depot from which glucose may be released by degradation. One of the cellular advantages of condensing many glucose molecules into a single large molecule of glycogen is

that the condensation reduces osmotic pressure. Additionally, glycogen is a very efficient storage form of glucose: one high-energy phosphate bond is spent in incorporating glucose-6-phosphate (in an activated form, as uridine diphosphate glucose, or UDP-glucose) into glycogen, but the energy yield from the breakdown of glycogen is high. About 90% of the residues are phosphorolytically cleaved to glucose-1-phosphate, which is converted at no energy cost into glucose-6-phosphate. The other 10% are branched residues, which are hydrolytically cleaved. One ATP is then used to phosphorylate each of these glucose molecules to glucose-6-phosphate. The complete oxidation of glucose-6-phosphate yields approximately 30 molecules of ATP, and storage consumes slightly more than one ATP per glucose-6-phosphate, so the overall efficiency of storage is nearly 97% (Stryer, 1995). **Figure 1.3** illustrates the basic mechanisms of glycogen synthesis and glycogenolysis. There are three enzymes of particular relevance in this study: glycogen synthase, which catalyzes the addition of UDP-glucosyl residues onto the existing glycogen polymer; glycogen phosphorylase, which enables the phosphorolytic cleavage of glycosyl residues from glycogen; and glucose-6-phosphatase, which removes the terminal phosphate from glucose-6-phosphate, thereby allowing the free glucose to diffuse out of the cell.

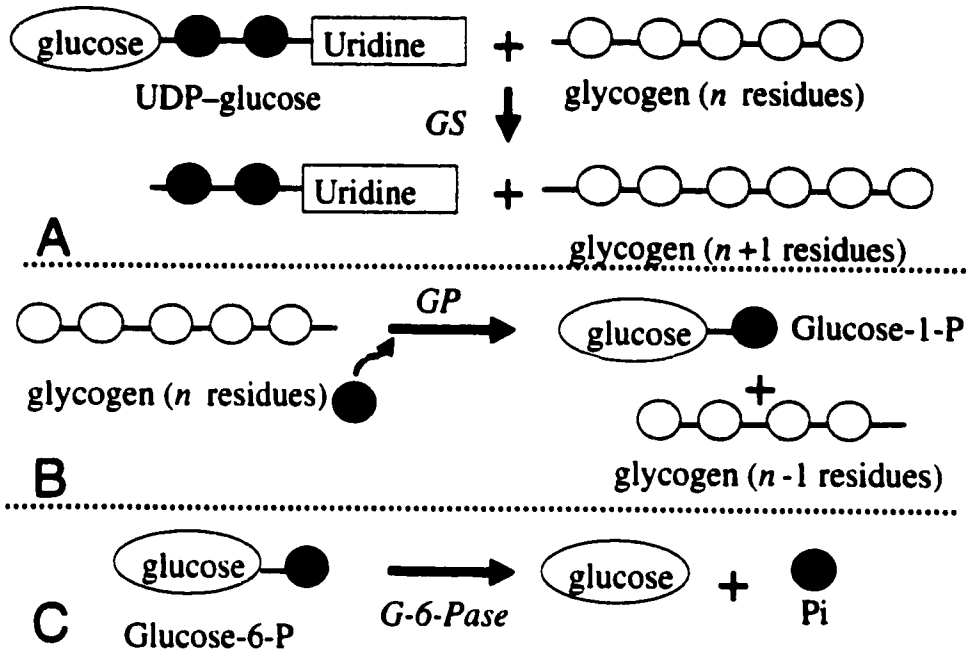


Figure 1.3. Glycogen dynamics: (**A**) Glycogen synthase (GS) catalyzes the transfer of glucose, in its activated form as UDP-glucose, to a growing glycogen chain. (**B**) Glycogen phosphorylase (GP) catalyzes the sequential phosphorolytic cleavage of glycosyl residues from the end of the glycogen chain. The glucose-1-phosphate that is released is subsequently isomerized to glucose-6-phosphate. (**C**) Glucose-6-phosphate is hydrolyzed by glucose-6-phosphatase (G-6-Pase), releasing free glucose and inorganic phosphate (P_i).

Expression in brain Glycogen was first shown in the vertebrate brain in 1905 (Koizumi, 1974). It is present at low levels in brain relative to liver (Villar-Palasi and Larner, 1960), and the levels vary with plasma glucose concentrations (Clarke and Sokoloff, 1999). Electron microscopy studies have shown glycogen granules primarily in astrocytes in adult brain (Cataldo and Broadwell, 1986; Clarke and Sokoloff, 1999), with some reports indicating that glycogen is especially abundant in astrocytic vascular end-feet (Maxwell and Kruger, 1965). In Maxwell's

study, most neurons were found to contain little or no glycogen. Indeed, the author used the demonstration of cytoplasmic glycogen granules as an indicating marker for astrocytes, and used the presence of cytoplasmic profiles containing filaments but consistently devoid of glycogen particles as determinative of a structure's being either an axon or dendrite. Moreover, *in vitro* experiments indicate that only astrocytes generate measurable quantities of glycogen (Dringen et al., 1993; Wiesinger et al., 1997).

At the cellular level, brain glycogen synthase mRNA is highly expressed in astrocytes (Pellegri et al., 1996), and rapid stimulation of glycogen synthesis occurs in astrocytes that are cultured in glucose-enriched medium (Lomako et al., 1993). Two neurotransmitters (vasoactive intestinal peptide and norepinephrine) that have been shown to induce delayed glycogen resynthesis in cultured astrocytes, upregulate the expression of glycogen synthase mRNA in astrocytes but not in neurons (Pellegri et al., 1996). Glycogen phosphorylase is reported to be located primarily in astrocytes and ependymal cells (Ohanian, 1972; Ignacio et al., 1990; Pfeiffer et al., 1990; Reinhart et al., 1990). These patterns of enzymatic expression are consistent with a primarily glial localization of glycogen. That said, glial cells, unlike hepatocytes, appear not to release glucose from glycogen breakdown; and the existence in brain of the key enzyme for glucose release, glucose-6-phosphatase, is controversial (Nelson et al., 1985; Wiesinger et al., 1997). The enzyme's catalytic subunit was shown to colocalize with GFAP (with no staining in neurons) (Bell et al., 1993), and further proof of the enzyme's activity in brain was reported by another

group (Forsyth et al., 1993). It is important to note, however, that contrary to these findings, in some reports no release of glucose from cultured astrocytes was observed (Dringen and Hamprecht, 1993), and it is often held that there is no evidence in any preparation that glial cells release glucose to supply adjacent neurons (Coles, 1995). Thus, while it is generally accepted that astrocytes *in vitro* cannot pass glucose to the extracellular space, some investigators have proposed that astrocyte-derived glucose is possible *in vivo* (Forsyth et al., 1996).

Lactate as a fuel for white matter

Lactate dehydrogenase (LDH)

LDH is the rate-limiting enzyme in lactate fluxes and controls the direction of lactate/pyruvate interconversion. The enzyme is composed of two distinct protein subunits, with catalytic properties adapted to the metabolic environment of the tissues in which each subunit predominates: the **M** ("muscle") subunit predominates in lactate-producing tissues (e.g., skeletal muscle). This subunit confers a very high affinity for pyruvate, which it preferentially reduces to lactate. The **H** ("heart") subunit is active in tissues that are heavily reliant on aerobic respiration (e.g., myocardium), where it preferentially oxidizes lactate to pyruvate. Active LDH is a tetramer composed of these subunits, such that at one extreme lies M_4 (in glycolytic tissue; also called "LDH5") and at the other end of the spectrum is found H_4 (in aerobic tissue; also called "LDH1"). Heterotetramers (e.g., M_3H) have intermediate

properties. It is possible to establish a correlation between the percentage of H subunits in a given tissue and the type of metabolism of the tissue. For example, it has been shown in human hippocampus and occipital cortex that neurons, which are known to have a high oxidative requirement, stain exclusively with anti-H (anti-LDH1) antibodies (Bittar et al., 1996); while astrocytes, which have the least activity of oxidative enzymes among all the cells of the CNS (Friede, 1962), are stained by both M (LDH5) and H antibodies. Anti-M immunoreactivity was most intense in astrocytes localized throughout the WM in the two regions studied. This finding supports a paradigm wherein astrocytes have the ability to continually convert pyruvate to lactate, because lactate would accumulate without binding M subunits (the K_m for M for lactate is high), which would reconvert the lactate back to pyruvate. If such a model is to be consistent with astrocytes' passing lactate to the extracellular space for putative uptake by neuronal elements, then it is reasonable to speculate that there may be a complementary expression of monocarboxylate transporters, i.e., glial expression of a transporter isoform that would preferentially export lactate and expression of a neuronal isoform that would favor lactate import.

Monocarboxylate transporters (MCTs)

MCTs are the proteins that shuttle lactate and pyruvate (among several other compounds), using proton symport, across cell and mitochondrial membranes. The transporter has been shown by hydropathy plots to be composed of 10–12 transmembrane α-helical domains with cytoplasmic N- and C-termini, a topology

shared by the GLUT family of glucose transporters. Observations suggest (Bröer et al., 1997; Jackson and Halestrap, 1996) that **MCT1** is expressed in tissue that preferentially releases lactate; **MCT2** is expressed in tissues that mainly consume lactate. Functional and molecular evidence demonstrate (Bröer et al., 1997; Koehler-Stec et al., 1998) that MCT1 is the only MCT expressed by astrocytes (in which MCT2 was barely detectable), whereas cultured neurons express the transporter isoform MCT2, with only very faint expression of MCT1. MCT immunostaining in microvascular endothelial cells is extremely weak in the adult, and passage of lactate across the blood–brain barrier is correspondingly limited (Gerhart et al., 1997; Koehler-Stec et al., 1998; Pellerin et al., 1998). It should be mentioned that new MCT isoforms have been identified, through MCT8 and possibly MCT9, and evidence suggests that isoform MCT8 (formerly classified as MCT7) is actually the transporter type that is most abundant in brain (Halestrap and Price, 1999). More specifically, however, this was shown in human fetal brain; whether MCT8 predominates in adult tissue and in other mammals remains to be determined.

These expression patterns raise an interesting question: Why would parenchymal brain cells continue to express in adulthood transporters for molecules that can no longer easily cross the blood–brain barrier to be used as energy? It has been postulated (Pellerin et al., 1998) that monocarboxylates in adult brain are predominantly from within the parenchyma and not from the circulation. Sustained intraparenchymal expression of MCT mRNA in adults, in face of the seemingly complete disappearance of such expression on endothelial cells, reinforces the view

that an intercellular exchange of lactate occurs within the adult brain. It may be concluded from these observations that the complementary distribution of LDH isoforms and MCTs in astrocytes and neurons supports the view that astrocytes may supply neurons with lactate.

Hypoglycemic brain injury

Hypoglycemia[1] causes brain dysfunction followed by brain injury (Siesjö, 1988; Clarke and Sokoloff, 1999). The question of how hypoglycemia affects the brain has been only partially answered, although it is well established that metabolic energy is required to maintain neurons' transmembrane Na^+ and K^+ gradients through activity of the electrogenic Na^+-K^+-ATPase pumps (Siesjö, 1988). This phenomenon establishes a direct relationship between metabolic activity and membrane potential, which is critical to axon conduction, and explains why pathological states that interfere with metabolism, e.g., hypoglycemia, may cause depolarization of cells and a massive efflux of K^+ to the extracellular space (Siesjö, 1988).

Neurons appear to be more sensitive to irreversible injury due to glucose deprivation than are axons (Auer, 1986; Siesjö, 1988). Neurons are injured by the excessive release of glutamate and consequent pathological calcium entry (i.e., excitotoxicity through activation of NMDA receptors), as is the case under ischemic

[1] The term "hypoglycemia" is most precisely defined as abnormally diminished concentration of glucose in the *blood*. Although all experiments described herein were done *in vitro*, the terms "hypoglycemia," "aglycemia," "glucose withdrawal," and "glucose deprivation" are used

conditions (Choi, 1988). In stark contrast, optic nerve axons, which are not injured even after prolonged exposure to high glutamate concentrations (Ransom et al., 1990), appear unlikely to be directly damaged by excitatory neurotransmitters during anoxia and ischemia (Stys, 1998). Despite the apparent noninvolvement of NMDA toxicity, the agent that appears to be common to anoxic/ischemic injury in both gray and WM is calcium, which can enter CNS axons through reverse Na^+/Ca^{2+} exchange or through voltage-gated calcium channels (Stys et al., 1991; Fern et al., 1995).

As is the fate that befalls gray matter regions, WM axons deprived of energy substrate suffer collapse of ion gradients as ATP is depleted and consequently activity of the Na^+-K^+-ATPase pumps ceases (Ransom and Fern, 1997). This injury caused by glucose withdrawal, as with anoxia/ischemia, appears to be mediated by pathological calcium entry (Brown and Ransom, 1999). Because central axons can extend for great distances from their cell bodies of origin, they must depend on local production of ATP to maintain ion gradients and sustain energy-consuming functions. This metabolic isolation also means that axons suffer energy withdrawal in a manner that is independent of neuron cell bodies (Ransom et al., 1993). A notable difference between hypoglycemia-induced brain injury and injury seen with ischemia or anoxia is that hypoglycemia injures in the absence of acidosis (Siesjö, 1988). This may be explained in part by the ability of tissue with adequate oxygen

---

interchangeably, in all instances implying superfusion of tissue with glucose-free artificial CSF (aCSF).

tension to metabolize lactic acid oxidatively by shuttling it through the citric acid cycle, thereby preventing a pathological accumulation of organic acid.

Brain energy metabolism: the role of astrocytic glycogen

A crucial question about brain energy metabolism remains unanswered: What is the role of glycogen in the brain? From a disease perspective, a corollary question is of great interest: Does astrocytic glycogen benefit neuronal function and survival during glucose withdrawal? Experiments described in this Dissertation tested these and related questions.

Brain glycogen content is highly regulated. Neurotransmitters, including norepinephrine, vasoactive intestinal peptide, adenosine, histamine, and serotonin, which increase intracellular cyclic AMP, all activate glycogen phosphorylase and cause a rapid drop in astrocyte glycogen content (Magistretti et al., 1993) in glia. Glutamate, on the other hand, increases glucose uptake, glycogen synthesis, and lactate release by glial cells (Swanson, 1992). Insulin and insulin-like growth factor increase astrocytic glycogen content (Dringen and Hamprecht, 1992). The content of glycogen in the brain can be substantially increased by anesthetics (Phelps, 1972), possibly because of diminished neural activity, and by methionine sulfoximine (Phelps, 1975; Swanson et al., 1990).

The ability of brain glycogen to serve as a physiologically relevant energy source is not understood. Glycogen turns over rapidly in the brain, however, and

turnover is enhanced when adjacent neural activity is increased [(Swanson, 1992); see also (Orkand et al., 1973; Pentreath and Kai-Kai, 1982)]. It may be postulated that glycogen serves to provide fuel to the brain when glucose is in short supply. Indeed, astrocytic glycogen *in vitro* is rapidly degraded when glucose is withdrawn (Dringen et al., 1993), and glycogen falls rapidly *in vivo* during ischemia, with a time course that is closely related to depletion of ATP and accumulation of lactate (Swanson et al., 1989). These observations are consistent with glycogen's acting as a fuel source during glucose shortage.

In light of these findings, it might be speculated that glycogen can enhance the survival and function of brain tissue in the absence of glucose. Surprisingly, only a single study, performed using cultured cells, has tested this question. It was shown that neurons grown in astrocyte-rich cultures are less severely injured by glucose withdrawal than are neurons in astrocyte-poor cultures (Swanson and Choi, 1993). This benefit appeared to derive from the presence of greater amounts of glycogen in the astrocyte-rich cultures, since depleting the astrocyte-rich cultures of glycogen by incubation in norepinephrine prevented the benefit (Swanson and Choi, 1993). Two possible mechanisms for this protective effect were suggested but not tested: (1) Astrocytes themselves utilize the energy from glycogen breakdown to prevent the accumulation of toxic levels of glutamate (removing it by a sodium-gradient-dependent transporter), or (2) glycogen provides fuel to neurons to sustain their energy metabolism. The mechanism of this effect remains unknown.

Rat optic nerve (RON) as a CNS white matter model

Preliminary evidence indicates that the role of astrocytic glycogen in the CNS can be fruitfully investigated using an *in vitro* preparation of CNS WM, the isolated RON. Motivated by the very high incidence of WM involvement in stroke (Fisher, 1979) and the fact that WM damage contributes to the resulting neurological disability, a large body of work has pioneered the use of the RON to analyze the molecular and ionic mechanisms of central axonal injury (Ransom et al., 1990; Ransom et al., 1990; Ransom et al., 1993; Stys, 1998).

Much of the work looking at metabolic coupling between glia and neurons in gray matter uses stimulus-evoked field potentials in hippocampus to monitor neuron function and to determine when energy withdrawal has led to the presumptive collapse of ion gradients across neuronal membranes to an extent that prevents conduction (see, for example, Schurr et al., 1988; Izumi et al., 1994; Izumi et al., 1997; Schurr et al., 1999). The experiments reported herein examining glial–axonal metabolic interaction CNS in WM use the adult RON. WM is attractive for these studies because of its relative simplicity. It contains no synapses or neuronal cell bodies and is homogeneously composed of glia and axons. The intact RON contains about 100,000 myelinated axons (Forrester and Peters, 1967; Foster et al., 1982) and is easily isolated and studied *in vitro*. A RON's function, in the form of compound action potentials (CAPs), can be monitored quantitatively throughout an experiment.

The use of CAPs to evaluate optic nerve function is based on a technique pioneered by Erlanger and Gasser (Erlanger and Gasser, 1937), who worked initially with frog sciatic nerve. They found that the action potentials of the individual axons in a nerve sum to give the CAP. It was later demonstrated that the area under the CAP reflects the number of active, conducting axons in the nerve (Cummins et al., 1979), and this method is used to monitor RON function (see, for example, Stys et al., 1991).

Chapter 2: Research design and methods

Preparation

Long Evans rats aged 50–80 days were deeply anesthetized with CO_2 and then decapitated. The optic nerves were exposed by lifting the cerebral hemispheres, and the nerves (5–10 mm long) were cut at the optic chiasm and behind the orbit. The optic nerves were gently freed from their dural sheaths and placed in an interface perfusion chamber (Medical Systems Corp, Greenvale, NY, USA; see also Stys et al., 1990). Nerves were maintained at 37°C and perfused with artificial cerebrospinal fluid (aCSF) that contained (in mM): 153 Na^+, 3 K^+, 2 Mg^{2+}, 2 Ca^{2+}, 143 Cl^-, 26 HCO_3^-, 1.25 HPO_4^{2-} and 10 glucose. The aCSF was bubbled with an O_2-free gas mixture (95% N_2, 5% CO_2) to maintain pH at 7.45. The tissue was oxygenated by a humidified gas mixture (95% O_2, 5% CO_2) that flowed over its surface. Suction electrodes filled with aCSF were attached to the nerve for stimulation and recording of the compound action potential (CAP). Stimulus strength was adjusted to evoke the maximum amplitude CAP and then increased another 25% to ensure that stimulus strength was always supramaximal. Nerves were allowed to equilibrate for 60 min before recording commenced. When nerves were exposed to glucose-free aCSF, the solution in the stimulating and recording electrodes was switched to glucose-free aCSF. The area under the triphasic supramaximal CAP (see Figs. 3.2.B and 4.1.C) was used as a quantitative estimate of all the contributing axons in a nerve (Cummins et al., 1979; Stys et al., 1991; Wijesinghe et al., 1991). CAP area was

represented as a normalized value: CAP area was set to 1.0 at the point just prior to the start of perfusion with a test solution. In most experiments, except as noted, nerves were allowed to equilibrate for 1 hr in control aCSF (i.e., containing 10 mM glucose) after dissection and before beginning recording.

For the experiments described in Chapter 3 that tested the ability of substrates to support axon conduction for 120 min, a supramaximal CAP was evoked and recorded every 30 s for the duration of the experiment. The suction electrodes were filled with the appropriate test aCSF used in that experiment. For this set of experiments, data were acquired online (Axon Instruments, Digidata 1200A) using proprietary software (Axon Instruments, Axotape). CAP area was calculated using Clampfit (Axon instruments). For experiments testing the ability of a substrate to substitute for glucose by measuring recovery, we continuously recorded from only one of six nerves placed in the recording chamber. The five other nerves from each of these experiments had CAPs recorded at two discrete times: immediately before the substitution (control CAP) and 120 min later (recovery CAP). CAP areas for these experiments were determined using an electrophysiological technique that corrected for variation in electrode impedance (Stys et al., 1991), and CAP recovery was expressed as a percentage of the baseline CAP area.

For experiments described in Chapter 4, optic nerve CAPs were monitored continuously by leaving suction electrodes attached to the ends of the nerve for the entire duration of each experiment (and thus only one nerve was used per experiment in this phase of the study). The suction electrodes were filled with whatever test

aCSF was used in that experiment, e.g., for control experiments (perfusion of nerves for 60 min with control aCSF followed by 60 min of glucose deprivation and a 60-min recovery period), the electrodes were filled with glucose-free aCSF. For these experiments, the control experiment led to 45% recovery as opposed to the 15% seen with the Chapter 3 "recovery" technique. We believe that this is explained by probable diffusion of some bath glucose up into the electrodes while a 10-min baseline was being acquired in control aCSF, which would expose the nerve to a moderate amount of glucose for some period into glucose withdrawal before the glucose in the electrodes diffused back out into the bath. We determined this to be an acceptable trade-off for the ability to obtain continuous CAP measurements, and all experiments in each series (i.e., Chapters 3 and 4) were internally consistent. For the Chapter 4 experiments, data were acquired online (Axon Instruments, Digidata 1200A) using proprietary software (Axon Instruments, Axotape). CAP area was calculated using Clampfit (Axon instruments). A curve-fitting protocol was used for data interpretation in these experiments, as described below.

Curve fitting

To standardize data interpretation for experiments described in Chapter 4, a mathematical approach was employed to analyze CAP area. This approach is based on the Boltzmann equation because, as illustrated in Fig. 4.1.D, plotting CAP area against time during glucose removal (our standard insult) resulted in a trace that can

be resolved into two sigmoidal curves, each of which can be fit by a Boltzmann function, one with a negative (falling) slope and the other with a positive (rising) slope. Our goal was to use the Bolzmann equation to precisely define the point of CAP decline and the maximum amount of CAP recovery (Fig. 4.1.D). The Boltzmann relationship is described by:

$$y = \frac{\max}{1 - \exp\left(\frac{V - t}{k}\right)}$$

where y is the area under the CAP curve, *max* is the maximum value of the described sigmoidal (designated max_1 for the first sigmoidal, of negative slope, and max_2 for the second sigmoidal, of positive slope), V is the time at which the CAP area is 50% of max, t is the time, and k is the slope at point V. It is important to note that max_1 and max_2 are not necessarily the highest CAP area values from the baseline and recovery periods, respectively, but rather are the maximum values of the described sigmoidals. The minimum value for any data set is defined by the function as zero. The solid red line superimposed on the data set shown in Fig. 4.1.D was generated by fitting the Boltzmann equation to the data. The break in the curve, indicated by the downward pointing vertical arrow, identifies the point at which the equation has identified the zero point. The equation is applied separately to the second curve (of positive slope). In this case, the maximum point as determined by curve fitting will be represented by max_2, which describes CAP recovery. By applying this equation to

every data set, it was possible to calculate the latency of onset of CAP decline, defined as $t = 0.95 \times max_1$, and CAP recovery, defined as $(max_2/max_1) \times 100\%$.

Test solutions

Test solutions contained variable amounts of glucose or glucose substitutes. No osmotic adjustment was made in solutions with decreased glucose concentration; maximum osmotic variation, however, was calculated to be no more than about 3%. When glucose substitutes were in the form of sodium salts (e.g., sodium lactate), the aCSF was adjusted to maintain constant osmolarity by decreasing the amount of NaCl as appropriate. In instances where a free acid was used, the solution's pH was brought back up to 7.45 using NaOH. t = 0 min was set as the time when solutions were changed (e.g., from control aCSF to some test solution). For experiments described in Chapter 3, using a dye solution, it was determined that perfusate exchange in the bath was complete within 5 min of changing the inflowing solution; therefore, continuous recordings for "recovery" experiments (see, for example, Figs. 3.4.A and 3.5.A) were started at t = 10 min. Glucose and other sugars and β-hydroxybutyrate were in all cases D-isomers, and monocarboxylates and amino acids were L-isomers

Lactate transport blockers used in the experiments described in Chapter 4 were dissolved as follows: quercetin, 50 mM stock in 100% ethanol, dissolved to a final concentration of 0.1% ethanol; α-cyano-4-hydroxycinnamic acid (4-CIN), 250

mM stock in 100% methanol, dissolved to a final concentration of 0.06% methanol; and *p*-chloromercuribenzene sulfonic acid (*p*CMBS), dissolved directly in aCSF with no other vehicle.

All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Ion-sensitive microelectrodes

Ion-sensitive microelectrodes were made with double-barreled piggyback glass. Electrodes were pulled on an upright puller, producing tips of about 1 μm in diameter. These were subsequently beveled to a tip diameter of 2–5 μm. The tip of the ion-sensitive barrel was filled with hexamethyldisilazane and baked at 160°C for 15 min. The indifferent barrel was back-filled with 1 M sodium acetate + 30 mM NaCl (pH electrodes) or 150 mM NaCl (K^+ electrodes), and the ion-sensitive barrel was back-filled with 140 mM NaCl + 20 mM HEPES adjusted to pH 7.0 with 1 M NaOH (pH electrodes) or 150 mM KCl (K^+ electrodes). The ion-sensitive barrel was filled at the tip by applying light suction with a short (100–400 μm) column of H^+-sensitive liquid ion sensor (Fluka ionophore I, cocktail A, 95291) for pH electrodes and K^+-sensitive liquid ion sensor (Fluka K^+ ionophore I, cocktail B, 60398) for potassium electrodes. Electrodes were calibrated in solutions of pH 7.0 and 8.0 containing 140 mM NaCl + 20 mM HEPES (pH electrodes) or in solutions of 2 mM KCl and 20 mM KCl in NaCl so that $[Cl^-]$ was a total of 150 mM (K^+ electrodes). All electrodes were individually calibrated, and only those showing stable, near-

Nernstian responses (i.e., 50–60 mV) to decade changes in pH or a 10-fold change in [K^+] were used for experimental measurements. Electrodes were recalibrated after the recording. The average between the initial and final calibration responses was used to evaluate the experimental measurement. Ion-sensitive microelectrodes were connected via chlorided wires to an amplifier. The ion-sensitive barrel was connected via a high-impedance headstage, and the indifferent signal was subtracted from the ion-sensitive signal using a differential amplifier. Signals were amplified 50×, filtered at 1 Hz, and acquired at 1 Hz. Time of rise of pH or K^+ was taken as the time at which pH had increased by +0.02 pH units over baseline or when [K^+] had increased by 0.15 mM over baseline.

Transmission electron microscopy

Adult male Long Evans rats were deeply anesthetized with ketamine/xylazine (40/2.5 mg/kg body weight, i.p.) and perfused transcardially, first with a phosphate-buffered saline solution and then with a fixative solution containing 2% paraformaldehyde and 2% glutaraldehyde in 0.14 M phosphate buffer, pH 7.4. Optic nerves were carefully freed and placed in fresh fixative overnight at 4°C. Tissue was then rinsed several times in 0.14 M phosphate buffer, post-fixed in 1% OsO_4 and 1.5% potassium ferrocyanide in 0.14 M phosphate buffer for 3 hr at 4°C, and rinsed several times in phosphate buffer. The nerves were then dehydrated in a graded

ethanol series and embedded in Epon. Silver-gray sections were cut with a Reichardt Ultrocut E and contrasted with uranyl acetate and lead citrate.

Glycogen and protein assays

Nerves were removed from the standard experimental chamber after various manipulations and were immediately placed in 3 mL ice-cold 85% ethanol / 15% 30 mM HCl. This instantly stops glycogen metabolism. The tissue (in solution) was stored at −20°C until assays were performed.

Assays were carried out as previously described (Swanson and Choi, 1993). Briefly, the nerves in the ethanol/HCl solution were warmed to room temperature, and the tissue was gently agitated for several hours to permit egress of all glucose. (Glucose is soluble in this solution but glycogen is not.) Each determination required four optic nerves (~ 8 mg tissue total). The nerves were transferred to 0.3 mL 30 mM HCl and sonicated to suspension. 50 μL of the suspension was removed and added to 200 μL of 0.1 N NaOH for protein assay using the Lowry method, in triplicate (Lowry et al., 1951). The remainder was divided into two 100-μL fractions. Glycogen was determined by the amyloglucosidase method of Passonneau and Lauderdale (Passonneau and Lauderdale, 1974). Amyloglucosidase completely hydrolyzes glycogen to glucose. One of the two 100-μL fractions (fraction A) was treated with amyloglucosidase, and the other (fraction B) was not. Glucose in both fractions was then quantified by the glucose-6-phosphate dehydrogenase / NADH

fluorescence method. Glucose in fraction B, which reflects endogenous true glucose in the nerves, was subtracted from glucose in fraction A, which reflects the sum of endogenous glucose and glucose derived from glycogen hydrolysis, to yield glycogen expressed as glucosyl equivalents. In practice, the soaking of the nerves in the ethanol/acid solution removes all detectable glucose, such that glucose measured in fraction B was negligible and all of the glucose measured in fraction A reflects hydrolyzed glycogen. Standards were prepared either from glucose or from rabbit liver glycogen after desiccation at 120°C. These are found to be equivalent, i.e., the desiccated glycogen digested with amyloglucosidase yields almost exactly the predicted amount of glucose.

Data analysis

For experiments described in Chapter 3, percent CAP recovery after 60 min exposure to a test solution followed by a 60-min recovery period was compared with percent CAP recovery after 60 min exposure to 0 mM glucose without an added metabolite, using analysis of variance (ANOVA) evaluation.

Data for all experiments are presented as means and standard error of the mean. Significance was determined in all cases by ANOVA using Tukey's post-test, where $p < 0.05$ was taken to indicate statistical significance. Analysis was performed using GraphPad Prism version 2.0.

Chapter 3: Metabolic substrates other than glucose support axon function and improve recovery from hypoglycemia in central white matter

INTRODUCTION

Energy metabolism in the adult mammalian brain is supported almost exclusively by the glucose extracted from blood (Siesjö, 1978; Auer, 1986; Clarke and Sokoloff, 1999). The adult human brain requires about 120 grams of glucose daily, accounting for most of the 160 grams of glucose needed by the whole body (Stryer, 1995). Not surprisingly, lack of blood glucose rapidly disturbs brain function and, if persistent, destroys brain tissue (Sieber and Traystman, 1992). Only mannose has been shown to be a direct substitute for glucose as a brain energy fuel (Sloviter and Kamimoto, 1970). The brain's inability to use other substrates to support its energy metabolism is a consequence of the blood-brain-barrier (BBB), which allows only certain substances, including glucose and mannose, to enter the brain parenchyma.

In the absence of the BBB, nervous tissue can use other substrates, including lactate, to maintain proper levels of high-energy phosphate compounds (McIlwain, 1953; Izumi et al., 1994; Larrabee, 1995; Larrabee, 1996; Tsacopoulos and Magistretti, 1996; Izumi et al., 1997; Schurr et al., 1997; Schurr et al., 1999). This is important because a number of substrates may be available within the brain to

support energy metabolism, including fatty acids, ketone bodies (Nehlig, 1996), amino acids, and Krebs cycle intermediates (Schousboe et al., 1997). For example, in the guinea pig retina glucose is converted by glial cells to lactate, which is passed via the extracellular space to neurons (Poitry-Yamate et al., 1995), which use the lactate as an energy source (see also Tsacopoulos et al., 1994). Moreover, in the brain, the carbohydrate storage molecule glycogen is localized in astrocytes (Cataldo and Broadwell, 1986) and is thought to be shared with neighboring neural elements. Current evidence suggests that astrocytic glycogen is exported to neural elements as something other than glucose (Poitry-Yamate et al., 1995; Tsacopoulos and Magistretti, 1996; Wiesinger et al., 1997).

Our laboratory studies energy metabolism in white matter (WM), an area of the brain consisting of axons and glial cells (Waxman and Ritchie, 1993; Ransom and Fern, 1997). Axons are independent of neuron cell bodies in the manufacture of ATP [i.e., they have mitochondria (Peters and Palay, 1976) and maintain ATP levels in the absence of their cell body of origin]. In contrast to neuronal cell bodies, little is known about what energy substrate molecules can fuel axons (Vega et al., 1998). This is not a trivial question because the expression of specific uptake mechanisms for substrates and metabolic enzymes may be different in axons compared with cell bodies. To address this question, we tested a wide range of substances for their ability to sustain axon function and prevent irreversible injury in the absence of glucose. The compounds chosen are theoretically able to enter primary energy pathways (**Fig. 3.1**). Our results indicate that several substrates, including lactate

and pyruvate, were able to maintain axon function and prevent injury in the absence of glucose.

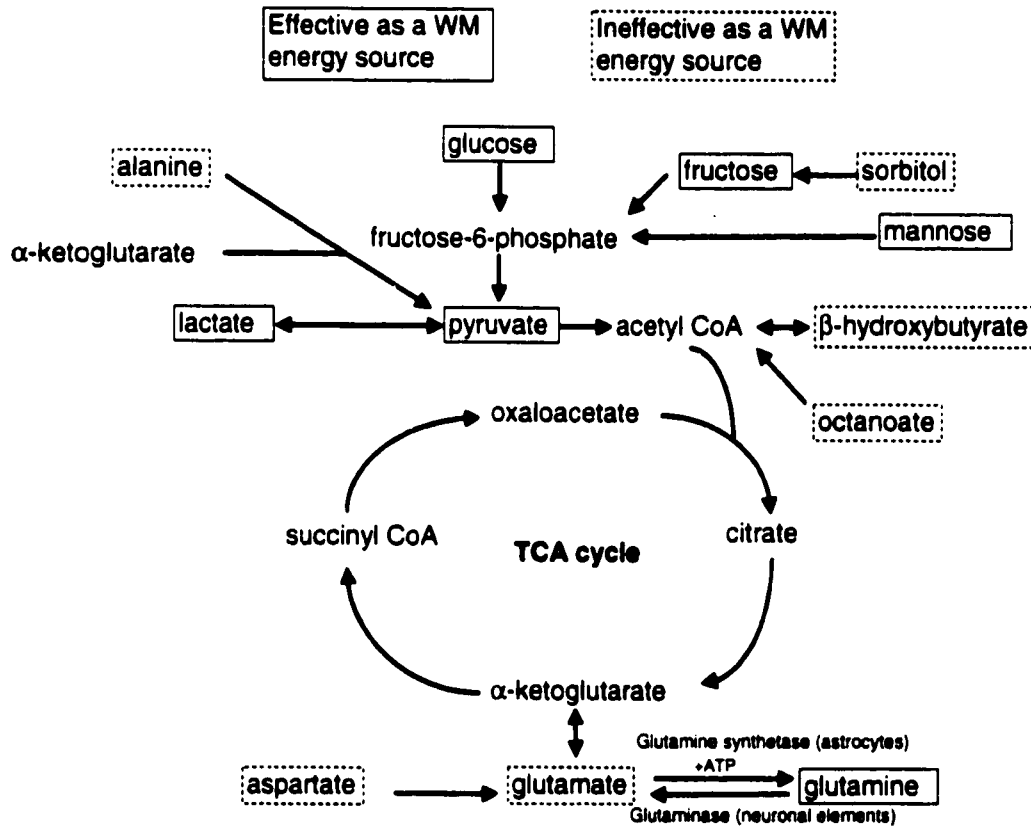


Figure 3.1. Biochemical pathways by which tested substrates might be used to generate energy in the RON in the absence of glucose.

RESULTS

Ability of glucose and other substrates to support CNS axon function

We determined the ability of glucose and other substrates to sustain RON axon function by monitoring CAP area every 30 s for 120 min in the presence of the test

substrate; as with all experiments reported here, temperature was maintained at 37° C. In the absence of glucose or another substrate, CAP area fell to zero in about 50 min (**Fig. 3.2.A**). Glucose (10 mM) supported RON axon function over the 120 min test period with no decline in CAP area (Fig. 3.2.A), as expected (Stys et al., 1991).

(continued on next page...)

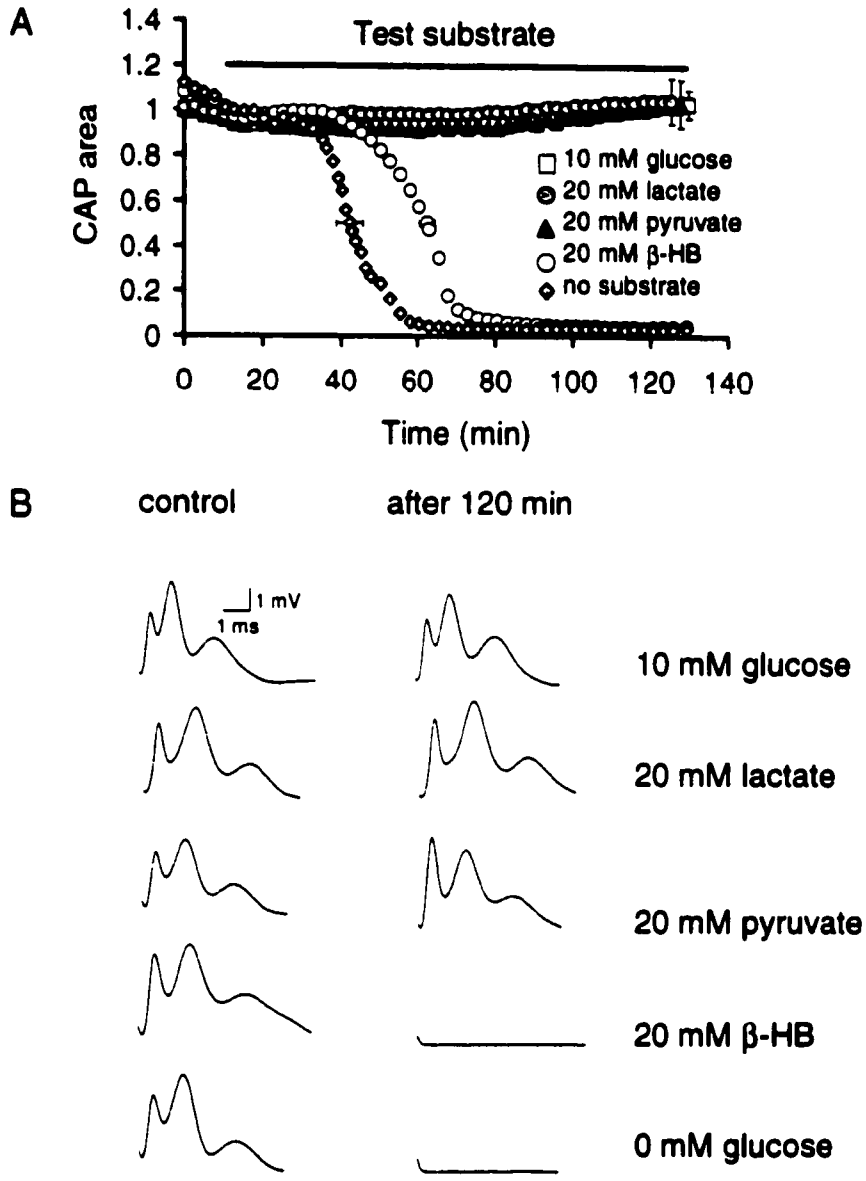


Figure 3.2. Comparison of the ability of monocarboxylates and glucose to support axon function. (A) In substrate-free aCSF, the CAP fell to zero by approximately 50 min. 10 mM glucose, 20 mM lactate, and 20 mM pyruvate supported axon function equally well for 120 min. 20 mM β-HB did not support conduction for the full period tested, but CAP area fell more slowly (latency to 0.5 CAP area = 50.9 ± 1.9 min with β-HB, 33.7 ± 3.4 min without substrate, $p < 0.01$). Traces are averages of four nerves in all conditions; SEMs at 130 min are shown in staggered fashion for clarity (SEM bars are too small to be visible for β-HB and "no substrate"). CAP area is shown as a normalized value (see Methods). **(B)** Representative CAPs recorded at 10 min ("control") and at 130 min ("after 120 min") demonstrating the effectiveness of each substrate to support axon function for 120 min.

Lactate (Larrabee, 1983; Schurr et al., 1988; Izumi et al., 1994), pyruvate (Izumi et al., 1997), or β-hydroxybutyrate (Edmond et al., 1987; Auestad et al., 1991) can be effective energy sources for gray matter or glia. We determined whether these compounds could serve as energy sources for CNS WM. Nerves were perfused with either 20 mM lactate, 20 mM pyruvate, or 20 mM β-hydroxybutyrate (β-HB) for 120 min in the absence of glucose. These compounds were tested at 20 mM concentrations because 10 mM glucose can be metabolized to 20 mM lactate or pyruvate. β-HB was used at 20 mM for consistency. Lactate or pyruvate at 20 mM sustained axon function as well as did 10 mM glucose (Fig 3.2.A and **3.2.B**). β-HB was not able to sustain the CAP for the duration of the test period: in the presence of 20 mM β-HB, CAP declined to zero in about 65 min (Fig. 3.2.A). The latency to 0.5 CAP area, however, was significantly prolonged in the presence of β-HB compared with no substrate ($p < 0.05$).

Fructose and mannose may also serve as energy sources under certain conditions (Siesjö, 1978; Wiesinger et al., 1997). We determined their effectiveness as energy sources in CNS WM. Fructose or mannose (10 mM) were able to sustain axon function as well as did 10 mM glucose **(Fig. 3.3.A).**

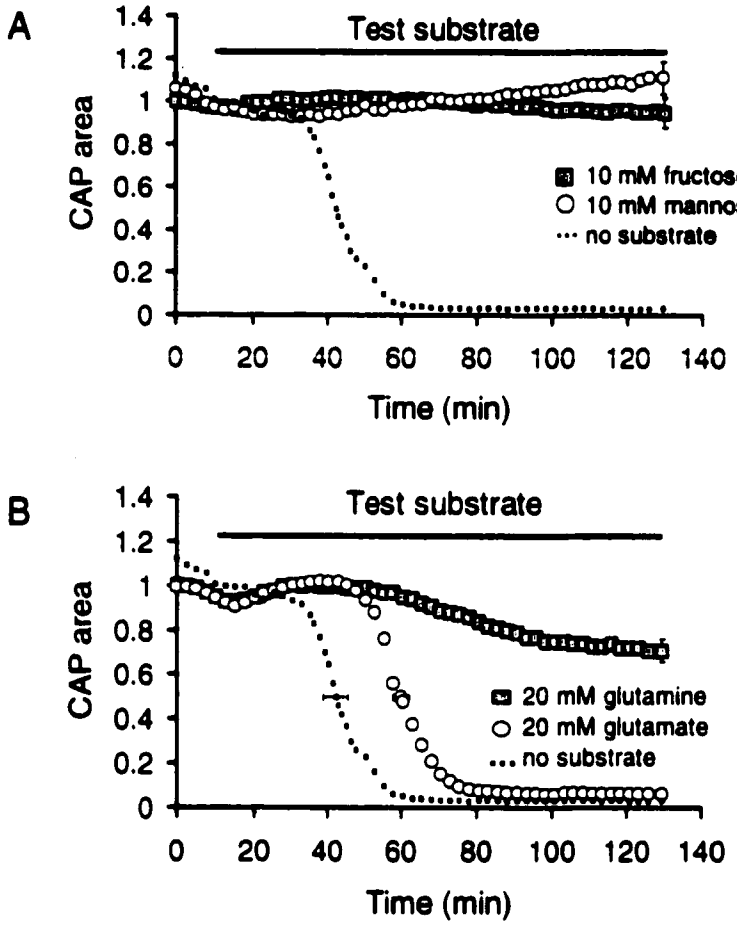


Figure 3.3. Comparison of the ability of monosaccharides and amino acids to support axon function. (A) 10 mM fructose or 10 mM mannose effectively supported axon function for 120 min. The behavior of CAP area in substrate-free aCSF is indicated by the dotted trace. (B) 20 mM glutamine partially supported axon function. 20 mM glutamate failed to sustain the CAP, but CAP amplitude fell more slowly with glutamate than in the absence of substrate (latency to 0.5 CAP area = 50.8 ± 2.2 min with glutamate, 33.7 ± 3.4 min without substrate, $p < 0.01$). Traces are averages of four nerves in all conditions.

The amino acids glutamine and glutamate can both enter the Krebs cycle, and transporters for both are expressed in neurons and glial cells (Lee et al., 1996; Tamarappoo et al., 1997). Glutamine provided partial support for axon function. After 120 min of perfusion with 20 mM glutamine, CAP area was 68.0 ± 5.0% of

baseline (n = 4, Fig. **3.3.B**), significantly less than with 10 mM glucose ($p < 0.05$). Although glutamate significantly delayed the decline of CAP area compared with no substrate ($p < 0.05$), CAP area in the presence of glutamate fell to zero in about 70 min (Fig. 3.3.B).

Other substances tested for their ability to sustain CAP function in the absence of glucose were sorbitol, octanoate, alanine, and aspartate. They were not able to sustain CAP area, which fell to zero within 60 min. **Table 1** summarizes these experimental results.

TABLE 1. CAP area after 120 min exposure to test substrates

Substrate	Percent CAP area at 120 min†
Glucose	102.8 ± 6.2***
Lactate	105.3 ± 10.0***
Pyruvate	103.0 ± 10.5***
β-hydroxybutyrate	4.0 ± 0.0n.s.
Fructose	95.0 ± 7.1***
Mannose	111.5 ± 7.6***
Glutamine	71.8 ± 5.2***
Glutamate	6.0 ± 0.7n.s.
Alanine	‡
Aspartate	‡
Octanoate	‡
Sorbitol	‡
No substrate	3.0 ± 0.4

†Percent CAP area at 120 min = [CAP area at 120 min / CAP area at 0 min] x 100

n.s.$p > 0.05$ and ***$p < 0.001$ versus no substrate

‡Nerves perfused with this compound had average CAP area = 0 by 60 min of perfusion and were not formally measured at 120 min

Ability of substrates to improve CAP recovery from glucose deprivation

Another approach was used to quantitatively evaluate a substrate's efficacy as an energy source for WM. Substrates, at different concentrations, were tested for their ability to prevent the functional injury that occurred when the RON was subjected to 60 min of glucose deprivation (**Fig. 3.4.A**; see also Ransom and Fern, 1997). After 60 min of complete glucose deprivation followed by 60 min in control aCSF, CAP area recovered to 14.6 ± 1.8% of control area (**Fig. 3.4.B**; n = 39).

(continued on next page...)

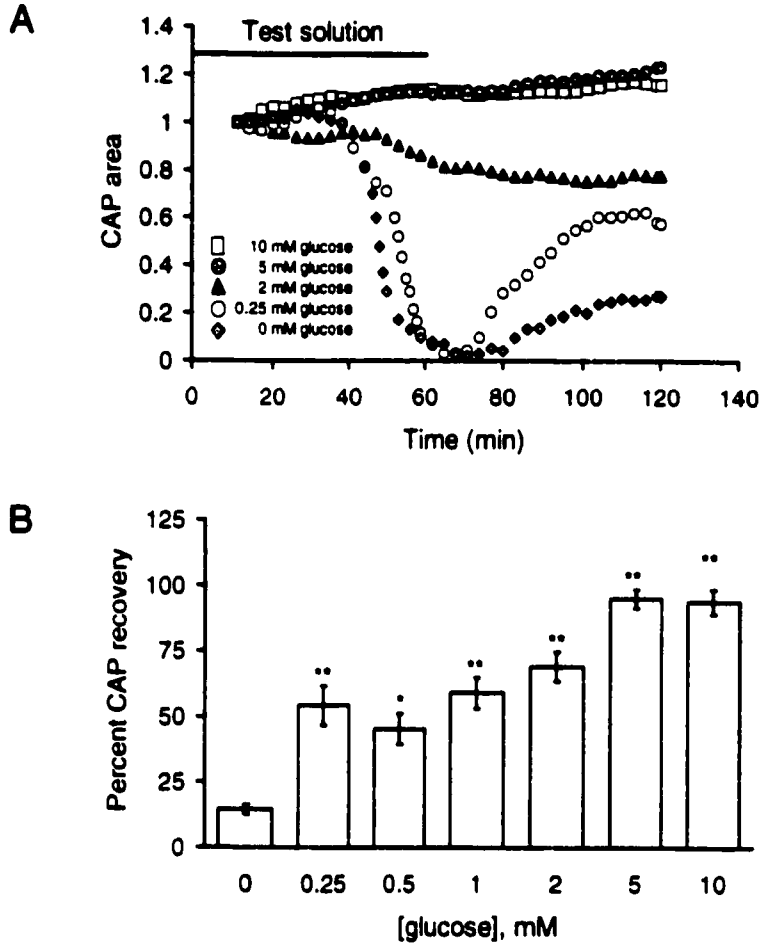


Figure 3.4. Efficacy of glucose at different concentrations to prevent functional injury when substituted for 60 min. (**A**) CAP areas were normalized at the beginning of the experiment (i.e., 10 min after starting perfusion with the test solution). Recordings from five representative nerves, each exposed to the indicated glucose concentration for 60 min ("Test solution"), are shown superimposed. Following the period of test solution exposure, the nerves were reperfused with 10 mM glucose. The amplitude of the CAP area at 120 min was operationally defined as "CAP recovery". (**B**) Graph showing percent CAP recovery [i.e., (CAP area at 120 min) / (CAP area at 0 min) x 100 = percent CAP area] measured at 120 min, 60 min after the exposure to the different glucose concentrations. Although 0.25 mM glucose did not sustain axon function, it significantly improved recovery. * indicates $p < 0.05$ and ** indicates $p < 0.001$ compared with 0 mM glucose. Each column represents a minimum of five nerves.

We first analyzed the ability of variable glucose concentrations during the deprivation period to affect the magnitude of CAP recovery (Fig. 3.4). Over the

range of concentrations tested, even 0.25 mM glucose significantly increased CAP recovery compared with 0 mM glucose ($p < 0.001$; Fig. 3.4.B). It should be noted that while low concentrations of glucose (e.g., 0.25 mM) improved CAP recovery, they did not maintain CAP area during the period of exposure (Fig. 3.4.A).

Using the protocol described above (see **Fig. 3.5.A**), RONs were perfused for 60 min with varying concentrations of lactate, pyruvate, or β-HB (2 mM to 20 mM) in glucose-free aCSF, followed by a 60-min period in control aCSF. **Figure 3.5.B** illustrates the relationship between monocarboxylate concentration and percent CAP recovery. Pyruvate and lactate significantly improved percent CAP recovery compared with no substrate at each concentration tested. β-HB also improved CAP recovery at all concentrations tested, except at 2 mM. Pyruvate and lactate produced significantly greater CAP improvement than did β-HB at each concentration ($p < 0.001$), except 5 mM.

(continued on next page...)

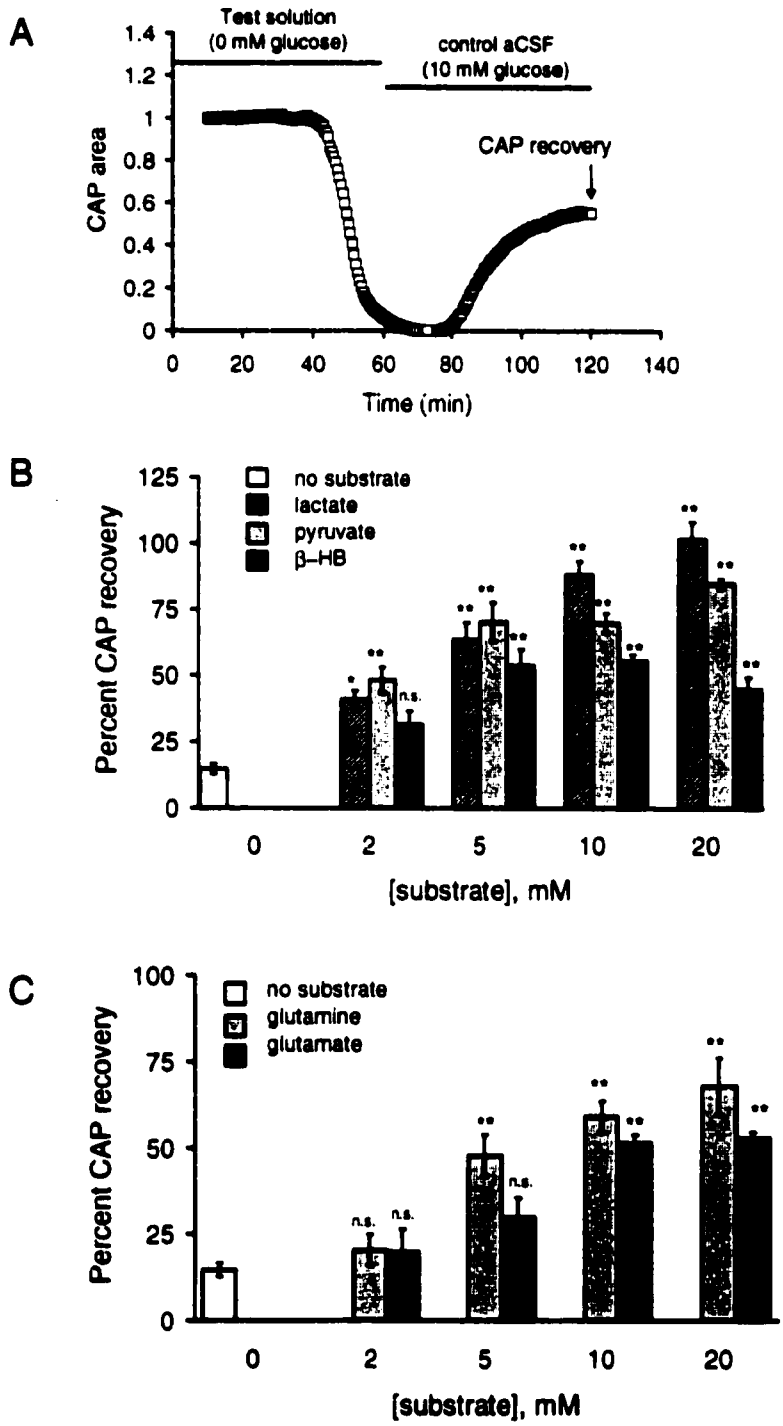


Figure 3.5. Efficacy of monocarboxylates or amino acids at different concentrations to prevent injury when substituted for glucose for 60 min. (A) The paradigm used in these experiments is illustrated. A substrate was substituted for glucose for 60 min ("Test solution"), and CAP area was measured at 120 min ("CAP recovery") after 60 min of recovery in aCSF with 10 mM glucose. (B) Graph showing percent CAP recovery after the 60 min exposure to different

monocarboxylate concentrations. (C) Graph showing percent CAP recovery measured at 120 min after exposure to different glutamine and glutamate concentrations. n.s. = not significant, * indicates $p < 0.05$, and ** indicates $p < 0.001$ compared with 0 mM glucose. Each column represents a minimum of five nerves.

Glutamine and glutamate were also tested for their ability to reduce irreversible injury after 60 min of glucose deprivation. The relationship between glutamine and glutamate concentration and percent CAP recovery is shown in **Fig. 3.5.C.** Glutamine significantly improved percent CAP recovery at concentrations of 5 mM and above. Glutamate improved percent CAP recovery at concentrations of 10 mM and above. There was no significant difference between glutamine's and glutamate's ability to support axon function at any concentration tested ($p > 0.05$). As with low concentrations of glucose, 10 mM or 20 mM glutamate failed to sustain CAP function but strongly improved percent CAP recovery. To allay concerns that glutamate may have induced some degree of receptor-mediated toxicity (Li et al., 1999), particularly in light of the high concentrations used, we perfused with 10 mM glutamate in the presence of the kainate/AMPA receptor antagonist CNQX (10 mM) and the NMDA receptor antagonist AP-5 (100 mM). CAP recovery was 35.1 ± 6.6% of baseline ($n = 10$) after perfusion with 10 mM glutamate/CNQX/AP-5, compared with 51.6 ± 6.4% ($n = 9$) after perfusion with 10 mM glutamate alone (data not shown; not statistically significant). The data for all tested substrates that improved CAP recovery are summarized in **Fig. 3.6.**

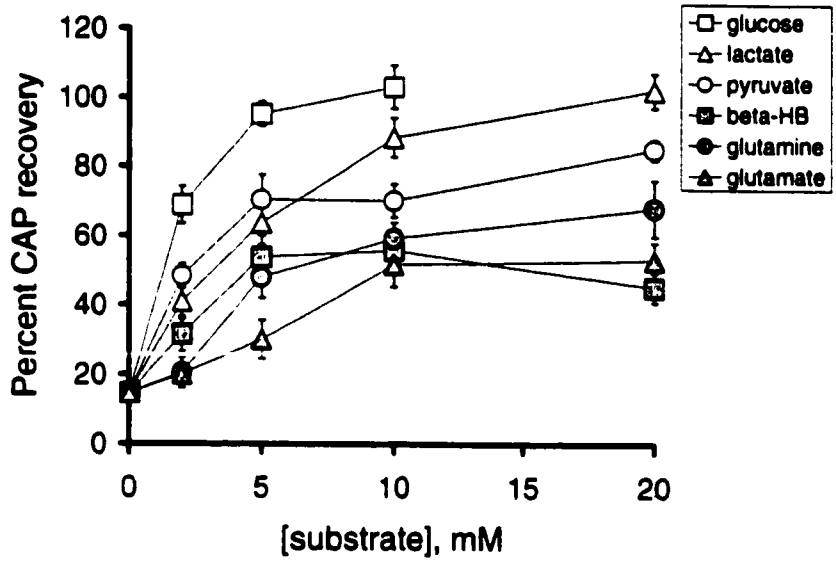


Figure 3.6. Comparison of percent CAP recoveries for different substrates at different concentrations. Percent CAP recovery is plotted against substrate concentration. Data taken from experiments such as those shown in Figs. 3.4 and 3.5.

A number of substrates failed to improve percent CAP recovery following aglycemia. Alanine (20 mM), which supports photoreceptor function in honeybee retina (Tsacopoulos et al., 1994), did not improve percent CAP recovery during glucose deprivation (percent CAP recovery = 25.7 ± 5.1%, n = 5, $p > 0.05$ vs. 0 mM glucose, data not shown). Similarly, L-aspartate (10 mM), which induces glycogen synthesis in cultured astrocytes (Swanson et al., 1990; Wiesinger et al., 1997), or octanoate (10 mM or 20 mM) or sorbitol (10 mM) were without benefit (data not shown).

DISCUSSION

We have hypothesized that in the absence of glucose, WM astrocytes provide energy substrate, perhaps in the form of lactate, to neighboring axons (Ransom and Fern, 1997). The goal of the present study was to advance this hypothesis by testing one of its important tenets, namely, that energy substrates other than glucose can support WM function. We tested compounds that were theoretically able to enter primary energy pathways if they could enter cells (Fig. 3.1). Our results indicated that WM axons could be functionally sustained for up to 120 min supported solely by lactate, pyruvate, fructose, or mannose. Glutamine was partially effective, which is to say that CAP area declined by about 25% over 120 min. While glutamine, glutamate, or β-HB was not able to sustain axon function as the sole energy source, these compounds clearly delayed CAP decline and improved CAP recovery following 60 min of aglycemia.

Glial-axonal interactions in the absence of glucose

Before considering our results further, it is important to briefly discuss the consequences of glucose deprivation on RON function as evaluated by changes in CAP area, because the effect of this aglycemic challenge on the CAP was the standard against which each of the alternative energy substrate molecules was compared. As discussed in detail elsewhere (Ransom and Fern, 1997), the persistence of normal RON function for 30–40 min following the onset of perfusion

with glucose-free aCSF (see Fig. 3.2) is evidence that an endogenous energy source can be utilized to temporarily support axon function. Evidence indicates that this energy source is glycogen (Dringen et al., 1993; Ransom and Fern, 1997; Wender et al., 1999). The consequences of glucose deprivation in RON appear to be impacted by astrocytic glycogen stores (Wender et al., 1999). Approximately 40 min after glucose withdrawal, the glycogen content of the RON falls to a low, stable level and the CAP is lost. Upregulation of RON glycogen content prolongs the CAP in the absence of exogenous glucose, while reducing glycogen accelerates CAP decline (Wender et al., 1999). These findings are among the observations that have led us to hypothesize that astrocytic glycogen is broken down to lactate and/or pyruvate, which is then shuttled to nearby axons where it serves as an energy source.

We tested a variety of molecules for their ability to sustain the CAP for 120 min in the absence of glucose. The persistence of the CAP depended upon axons' having access to a usable energy substrate, thereby allowing them to synthesize appropriate amounts of ATP to maintain normal ion gradients and membrane potentials. Our experiments did not allow us to determine whether the applied substrate was taken directly into axons and used to generate ATP, or whether the compound was taken up by astrocytes which then, secondarily, provided a usable energy substrate to the axons. The latter scheme is a realistic possibility, based on a variety of considerations. Some neural elements appear to prefer lactate to glucose for energy metabolism (Poitry-Yamate et al., 1995). Glial cells more than neurons have the capability of participating in gluconeogenesis, i.e., the production of glucose

residues from other compounds such as amino acids, lactate, or pyruvate (Wiesinger et al., 1997). Finally, the transport mechanisms for the uptake of potential energy substrate molecules, as well as the enzymes for metabolizing them, are probably differently distributed between neurons and glial cells (Wiesinger et al., 1997). In some instances, therefore, the substrate molecule may be taken up only by astrocytes and then be converted to a compound that can be exported to axons via the extracellular space. It is unlikely, however, that astrocytes would convert other substrate compounds to glucose. When glucose is formed inside (or enters) astrocytes it is immediately phosphorlyated to glucose-6-phosphate, which is impermeable and trapped within the cell (Stryer, 1995). Glucose release can proceed only in the presence of glucose-6-phosphatase (EC 3.1.3.9., D-Glucose-6-phosphate phosphohydrolase), which cleaves the terminal phosphate from glucose-6-phosphate. While astrocytes appear to possess glucose-6-phosphatase, the astrocyte form of this enzyme has a very high K_m value, in the millimolar range (Forsyth et al., 1993; Schmoll et al., 1997). It is unlikely that the concentrations of glucose-6-phosphate would ever reach concentrations suitable for the activation of the enzyme (Wiesinger et al., 1997).

Alternative energy substrates

The three-carbon monocarboxylic acids lactate and pyruvate, and the monosaccarides fructose and mannose fully supported axon function for 120 min in the absence of glucose. The capacity of pyruvate or lactate to support axon function was

concentration dependent; concentrations greater than 2 mM were effective, consistent with findings in hippocampus (Schurr et al., 1988). Neurons appear to express a powerful transport system for monocarboxylates (Bröer et al., 1997; Koehler-Stec et al., 1998). This transport system has also been demonstrated in peripheral axons (Schneider et al., 1993). Although it has not been specifically demonstrated in optic nerve axons, it is reasonable to assume that pyruvate and lactate were transported via this system directly into axons. Neurons also appear to express an isoform of lactate dehydrogenase [EC 1.1.1.27. *(S)*-Lactate:NAD^+ oxidoreductase; LDH1] that preferentially oxidizes lactate to pyruvate (Bittar et al., 1996). Thus lactate, as well as pyruvate, would have immediate access to the Krebs cycle in axons. These two compounds constitute desirable fuels because two molecules of either pyruvate or lactate provide nearly as much ATP under aerobic conditions as does one molecule of glucose. Our findings are compatible with the hypothesis that astrocytic glycogen is broken down to lactate for use by neighboring axons (Pellerin and Magistretti, 1994; Pellerin and Magistretti, 1996; Pellerin et al., 1998). It is also true that pyruvate could serve equally well as the shuttle molecule.

The ketone body β-hydroxybutyrate (β-HB) partially supported axon function, as might have been predicted from previous studies in gray matter and in culture (Edmond et al., 1987; Nehlig, 1996). This compound is also transported by the monocarboxylate transporter (Poole et al., 1996). It can be taken directly into axons, where it could combine with acetyl CoA to enter the Krebs cycle. Our studies

do not allow comment about whether β-HB would enter astrocytes in the RON preparation. It is known, however, that neurons and oligodendrocytes use ketone bodies threefold more efficiently than do astrocytes in neonatal cultures (Edmond et al., 1987). In contrast to the efficacy of β-HB, the fatty acid octanoate was ineffective in enhancing CAP recovery from glucose deprivation, consistent with prior findings that only astrocytes metabolize fatty acids (Bourre et al., 1983; Edmond et al., 1987; Auestad et al., 1991).

Glutamine and glutamate can provide energy by entering the Krebs cycle (Fig. 3.1). Transporters for both of these compounds are known to be expressed in both neurons and glial cells (Lee et al., 1996; Tamarappoo et al., 1997). Recent indirect evidence suggests that sodium-dependent glutamate transport may also occur in central axons (Li et al., 1999). We are unaware, however, of studies that have directly investigated the uptake of either glutamine or glutamate into central myelinated axons. Glutamine, but not glutamate, partially supported WM function during glucose deprivation for 120 min. Both compounds, however, improved CAP recovery when substituted for glucose for 60 min. This suggested that axons derived some metabolic benefit from these compounds, even if not sufficient to maintain full function. While it is possible that these compounds entered axons directly, it seems to us more likely that they were taken up predominantly by astrocytes. Glutamate is believed to be taken up and metabolized predominantly by astrocytes (Schousboe and Westergaard, 1995). Inside astrocytes glutamate and glutamine may be

converted to lactate. When 0.5 mM glutamate is applied to cultured astrocytes, even in the presence of glucose, about 10% of the exported lactate is derived from the glutamate (Sonnewald et al., 1996). A far greater amount of extracellular lactate may be generated by high concentrations of glutamate (McKenna et al., 1998), especially in the absence of glucose. To the extent that neurons generate more extracellular glutamate with activity, a linkage would exist between neuronal energy needs and lactate export by astrocytes. In this regard, it is of interest that glutamate is released from optic nerve axons with activity (Kriegler and Chiu, 1993). The failure of glutamate to support the CAP for 120 min could be a consequence of slowly developing toxicity (Li et al., 1999), but no toxicity was apparent for 60 min application periods (see Results).

Mannose and fructose were effective substitutes for glucose in maintaining normal WM function. The result with mannose was expected, given its known efficacy in sustaining whole brain function (Sloviter and Kamimoto, 1970). Mannose is taken up by the glucose transporter, which is expressed in both astrocytes and neurons (Dringen et al., 1994). Accordingly, mannose would be directly accessible to axons. Fructose, on the other hand, probably enters brain cells, including astrocytes, by a diffusional mechanism (Wiesinger et al., 1997). Fructose is actively transported only by the GLUT 5 isoform of the glucose transporter, which is expressed at very low levels in brain, mainly in microglia (Vannucci et al., 1997). While fructose and mannose might enter axons directly, it is well established that they enter astrocytes (Wiesinger et al., 1997). Astrocytes fed fructose or mannose

release lactate, which could support nearby axons. Lactate released from astrocytes in the presence of mannose or fructose was 90% or 30%, respectively, of that released in the presence of glucose (Dringen et al., 1994; Bergbauer et al., 1996). Finally, it is noteworthy that only mannose is able to substitute for glucose as a substrate for glycogen synthesis (Dringen and Hamprecht, 1993).

Sorbitol (10 mM) failed to support CAP function in the absence of glucose. This result was somewhat puzzling. Sorbitol can sustain cultured astrocytes by enzymatic conversion to fructose (Wiesinger et al., 1997) and fructose, in turn, should create lactate for export to the extracellular space. It remains unclear, therefore, why sorbitol was of no apparent metabolic benefit to the optic nerve. It may be that the enzyme sorbitol dehydrogenase (EC 1.1.1.14., L-Iditol:NAD^+ 2-oxidoreductase), which oxidizes sorbitol to fructose, is expressed more robustly in cultured astrocytes than *in vivo*.

Thus, we conclude that CNS myelinated axons can derive benefit from fuels other than glucose, as indicated by the ability of these substrates to support axon function during glucose withdrawal. The specific pathways by which these carbon sources are metabolized, i.e., whether they are used directly by axons or are first processed by glia, remains to be elucidated.

Chapter 4: Astrocytic glycogen stores influence axon function and survival during glucose deprivation in central white matter

INTRODUCTION

The ability of brain glycogen to serve as a physiologically relevant energy source is not understood. Glycogen turns over rapidly in the brain, however, and turnover is enhanced when adjacent neural activity is increased (Orkand et al., 1973; Pentreath and Kai-Kai, 1982; Swanson et al., 1992). It may be postulated that glycogen serves to provide fuel to the brain when glucose is in short supply. Indeed, astrocytic glycogen *in vitro* is rapidly degraded when glucose is withdrawn (Dringen et al., 1993), and glycogen falls rapidly *in vivo* during ischemia, with a time course that is closely related to depletion of ATP and accumulation of lactate (Swanson et al., 1989). These observations are consistent with glycogen's acting as a fuel source during glucose shortage but do not prove this hypothesis. Glycogen content varies by a factor of two or more between brain regions (it is highest in the brain stem and cerebellum and lowest in the striatum and WM (Swanson et al., 1989)). Energy metabolism also varies significantly between different brain regions (Sokoloff et al., 1977). Therefore, glycogen could be more protective in some areas than in others.

In light of these findings, it might be speculated that glycogen can enhance the survival and function of brain tissue in the absence of glucose. Surprisingly, only a single study, on cultured cells, has tested this question. It was shown that neurons grown in astrocyte-rich cultures are less severely injured by glucose withdrawal than are neurons in astrocyte-poor cultures (Swanson and Choi, 1993). This benefit appeared to derive from the presence of greater amounts of glycogen in the astrocyte-rich cultures. Depleting the astrocyte-rich cultures of glycogen negated the benefit (Swanson and Choi, 1993). Two possible mechanisms for this benefit were suggested but not tested: (1) Astrocytes themselves utilize the energy from glycogen breakdown to prevent the accumulation of toxic levels of glutamate (removing it by a sodium-gradient-dependent transporter); or (2) Glycogen provides fuel to neurons to sustain their energy metabolism.

We have studied the role of astrocytic glycogen in an *in vitro* preparation of CNS WM, the isolated rat optic nerve (RON). An advantage of this preparation is that axon function, as measured by CAP area, can be continuously monitored. Axon function persists for about 30 min in the absence of glucose (Ransom and Fern, 1997; Fern et al., 1998), suggesting the presence of an intrinsic energy reserve such as astrocytic glycogen. It is also known that the optic nerve, like other neural tissues, can survive on substrates other than glucose, making it feasible that a breakdown product of glycogen other than glucose could mediate energy transfer between astrocytes and axons (Schurr et al., 1988; Larrabee, 1995; Ransom and Fern, 1997). We tested the hypothesis that axon function and survival depend on astrocytic

glycogen when glucose is withdrawn. Our results indicated that glycogen content strongly affected the duration of function and survival of axons following glucose removal and that lactate was probably the molecule that shuttled from astrocytes to axons to mediate energy transfer.

RESULTS

The effects of glucose deprivation on CAP area in adult RONs are shown in Fig. 4.1. CAPs were evoked every 30 s. During a 60-min period of glucose withdrawal, the CAP was maintained for 28.8 ± 2.1 min (n = 15) before it began to fail (**Fig. 4.1.A**). It fell rapidly from that point to zero. The CAP recovered to 45.3 ± 3.7% (n = 15) of the control CAP after a 60-min recovery period in normal aCSF (i.e., containing 10 mM glucose), indicating that irreversible injury had occurred. This agreed with previously published results (Ransom and Fern, 1997). Glucose deprivation led to a prominent rise in extracellular potassium, $[K^+]_o$, suggesting failure of Na^+/K^+ ATPase pumps, collapse of ion gradients, and resultant axon depolarization (**Fig. 4.1.B**). This coincided almost exactly with decline of the CAP: $[K^+]_o$ began to rise at 28.8 ± 2.0 min from a baseline of 2.55 ± 0.04 mM to a peak of 6.83 ± 0.64 mM (n = 6; error bars show s.e.m.). **Figure 4.1.C** shows representative CAPs from a nerve represented in Fig. 4.1.A (a) before removing glucose, (b) at the conclusion of 60 min of glucose deprivation, and (c) following maximum recovery. To quantify the effects of glucose withdrawal on the CAP, a curve-fitting protocol was adopted to

standardize analysis of latency to CAP decline and CAP recovery magnitude (**Fig. 4.1.D**; see Chapter 2 for details).

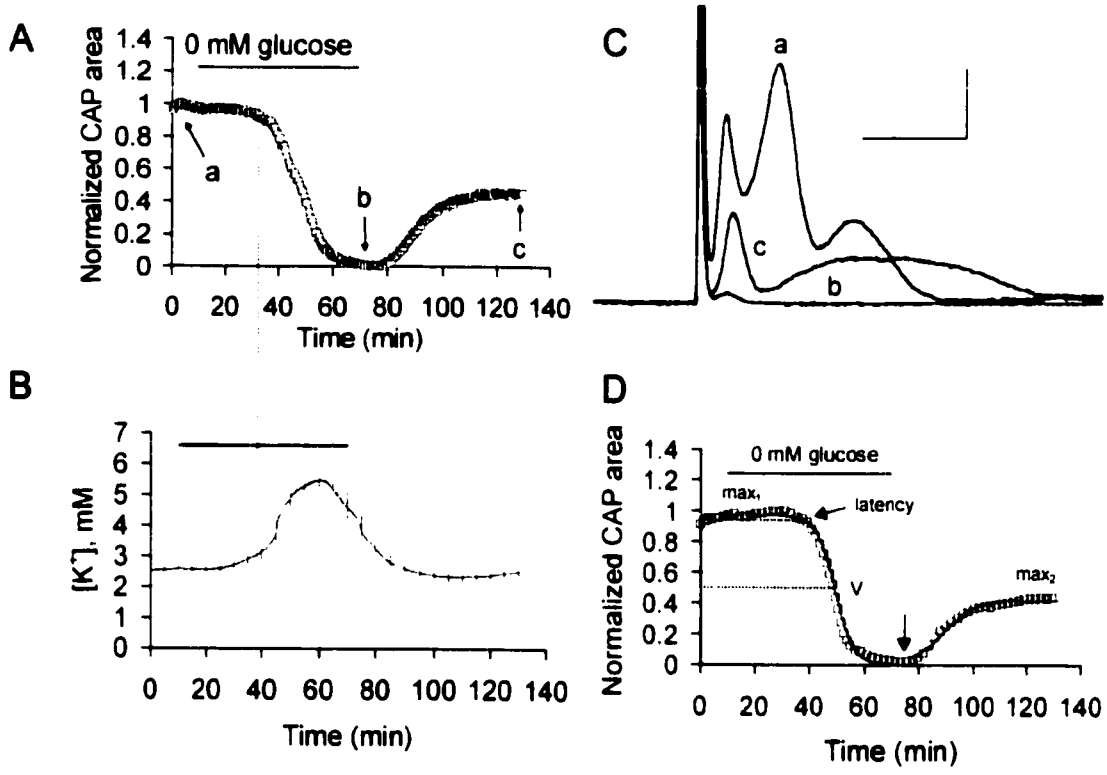


Figure 4.1. Effects of glucose withdrawal for 60 min on the rat optic nerve CAP area. **(A)** A 60-min period of glucose withdrawal caused failure of the CAP after about 30 min and resulted in incomplete CAP recovery. Each symbol represents the area of an individual CAP (evoked every 30 s). Averaged data from 15 nerves. **(B)** Glucose deprivation induced a steep rise in $[K^+]_o$, suggesting axon depolarization. The overbar indicates the period of glucose withdrawal. **(C)** Representative CAPs recorded from one of the nerves averaged in A. The recordings were taken at the time points indicated. Scale bars, 0.5 mV:1 ms. **(D)** Representative trace from an individual nerve included in A to illustrate the curve-fitting protocol used to quantify latency and recovery. For clarity, only every fourth data point is shown.

Ultrastructural identification of astrocytic glycogen in the RON

Electron-microscopy (EM) studies performed on perfusion-fixed RONs (**Fig. 4.2**) showed granules of glycogen located within most astrocytes. No glycogen was seen in axons or oligodendrocytes, and no attempts were made to quantify glycogen seen at the EM level. These results agreed with previous studies on other neural areas (Cataldo and Broadwell, 1986; Magistretti et al., 1993).

Figure 4.2. Ultrastructural evidence of glycogen deposition in astrocytes in adult rat optic nerve. Electron micrograph showing accumulations of glycogen granules (gly), which are approximately 20–25 nm in diameter, within processes of astrocytes (As) at the glia limitans (GL). Glycogen granules were not seen in axons or oligodendrocyges. Scale bar, 0.25 μm.

We feel confident that the electrondense particles located in glial end-feet are indeed glycogen, based on two observations. First, the only other structure with

which these might be confused is free ribosomes, which at 10–15 nm are too small to have accounted for the structures in Fig. 4.2 (which are 20–40 nm in diameter). Second, the pattern of glycogen distribution illustrated in Fig. 4.2 is consistent with what has been shown in previous ultrastructural studies (Peters and Palay, 1976; Cataldo and Broadwell, 1986; Clarke and Sokoloff, 1999), with some reports indicating that glycogen is especially abundant in astrocytic vascular end-feet (Maxwell and Kruger, 1965). This ultrastructural picture is consistent with *in vitro* experiments indicating that only astrocytes generate measurable quantities of glycogen (Dringen et al., 1993; Wiesinger et al., 1997).

Glycogen content of RON

The levels of glycogen in RONs under different conditions were determined by biochemical assay (**Fig. 4.3).** While glycogen content of RONs was relatively stable after 60 min of incubation in control aCSF containing 10 mM glucose (**Fig. 4.3.A:** compare the first and last bars), it declined sharply immediately after removal from the animal. In one set of experiments, it fell from 10.10 ± 0.72 pmol glycogen / μg protein ($n = 6$; data not shown) just after dissection to 4.85 ± 0.31 pmol glycogen / μg protein ($n = 6$; $p < 0.001$) in companion nerves that were perfused with control aCSF for 60 min. The absolute values of glycogen in the nerves under control conditions were sometimes variable between assays, but qualitatively similar results were obtained in all studies.

In one set of experiments, we investigated the effects of glucose removal on glycogen content. The glycogen content fell rapidly with glucose removal (Fig. 4.3.A). From a control value of 10.70 ± 0.45 pmol glycogen / μg protein (n = 6), glycogen fell to a low stable level at 30 min (2.69 ± 0.30 pmol glycogen / μg protein, n = 6). One group of nerves was allowed to recover for 60 min in control aCSF after the 60-min period of glucose withdrawal (cross-hatched bar at "120 min" in Fig. 4.3.A). Glycogen content in this group was not significantly higher than in nerves that were not given a recovery period (3.06 ± 0.33 pmol glycogen / μg protein, n = 6; $p > 0.05$ compared with nerves collected at 30, 45, and 60 min). The glycogen content of nerves that were simply perfused with control aCSF for the entire 120-min test period, with no period of glucose deprivation (right-hand bar at "120 min" in Fig. 4.3.A), was not significantly different from the control nerve glycogen content, but much greater than the glycogen content of nerves that had been exposed to glucose withdrawal (11.1 ± 0.89 pmol glycogen / μg protein, n = 6; $p < 0.001$ compared with the cross-hatched bar).

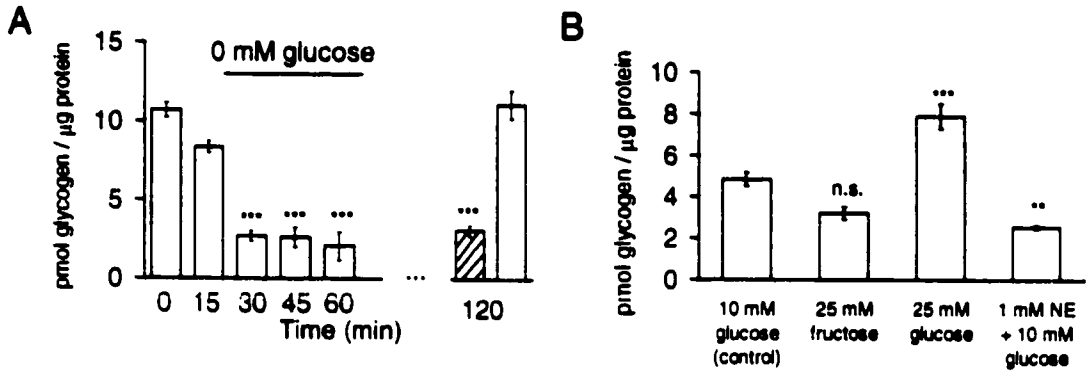


Figure 4.3. Glycogen content of rat optic nerves following glucose deprivation and pharmacological manipulation. **(A)** In the absence of glucose, glycogen content declined over time and reached a low stable level at 30 min. n = 6 for all conditions except 60 min (n = 4). Error bars indicate s.e.m. ***$p < 0.001$ compared with "0 min" and "15 min" groups. **(B)** Incubation of nerves with 25 mM glucose increased glycogen content, and incubation with NE caused glycogen to decline. Glycogen content in nerves pretreated with 25 mM fructose was not significantly different from control. n = 6 for each group except NE (n = 7). Error bars indicate s.e.m. n.s. = not significant, **$p < 0.01$, and ***$p < 0.001$ compared with control. Each measured value for all assays is from four pooled nerves.

As a crucial step to testing the effects of glycogen on RON function, we determined whether RON glycogen content could be modulated. These results are shown in **Fig. 4.3.B.** For this set of nerves, the control population incubated for 60 min in normal aCSF contained 4.85 ± 0.31 pmol glycogen / µg protein (n = 6). In other preparations, exposure to high glucose concentration increases glycogen content (Prasannan and Subrahmanyam, 1966; Swanson et al., 1989; Dringen and Hamprecht, 1992), while exposure to norepinephrine causes glycogen content to fall (Quach et al., 1978; Magistretti, 1988; Magistretti et al., 1993). Incubation of nerves in 25 mM glucose for 60 min caused up-regulation of glycogen stores to 7.90 ± 0.59 pmol glycogen / µg protein; conversely, pretreatment for 60 min with 1 mM

norepinephrine led to a decline in RON glycogen (2.56 ± 0.08 pmol glycogen / μg protein). Fructose can sustain the CAP in the absence of glucose (see Fig. 3.3.A), but does not lead to glycogen formation in astrocytes *in vitro* (Wiesinger et al., 1997). Nerves equilibrated for 60 min in 25 mM fructose had no change in glycogen content (3.23 ± 0.29 pmol glycogen / μg protein, $p > 0.05$) compared with control; the significance of this observation is discussed later.

Glycogen content and axon function

To determine whether RON glycogen content affected axon function during glucose withdrawal, the CAP was assessed during glucose withdrawal in nerves whose glycogen was up- or down-regulated (**Fig. 4.4**). Nerves with increased glycogen (i.e., pre-incubated with 25 mM glucose), compared with control nerves, showed increased latency to CAP area decline during glucose deprivation (41.5 ± 4.9 min, $n = 6$, versus 28.9 ± 2.1 min, $n = 15$, for control; $p < 0.05$). The CAP never fell to zero during aglycemia in the high-glycogen nerves (**Fig. 4.4.A**). Nerves with decreased glycogen (i.e., pre-incubated with 1 mM norepinephrine) showed decreased latency to CAP decline (23.2 ± 1.1 min, $n = 9$, $p > 0.05$ versus control). This change was prominent but not significant compared with control, although all of these nerves had latencies of less than 28 min. Magnitude of post-aglycemia CAP recovery for nerves with variable glycogen content is illustrated in **Fig. 4.4.B**. CAP recovery after glucose withdrawal was significantly greater in high-glycogen nerves (85.8 ± 7.2%,

n = 6) and significantly less in low-glycogen nerves (20.2 ± 3.3%, n = 9), compared with control tissue (45.3 ± 3.7%, n = 15).

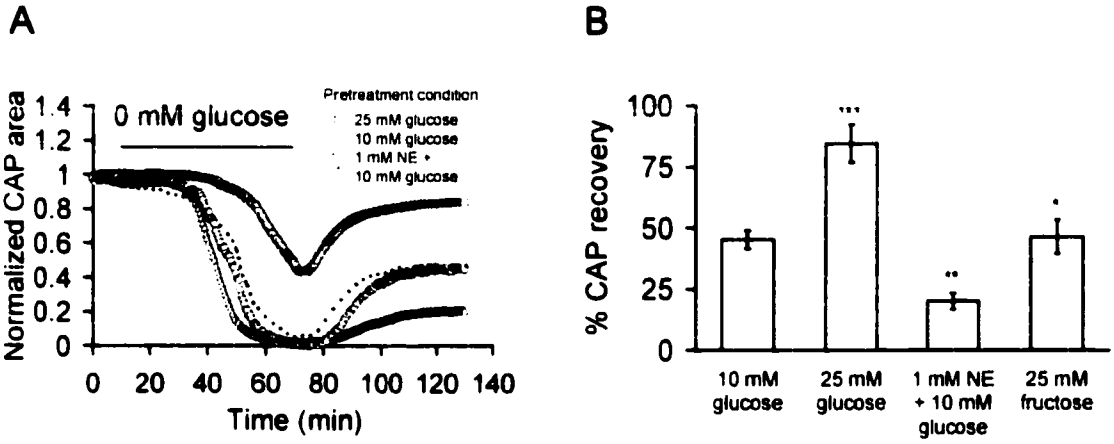


Figure 4.4. Effect of nerve glycogen content on axon function during, and recovery from, a 60-min period of glucose deprivation. **(A)** Up-regulation of glycogen by pretreatment of optic nerves with 25 mM glucose delayed onset of CAP area decline during 60 min of glucose deprivation, compared with control. Under these conditions, magnitude of CAP recovery was greater. Down-regulation of glycogen by pretreatment with 1 mM norepinephrine decreased latency to CAP decline and decreased extent of CAP recovery. Pretreatment of nerves with 25 mM fructose (dotted red line) had no effect on either latency or CAP recovery compared with control. **(B)** Percent CAP recovery 60 min after exposure to glucose-free aCSF for nerves represented in A. * $p > 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with "10 mM glucose" group.

To confirm that the results observed with 25 mM glucose were due to the presence of increased glycogen stores and were not merely a consequence of "loading" of the extracellular space with elevated glucose, we carried out a series of experiments with 25 mM fructose. Fructose, like glucose, sustains the CAP but it does not induce glycogen synthesis (Wiesinger et al., 1997, Fig. 4.3.B). There was

no statistically significant difference in either latency or recovery between nerves pretreated with 25 mM fructose versus control nerves pretreated with 10 mM glucose (latency 27.6 ± 2.2 min; recovery, 46.5 ± 7.0%; n = 4, p > 0.05; Fig. 4.4). This result with fructose suggests that the effects of 25 mM glucose were due to glycogen and not to lingering amounts of substrate in the extracellular space.

Blockade of lactate transport and axon function during glucose withdrawal

Extracellular concentration of protons ($[H^+]_o$) has been used as a qualitative proxy for extracellular concentration of lactic acid in neural tissue (Kraig and Chesler, 1990; Ransom et al., 1992). Using pH-sensitive microelectrodes, we measured extracellular pH (pH_o) in the isolated RON. A standing acid shift in the extracellular space, compared with perfusate pH of 7.45, was observed under control conditions **(Fig. 4.5.A).** Although the origin of this standing proton gradient may be complex, it is likely contributed to by lactic acid secretion into the extracellular space. After initiating perfusion with glucose-free aCSF, thereby removing the probable origin of most of the lactate produced within the optic nerve, there was an extracellular alkaline shift: pH_o began to rise at 24.2 ± 3.1 min from a baseline of 7.27 ± 0.02 to a peak of 7.44 ± 0.03 (n = 7; error bars show s.e.m.). This occurred on a time-course similar to CAP failure, as indicated in the lower panel of the figure, which shows a sample control experiment (one of the 15 experiments from the averaged trace shown

in Fig. 4.1.A.). pH_o rapidly acidified after 10 mM glucose was reintroduced at t = 70 min (75.2 ± 0.66 min).

Nerves in control aCSF containing 10 mM glucose maintained robust CAPs for several hours (**Fig. 4.5.B;** see also Fig. 3.2.A), in agreement with Stys et al. (Stys et al., 1991). A metabolically equivalent concentration of lactate (i.e., 20 mM) could be substituted for glucose for a 60-min test period with no loss of CAP area (Fig. 4.5.B; see also Fig. 3.2.A). These data strongly supported the hypothesis that lactate can support axon function as effectively as can glucose, at least for the period tested.

(continued on next page...)

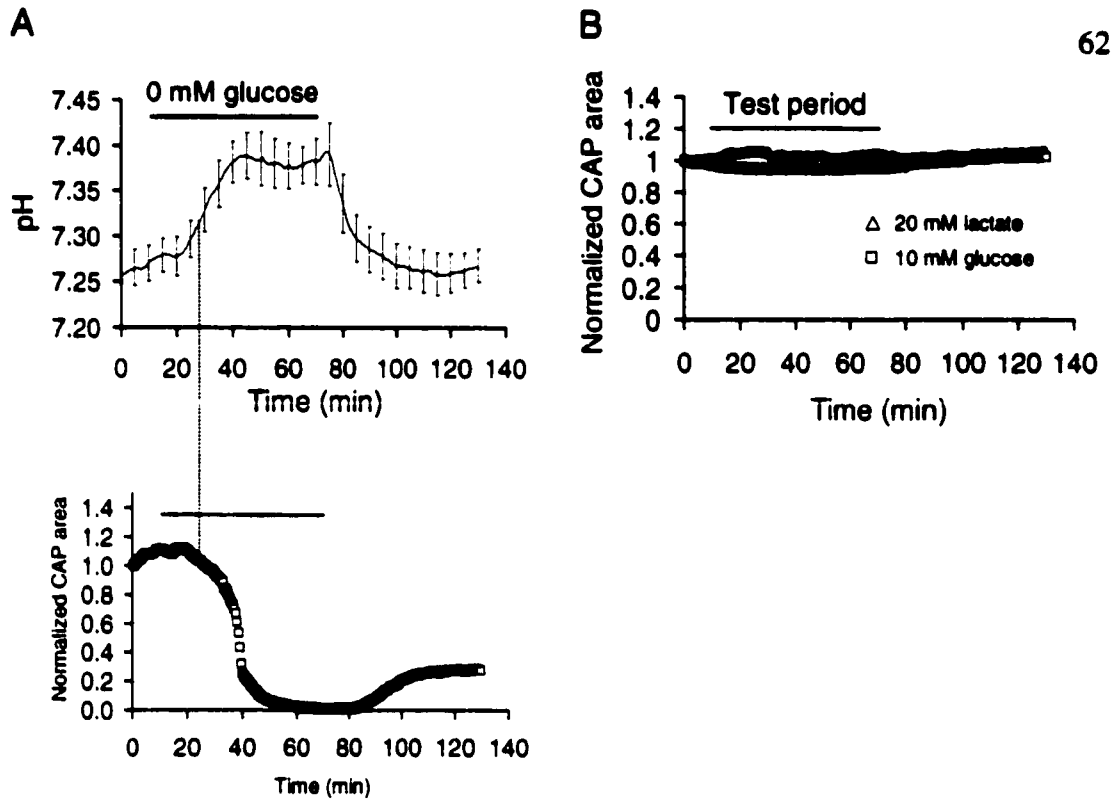


Figure 4.5. Glucose deprivation caused extracellular alkalosis, and lactic acid supported RON function in the absence of glucose. (**A**) Glucose deprivation induced a rise in pH_o, suggesting exhaustion of lactate precursors (glucose or glycogen) and hence arrest of presumptive efflux of H^+ and lactate anion. (**B**) Nerves continually perfused with control aCSF showed no decline in CAP area over 120 min (n = 6). Lactate could substitute for glucose in maintaining the CAP for the 60-min period shown by the bar (n = 6, 106 ± 8.9% of baseline CAP area at t = 130 min).

Because lactic acid appeared from pH recordings to be exported to the extracellular space in RON, as occurs with glycogen breakdown in cultured astrocytes (Dringen et al., 1993; Wiesinger et al., 1997), and because lactate served as an effective energy source for axon function, we attempted to interfere with lactate transport to test the theory that lactate was transferred from astrocytes to axons during glucose deprivation. We first tested the bioflavenoid quercetin, which preferentially blocks extrusion of lactate (Belt et al., 1979; Volk et al., 1997). Nerves

were perfused with 50 μM quercetin for 20 min prior to, and during, the 60-min period of glucose withdrawal. In the presence of quercetin, the latency to CAP failure was decreased compared with control (**Fig. 4.6.A;** 23.3 ± 2.8 min, n = 7, $p > 0.05$), although this did not reach statistical significance. These nerves sustained a greater degree of irreversible injury than did control nerves (12.3 ± 3.4% of baseline CAP area, $p < 0.001$). It appeared that quercetin blocked lactate *efflux* in the RON, because quercetin did not prevent lactate, exogenously applied, from supporting the CAP in the absence of glucose (105 ± 7.1% at t = 130 min; Fig. 4.6.A). As a control, quercetin was applied during continuous perfusion with glucose-containing aCSF. It was without effect under these conditions (Fig. 4.6.A).

(continued on next page...)

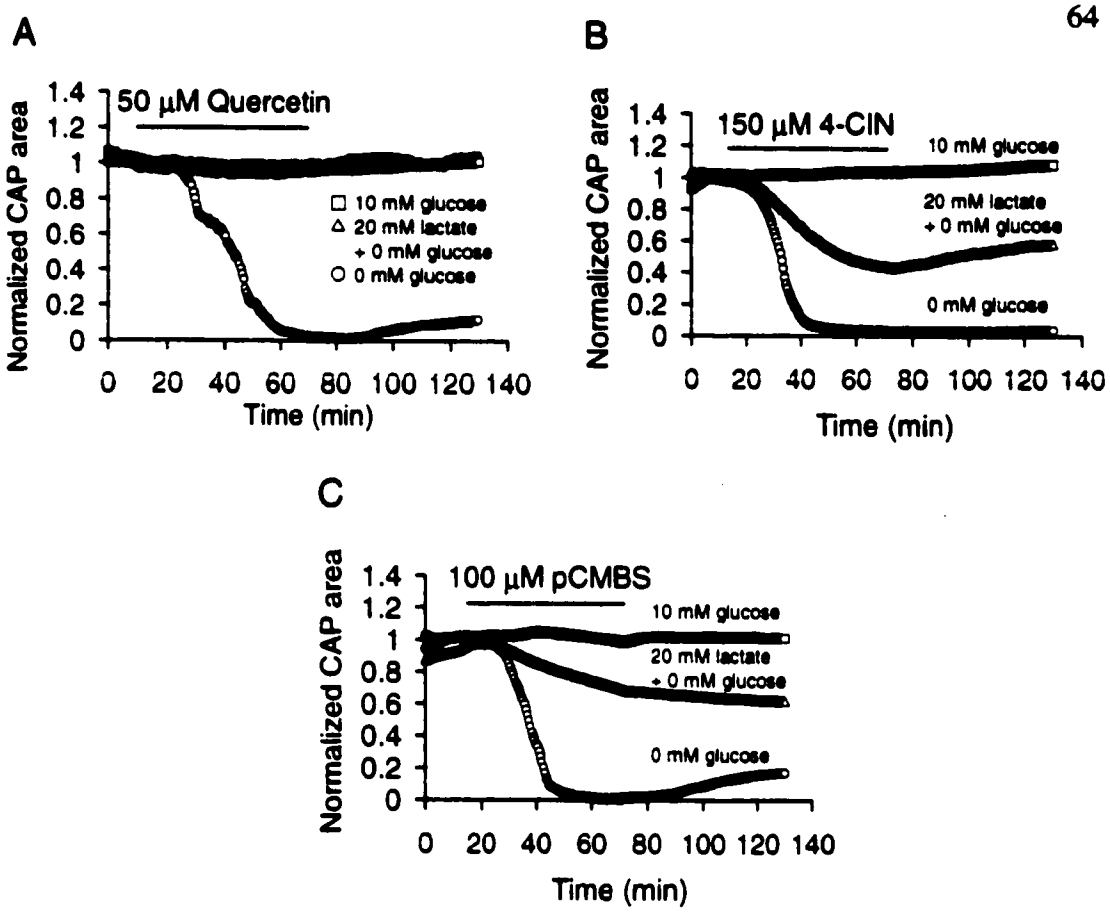


Figure 4.6. Effect of the lactate transport inhibitors quercetin, 4-CIN, and *p*CMBS on RONs exposed to 0 mM glucose or 20 mM lactate for 60 min. (A) Effect of quercetin (50 µM) on RONs subjected to 60 min of glucose withdrawal. Quercetin had no effect on the CAP of either control nerves perfused with 10 mM glucose or nerves exposed to 20 mM lactate during glucose withdrawal. Quercetin applied during 0 mM glucose exposure, however, caused more rapid decline in the CAP and reduced recovery. (B) 150 µM α-cyano-4-hydroxycinnamic acid (4-CIN) had no effect on the CAP in the presence of glucose, but led to a loss of function in nerves substituted with 20 mM lactate. 4-CIN caused rapid CAP failure and lower recovery of baseline CAP area in nerves exposed to 0 mM glucose. (C) 100 µM *p*-chloromercuribenzene sulfonic acid (*p*CMBS) had no effect on the CAP in the presence of glucose but led to a loss of function in nerves substituted with 20 mM lactate. *p*CMBS caused rapid CAP failure and lower recovery in nerves exposed to unsupplemented 0 mM glucose. All traces (panels A, B, and C) represent an average of six experiments, except as noted in the text for quercetin.

Two other lactate transport inhibitors, α-cyano-4-hydroxycinnamic acid (4-CIN) and *p*-chloromercuribenzene sulfonic acid (*p*CMBS), were tested for their effects on axon function during glucose deprivation (Fig. 4.6). Both compounds had no effect on the CAP in the continuous presence of glucose. 4-CIN (150 μM), applied 20 min prior to, and during, 60 min of glucose deprivation, decreased latency to CAP decline (**Fig. 4.6.B;** 17.5 ± 3.2 min, $p < 0.05$ vs. control). Although this did not reach significance, the CAP of every 4-CIN-treated nerve fell earlier than 28 min, the control value. 4-CIN-treated nerves recovered only minimally (3.5 ± 1.0%, $p < 0.001$ vs. control). It appeared that 4-CIN blocked lactate *uptake* by RON axons because in the presence of 4-CIN, 20 mM lactate in glucose-free aCSF was not able to fully support the CAP (Fig. 4.6.B). Even in the presence of lactate, CAP area declined and showed irreversible injury (latency = 24.7 ± 2.0 min, CAP area recovery = 58.2 ± 5.4%, $p < 0.001$ compared to nerves perfused with lactate in the absence of 4-CIN).

*p*CMBS, a specific blocker of the monocarboxylate transporter isoform MCT1 (Halestrap and Price, 1999; Juel and Halestrap, 1999), when applied during glucose withdrawal, shortened latency to CAP decline and reduced CAP recovery (**Fig. 4.6.C;** 19.5 ± 2.6 min, $p > 0.05$ vs. 0 mM glucose control; CAP area recovery = 17.1 ± 1.9% of baseline CAP area, $p < 0.01$ compared with recovery under control conditions). When applied in the presence of 20 mM lactate for the test period, *p*CMBS caused a fall in CAP area (Fig. 4.6.C; 29.5 ± 2.9 min, $p < 0.001$ compared

with nerves perfused with lactate in the absence of *p*CMBS) and reduced recovery (62.0 ± 4.4% of baseline CAP area, $p < 0.001$). These results suggested that MCT1 must be present on axons in adult RON. The effects of the lactate transport blockers on percent CAP recovery from 60 min of aglycemia or on exposure to 20 mM lactate are summarized in **Fig. 4.7.**

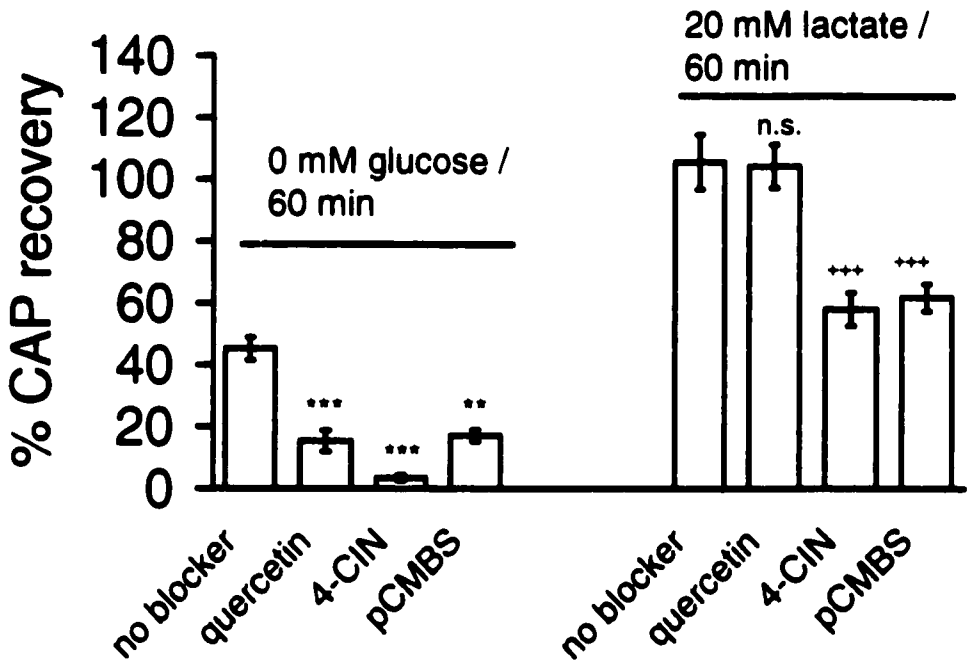


Figure 4.7. Summary of CAP recoveries following aglycemia with inhibition of lactate transport, with and without lactate supplementation. The set of bars on the left represent nerves that were subjected to a 60-min period of glucose withdrawal, i.e., no exogenous substrate was provided. A second set of nerves, represented by the four bars to the right, was exposed to the indicated lactate-transport blockers during aglycemia but in the presence of 20 mM lactate. Each data point represents a minimum of six experiments. Error bars indicate s.e.m. **$p < 0.01$ and ***$p < 0.001$ compared with "no blocker" in "0 mM glucose" group; $^{+++}p < 0.001$ and n.s. = not significant compared with "no blocker" in "20 mM lactate" group.

DISCUSSION

Our results support the hypothesis that during glucose deprivation in central WM, astrocytes supplied energy substrate to axons in the form of lactate derived from glycogen. This is, to our knowledge, the first demonstration of the importance of astrocytic glycogen for axon function and survival under conditions of glucose withdrawal. Our conclusions were based on the following observations: (1) The RON contained glycogen localized exclusively in astrocytes; (2) in the absence of glucose, RON axons remained functional for 30 min and then failed with a time-course that mirrored glycogen loss; (3) RON function during and after 60 min of glucose removal was enhanced by increasing glycogen content and decreased by decreasing glycogen content; (4) extracellular pH, serving as a proxy for lactic acid, became alkaline on elimination of an effectively unlimited source of glucose and consequent exhaustion of astrocytic glycogen as the presumptive remaining source of lactic acid; (5) lactate supported RON function in the absence of glucose; and (6) blockade of transmembrane lactate transport decreased RON function in the absence of glucose.

The persistence of the CAP for 30 min without glucose suggested the presence in this central WM tract of an intrinsic energy reserve. Brain glycogen, which is localized almost exclusively in astrocytes (see Fig. 4.2), is a prime candidate to fill this role (Cataldo and Broadwell, 1986; Magistretti et al., 1993). The preservation of the CAP in glucose-free aCSF was not due to persistent glucose within the tissue.

The glucose concentration in the extracellular space ($[glucose]_o$) will be less than in the bulk perfusate because the rate of diffusion of glucose is likely to be slow compared with glucose utilization. Even *in vivo*, where diffusion distances would be much less than in the isolated RON, the $[glucose]_o$ is only about one third of that in blood (Silver and Erecinska, 1994). When bath glucose is switched to zero, additional glucose would tend to diffuse from the nerve, further lowering $[glucose]_o$. Direct measurements of $[glucose]_o$ in mammalian cortex indicate that it falls within minutes to unmeasurable levels when the exogenous supply is interrupted (Siesjö, 1978; Silver and Erecinska, 1994). This is consistent with the high rate of glucose consumption by brain tissue at 37°C [0.5–1.0 mM/min/kg brain tissue (Siesjö, 1978)]. The persistence of CAP function in the absence of glucose can reasonably be attributed to the availability of another energy source, probably glycogen. Other possibilities exist, most importantly amino acids, lipids, and TCA cycle intermediates in axons.

In the absence of glucose, glycogen content fell to a low, stable level by 30 min (Fig. 4.3.A), closely corresponding to the time at which axonal conduction failed. Glucose controls glycogen content by binding and inactivating the glycogen breakdown enzyme, phosphorylase A (Stryer, 1995). It is not surprising that glycogen content remained at a low plateau level after 30 min of glucose withdrawal, rather than falling to zero. Cultured astrocytes are not able to completely mobilize their glycogen stores in the absence of glucose (Lomako et al., 1993; Lomako et al., 1995). Glycogen is composed of a protein core, glycogenin, with many attached glucose residues. Astrocytes seem to oscillate between low- and high-molecular

weight forms of glycogen and do not degrade glycogen all the way to the free glycogenin (Wiesinger et al., 1997).

Changes in RON glycogen content had significant functional consequences. Nerves with increased glycogen stores maintained normal conduction for 12 min longer than did control nerves during glucose deprivation, and showed much higher levels of CAP recovery following the insult. Moreover, CAP area never fell to zero in the high glycogen-containing nerves during aglycemia. In NE-treated nerves, with diminished glycogen, CAP recovery was much less than in control nerves and there was a trend to earlier CAP failure.

$[H^+]_o$ has been used as a qualitative proxy for extracellular concentration of lactic acid (Schneider et al., 1993). This qualitative relationship has been verified by showing that changes in pH_o appropriately mirror direct measurements of lactic acid and that pH_o changes predictably when tissue lactic acid concentration is modulated up or down (Kraig and Chesler, 1990; Ransom et al., 1992). The standing pH gradient of 0.18 pH units between the RON extracellular space and the aCSF perfusate supported the observation that astrocytes extrude lactic acid (Walz and Mukerji, 1990; Dringen et al., 1993). Perfusion of RONs with glucose-free aCSF led to an almost complete reversal of this gradient (0.17 pH units; Fig. 4.5.A) temporally coincident with CAP failure, and acid pH_o was rapidly re-established on reintroduction of control aCSF.

Our results supported the model shown in **Fig. 4.8.** The model asserts that WM astrocytes contain glycogen, which is quickly converted into lactate for transport

to nearby axons under conditions of glucose deprivation. [It should be noted that such a shuttle seems to occur under other conditions as well (Magistretti et al., 1999)]. Lactate is the most likely fuel substrate to be transferred from astrocytes to axons for the following reasons: (1) Astrocytes, but not neurons, are known to extrude large amounts of lactate (Walz and Mukerji, 1990). (2) Astrocytes, but not neurons, can survive on anaerobic metabolism alone (Goldberg and Choi, 1993; Pappas and Ransom, 1995; Ransom and Fern, 1996), and thus astrocytes could 'afford' to export large amounts of lactate. (3) Lactate, but not glucose, is released from astrocytes when glucose is removed (Dringen et al., 1993). (4) Lactate has been shown to be an effective fuel in numerous types of CNS tissue (Larrabee, 1983; Schurr et al., 1988; Izumi et al., 1994; Izumi et al., 1997), including RON axons (Wender et al., 1999). (5) Lactate from Müller cells (specialized retinal astrocytes) appears to fuel the neighboring photoreceptors in the guinea pig retina (Poitry-Yamate et al., 1995). According to the model, axons import lactate for subsequent oxidative metabolism to generate ATP. This model reflects the constraint that lactate could be metabolized by axons *only* in the presence of oxygen.

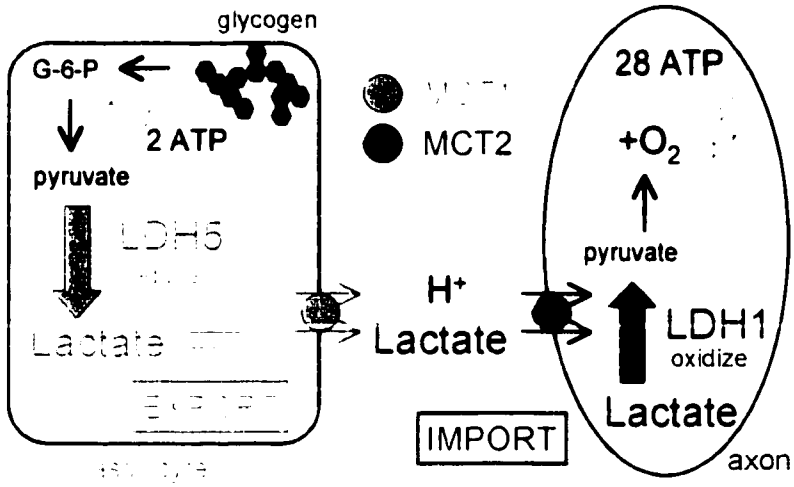


Figure 4.8. Schematic illustration of how astrocytic glycogen appears to fuel axons in the absence of glucose. In the absence of glucose, astrocytic glycogen is broken down to lactate, which is transported to the extracellular space via a MCT. It is then taken up by MCTs on axonal membranes and is oxidatively metabolized to produce energy needed to sustain excitability. LDH5 preferentially reduces pyruvate to lactate, while LDH1 preferentially oxidizes lactate to pyruvate. Quercetin preferentially blocks efflux of lactate from astrocytes. 4-CIN inhibits lactate transport by primarily MCT2, and *p*CMBS blocks only MCT1; both of the isoforms may be present on RON axons. It should be possible to override the effects of quercetin, but not of 4-CIN or *p*CMBS, with exogenous lactate supplementation. This scheme recognizes that astrocytes can subsist on glycolytic energy metabolism while axons require oxidative metabolism. G-6-P = glucose-6-phosphate, LDH = lactate dehydrogenase, and MCT = monocarboxylate transporter.

For astrocytes to convert glycogen to lactate for transfer to axons as a fuel source in the absence of glucose, several conditions must be met. There must be appropriate enzymes for the creation of lactate in astrocytes and for its use in axons, and also appropriate transport mechanisms for the movement of lactate. In fact, the expression patterns in the CNS of lactate dehydrogenase (LDH) and the monocarboxylate transporter (MCT) seem well suited to accommodate these needs.

LDH is the rate-limiting enzyme in the interconversion of pyruvate and lactate (Stryer, 1995). The enzyme is composed of various combinations of two subunits, H (or LDH1) and M (or LDH5). The former preferentially oxidizes lactate to pyruvate, and the latter predominantly reduces pyruvate to lactate (Stryer, 1995); see also (Gerhardt-Hansen, 1968). Neurons, which are highly dependent on oxidative metabolism (Siesjö, 1978), stain exclusively with anti-H (anti-LDH1) antibodies (Bittar et al., 1996); while astrocytes, which have the least activity of oxidative enzymes among all the cells of the CNS (Friede, 1962), are stained by both M (LDH5) and H antibodies. Thus, astrocytes, expressing at least some LDH5, can readily convert pyruvate to lactate, and neurons, expressing LDH1, are specialized to oxidize lactate to pyruvate in preparation for entry into the Kreb's cycle and oxidative phosphorylation.

MCTs, of which there are several isoforms, transport lactate and pyruvate across cell membranes using proton symport (Poole and Halestrap, 1993). There are several isoforms of MCTs. MCT1 appears to be expressed in tissue that preferentially releases lactate and MCT2 is expressed in tissues that mainly consume lactate (Jackson and Halestrap, 1996; Bröer et al., 1997). MCT1 is the only MCT expressed by astrocytes (in which MCT2 is barely detectable), whereas neurons express MCT2, with only faint expression of MCT1 (Bröer et al., 1997; Koehler-Stec et al., 1998). MCT2 has a ten-fold higher affinity for substrates than does MCT1 and is, therefore, ideally suited for rapid uptake at low substrate concentrations (Halestrap and Price, 1999). Additionally, because a pH gradient can drive the

extrusion or uptake of the lactate anion (Poole and Halestrap, 1993; Juel, 1997), glycolytically generated lactic acid could initiate its own export to the extracellular space. The expression patterns of LDH and MCT isoforms would tend to make astrocytes a lactate source and axons a lactate sink, consistent with the model.

The model is supported by the results of inhibiting lactate transport. This would be predicted to block the movement of lactate between astrocytes and axons. Indeed, all three MCT blockers tested reduced CAP recovery following a 60-min period of glucose withdrawal. Quercetin, a competitive inhibitor of MCTs, interacts with the transporter on the extracellular face of the plasma membrane to inhibit preferentially lactate efflux from cells (Belt, 1979 #966; see also McKenna et al., 1998). When 20 mM lactate was added to the glucose-free perfusate, quercetin had no effect on the CAP (Fig. 4.6.A), consistent with the idea that lactate influx into axons was relatively unaffected. Another competitive inhibitor of lactate transport, 4-CIN, preferentially blocks MCT2; the K_i for the 4-CIN/MCT2 complex is nearly 20 times lower than is the K_i for the 4-CIN/MCT1 complex (Halestrap and Price, 1999). In contrast to quercetin, and consistent with its known preference for blockade of MCT2, 4-CIN partially blocked the ability of exogenous lactate to support axon function during glucose removal (Fig. 4.6.B). Because 4-CIN is a competitive inhibitor, it is not surprising that axon function was maintained to some degree during perfusion with 20 mM lactate + 4-CIN. It has been shown that at > 10 mM lactate, even 5 mM 4-CIN could not inhibit all lactate transport (Edlund and Halestrap, 1988).

Although 4-CIN is considered to be a classical inhibitor of lactate transport (Halestrap and Price, 1999), its possible effects on mitochondrial pyruvate transport (Juel and Halestrap, 1999) can lead to misinterpretation of results. For this reason, we used a third lactate transport blocker, from a different class of drugs. *p*CMBS, a thiol reagent that is highly specific for MCT1 (Bröer et al., 1998; Bröer et al., 1999; Halestrap and Price, 1999; Juel and Halestrap, 1999), gave similar results to those observed with 4-CIN (Figs. 4.6.C and 4.7). Considering that *p*CMBS partially blocked exogenous lactate's ability to sustain the CAP, our data suggested that axon membranes express some MCT1.

Our results support the idea that astrocytic glycogen acted as a readily available source of energy (i.e., lactate) for axons when glucose was withdrawn. This glial–neuronal interaction, although long a theoretical possibility and suggested by earlier tissue culture experiments (Swanson and Choi, 1993), had not previously been demonstrated. It was surprising that glycogen was able to sustain nerve function for up to 30 min. It had been assumed that glycogen content in the brain could sustain neural function for less than 5 min (Clarke and Sokoloff, 1999). It may be that WM, which has a lower metabolic rate than gray matter, is unique in this regard.

References

Auer RN (1986) Progress review: hypoglycemic brain damage. Stroke 17:699-708.

Auestad N, Korsak RA, Morrow JW, Edmond J (1991) Fatty acid oxidation and ketogenesis by astrocytes in primary culture. J Neurochem 56:1376-1386.

Bell JE, Hume R, Busuttil A, Burchell A (1993) Immunocytochemical detection of the microsomal glucose-6-phosphatase in human brain astrocytes. Neuropathol Appl Neurobiol 19:429-35.

Belt JA, Thomas JA, Buchsbaum RN, Racker E (1979) Inhibition of lactate transport and glycolysis in Ehrlich ascites tumor cells by bioflavonoids. Biochemistry 18:3506-3511.

Bergbauer K, Dringen R, Verleysdonk S, Gebhardt R, Hamprecht B, Wiesinger H (1996) Studies on fructose metabolism in cultured astroglial cells and control hepatocytes: lack of fructokinase activity and immunoreactivity in astrocytes. Dev Neurosci 18:371-379.

Bittar PG, Charnay Y, Pellerin L, Bouras C, Magistretti PJ (1996) Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain. J Cereb Blood Flow Metab 16:1079-1089.

Borke RC, Nau ME (1984) Glycogen, its transient occurrence in neurons of the rat CNS during normal postnatal development. Brain Res 318:277-84.

Bourre JM, Faivre A, Dumont O, Nouvelot A, Loudes C, Puymirat J, Tixier-Vidal A (1983) Effect of polyunsaturated fatty acids on fetal mouse brain cells in culture in a chemically defined medium. J Neurochem 41:1234-1242.

Bröer S, Bröer A, Schneider HP, Stegen C, Halestrap AP, Deitmer JW (1999) Characterization of the high-affinity monocarboxylate transporter MCT2 in Xenopus laevis oocytes. Biochem J 341:529-535.

Bröer S, Rahman B, Pellegri G, Pellerin L, Martin JL, Verleysdonk S, Hamprecht B, Magistretti PJ (1997) Comparison of lactate transport in astroglial cells and monocarboxylate transporter 1 (MCT 1) expressing *Xenopus laevis* oocytes. Expression of two different monocarboxylate transporters in astroglial cells and neurons. J Biol Chem 272:30096-30102.

Bröer S, Schneider HP, Bröer A, Rahman B, Hamprecht B, Deitmer JW (1998) Characterization of the monocarboxylate transporter 1 expressed in *Xenopus laevis* oocytes by changes in cytosolic pH. Biochem J 333:167-174.

Brown AM, Ransom BR (1999) The role of extracellular calcium in hypoglycemic injury to the rat optic nerve, a central white matter tract. Soc Neurosci Abstr 29:841.2.

Cardinaud B, Coles JA, Perrottet P, Spencer AJ, Osborne MP, Tsacopoulos M (1994) Composition of the interstitial fluid in the retina of the honeybee drone: implications for the supply of substrates of energy metabolism from blood to neurons. Proc. R. Soc. Lond. [Biol.] 257:49-58.

Cataldo AM, Broadwell RD (1986) Cytochemical identification of cerebral glycogen and glucose-6- phosphatase activity under normal and experimental conditions. I. Neurons and glia. J of Electron Microscopy Technique 3:413-437.

Choi DW (1988) Glutamate neurotoxicity and diseases of the nervous system. Neuron 1:623-634.

Clarke DD, Sokoloff L (1999) Circulation and energy metabolism of the brain. In: Basic Neurochem: Molecular, Cellular, and Medical Aspects, 6th edition (Siegel GJ, Agranoff BW, Albers RW, Fisher SK, Uhler MD, eds), pp 637-669. Philadelphia: Lippincott-Raven Pubs.

Coles JA (1995) Glial cells and the supply of substrates of energy metabolism to neurons. In: Neuroglia (Kettenmann HaR, B. R., eds), pp 793-804. New York: Oxford Univ. Press.

Cummins KL, Perkel DH, Dorfman LJ (1979) Nerve fiber conduction-velocity distributions. I. Estimation based on the single-fiber and compound action potentials. Electroencephalogr Clin Neurophysiol 46:634-646.

Dringen R, Bergbauer K, Wiesinger H, Hamprecht B (1994) Utilization of mannose by astroglial cells. Neurochem Res 19:23-30.

Dringen R, Gebhardt R, Hamprecht B (1993) Glycogen in astrocytes: possible function as lactate supply for neighboring cells. Brain Res 623:208-214.

Dringen R, Hamprecht B (1993) Differences in glycogen metabolism in astroglia-rich primary cultures and sorbitol-selected astroglial cultures derived from mouse brain. Glia 8:143-149.

Dringen R, Hamprecht B (1992) Glucose, insulin, and insulin-like growth factor I regulate the glycogen content of astroglia-rich primary cultures. J Neurochem 58:511-517.

Edlund GL, Halestrap AP (1988) The kinetics of transport of lactate and pyruvate into rat hepatocytes. Evidence for the presence of a specific carrier similar to that in erythrocytes. Biochem J. 249:117-126.

Edmond J, Robbins RA, Bergstrom JD, Cole RA, de Vellis J (1987) Capacity for substrate utilization in oxidative metabolism by neurons, astrocytes, and oligodendrocytes from developing brain in primary culture. J Neurosci Res 18:551-561.

Erlanger J, Gasser HS (1937) Electrical Signs of Nervous Activity, Univ. of Pennsylvania Press.

Fern R, Davis P, Waxman SG, Ransom BR (1998) Axon conduction and survival in CNS white matter during energy deprivation: A Dev study. J Neurophysiol 79:95-105.

Fern R, Ransom BR, Waxman SG (1995) Voltage-gated calcium channels in CNS white matter: role in anoxic injury. J Neurophysiol 74:369-377.

Fisher CM (1979) Capsular infarcts: the underlying vascular lesions. Arch Neurol 36:65-73.

Forrester J, Peters A (1967) Nerve fibres in optic nerve of rat. Nature 214:245-7.

Forsyth RJ, Bartlett K, Burchell A, Scott HM, Eyre JA (1993) Astrocytic glucose-6-phosphatase and the permeability of brain microsomes to glucose 6-phosphate. Biochem J 294:145-151.

Forsyth RJ, Bartlett K, Eyre J (1996) Dephosphorylation of 2-deoxyglucose 6-phosphate and 2-deoxyglucose export from cultured astrocytes. Neurochem Int 28:243-50.

Foster RE, Connors BW, Waxman SG (1982) Rat optic nerve: electrophysiological, pharmacological and anatomical studies during development. Dev Brain Res 3:371-386.

Friede RL (1962) Cytochemistry of normal and reactive astrocytes. J Neuropath Exp Neurol 21:471-478.

Gerhardt-Hansen W (1968) Lactate dehydrogenase isoenzymes in the central nervous system. Danish Med Bull 15:1-112.

Gerhart DZ, Enerson BE, Zhdankina OY, Leino RL, Drewes LR (1997) Expression of monocarboxylate transporter MCT1 by brain endothelium and glia in adult and suckling rats. Am J Physiol 273:E207-13.

Goldberg MP, Choi DW (1993) Combined oxygen and glucose deprivation in cortical cell culture: calcium-dependent and calcium-independent mechanisms of neuronal injury. J Neurosci 13:3510-3524.

Halestrap AP, Price NT (1999) The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. Biochem J 343:281-299.

Hinkle PC, Kumar MA, Resetar A, Harris DL (1991) Mechanistic stoichiometry of mitochondrial oxidative phosphorylation. Biochemistry 30:3576-82.

Ibrahim MZ (1975) Glycogen and its related enzymes of metabolism in the central nervous system. Adv Anat Embryol Cell Biol 52:3-89.

Ignacio PC, Baldwin BA, Vijayan VK, Tait RC, Gorin FA (1990) Brain isozyme of glycogen phosphorylase: immunohistological localization within the central nervous system. Brain Res 529:42-9.

Izumi Y, Benz AM, Katsuki H, Zorumski CF (1997) Endogenous monocarboxylates sustain hippocampal synaptic function and morphological integrity during energy deprivation. J Neurosci 17:9448-9457.

Izumi Y, Benz AM, Zorumski CF, Olney JW (1994) Effects of lactate and pyruvate on glucose deprivation in rat hippocampal slices. Neuroreport 5:617-620.

Jackson VN, Halestrap AP (1996) The kinetics, substrate, and inhibitor specificity of the monocarboxylate (lactate) transporter of rat liver cells determined using the fluorescent intracellular pH indicator, 2',7'- bis(carboxyethyl)-5(6)-carboxyfluorescein. J Biol Chem 271:861-868.

Juel C (1997) Lactate-proton cotransport in skeletal muscle. Physiol Rev 77:321-358.

Juel C, Halestrap AP (1999) Lactate transport in skeletal muscle - role and regulation of the monocarboxylate transporter. J Physiol 517:633-642.

Koehler-Stec EM, Simpson IA, Vannucci SJ, Landschulz KT, Landschulz WH (1998) Monocarboxylate transporter expression in mouse brain. Am J Physiol 275:E516-E524.

Koizumi J (1974) Glycogen in the central nervous system. Prog Histochem Cytochem 6:1-37.

Kraig RP, Chesler M (1990) Astrocytic acidosis in hyperglycemic and complete ischemia. J Cereb Blood Flow Metab 10:104-14.

Kriegler S, Chiu SY (1993) Calcium signaling of glial cells along mammalian axons. J Neurosci 13:4229-4245.

Kuffler SW, Nicholls JG (1966) Physiology of neuroglial cells. Ergeb. Physiol. 57:1-90.

Larrabee MG (1995) Lactate metabolism and its effects on glucose metabolism in an excised neural tissue. J Neurochem 64:1734-1741.

Larrabee MG (1983) Lactate uptake and release in the presence of glucose by sympathetic ganglia of chicken embryos and by neuronal and nonneuronal cultures prepared from these ganglia. J Neurochem 40:1237-1250.

Larrabee MG (1996) Partitioning of CO_2 production between glucose and lactate in excised sympathetic ganglia, with implications for brain. J Neurochem 67:1726-1734.

Lee WN, Edmond J, Bassilian S, Morrow JW (1996) Mass isotopomer study of glutamine oxidation and synthesis in primary culture of astrocytes. Dev Neurosci 18:469-477.

Leloup C, Arluison M, Lepetit N, Cartier N, Marfaing-Jallat P, Ferre P, Penicaud L (1994) Glucose transporter 2 (GLUT 2): expression in specific brain nuclei. Brain Res 638:221-6.

Li S, Mealing GA, Morley P, Stys PK (1999) Novel injury mechanism in anoxia and trauma of spinal cord white matter: glutamate release via reverse Na^+-dependent glutamate transport. J Neurosci 19:RC 1-9.

Lomako J, Lomako WM, Whelan WJ (1995) Glycogen metabolism in quail embryo muscle. The role of the glycogenin primer and the intermediate proglycogen. European J Biochem 234:343-349.

Lomako J, Lomako WM, Whelan WJ, Dombro RS, Neary JT, Norenberg MD (1993) Glycogen synthesis in the astrocyte: from glycogenin to proglycogen to glycogen. FASEB J 7:1386-1393.

Lopes-Cardozo M, Larsson OM, Schousboe A (1986) Acetoacetate and glucose as lipid precursors and energy substrates in primary cultures of astrocytes and neurons from mouse cerebral cortex J Neurochem 46:773-8.

Lowry OH (1964) Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. J Biol Chem 239:18-30.

Lowry OH, Rosebrough NJ, Farr AL, Randall AR (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275.

Magistretti PJ (1988) Regulation of glycogenolysis by neurotransmitters in the central nervous system. Diabetes Metabolism 14:237-246.

Magistretti PJ, Pellerin L, Rothman DL, Shulman RG (1999) Energy on demand. Science 283:496-497.

Magistretti PJ, Sorg O, Martin J-L (1993) Regulation of glycogen metabolism in astrocytes: physiological, pharmacological, and pathological aspects. In: Astrocytes: Pharmacology and Function (Murphy S, eds), pp 243-265. San Diego, Calif.: Academic Press, Inc.

Magistretti PJ, Sorg O, Yu N, Martin JL, Pellerin L (1993) Neurotransmitters regulate energy metabolism in astrocytes: implications for the metabolic trafficking between neural cells. Dev Neurosci 15:306-12.

Maxwell DS, Kruger L (1965) Fine structure of astrocytes in the cerebral cortex and their response to focal injury produced by heavy ionizing particles. J Cell Biol 25:141-157.

McCall AL, Van Bueren AM, Moholt-Siebert M, Cherry NJ, Woodward WR (1994) Immunohistochemical localization of the neuron-specific glucose transporter (GLUT3) to neuropil in adult rat brain. Brain Res 659:292-7.

McIlwain H (1953) Substances which support respiration and metabolic response to electrical impulses in human cerebral tissues. J Neurol Neurosurg Psychiat 16:257-266.

McKenna MC, Tildon JT, Stevenson JH, Hopkins IB, Huang X, Couto R (1998) Lactate transport by cortical synaptosomes from adult rat brain: characterization of kinetics and inhibitor specificity. Dev Neurosci 20:300-309.

Nehlig A (1996) Respective roles of glucose and ketone bodies as substrates for cerebral energy metabolism in the suckling rat. Dev Neurosci 18:426-433.

Nelson T, Lucignani G, Atlas S, Crane AM, Dienel GA, Sokoloff L (1985) Reexamination of glucose-6-phosphatase activity in the brain in vivo: no evidence for a futile cycle. Science 229:60-2.

Nicholson C (1995) Extracellular space as the pathway for neuron-glial cell interaction. In: Neuroglia (Kettenmann HaR, B. R., eds), pp New York: Oxford Univ. Press.

Ohanian C (1972) Histochemical studies on phosporylase activity in the tissues of the albino rat under normal and experimental conditions. II. Normal distribution. Acta Histochem 44:244-63.

Orkand PM, Bracho H, Orkand RK (1973) Glial metabolism: alteration by potassium levels comparable to those during neural activity. Brain Res 55:467-471.

Pappas CA, Ransom BR (1995) The effects of anoxia and simulated ischemia on the viability of cultured rat hippocampal astrocytes. Soc Neurosci Abstr 21:211.

Passonneau JV, Lauderdale VR (1974) A comparison of three methods of glycogen measurement in tissues. Anal Biochem 60:405-412.

Pellegri G, Rossier C, Magistretti PJ, Martin JL (1996) Cloning, localization and induction of mouse brain glycogen synthase. Brain Res Mol Brain Res 38:191-9.

Pellerin L, Magistretti PJ (1996) Excitatory amino acids stimulate aerobic glycolysis in astrocytes via an activation of the Na^+/K^+ ATPase. Dev Neurosci 18:336-342.

Pellerin L, Magistretti PJ (1994) Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc Natl Acad Sci U S A 91:10625-10629.

Pellerin L, Pellegri G, Bittar PG, Charnay Y, Bouras C, Martin JL, Stella N, Magistretti PJ (1998) Evidence supporting the existence of an activity-dependent astrocyte- neuron lactate shuttle. Dev Neurosci 20:291-299.

Pellerin L, Pellegri G, Martin JL, Magistretti PJ (1998) Expression of monocarboxylate transporter mRNAs in mouse brain: support for a distinct role of lactate as an energy substrate for the neonatal vs. adult brain. Proc Natl Acad Sci U S A 95:3990-5.

Pentreath VW, Kai-Kai MA (1982) Significance of the potassium signal from neurones to glial cells. Nature 295:59-61.

Peters A, Palay SL (1976) Fine structure of the Nervous System: The Neurons and Supporting Cells, Philadelphia: W.B. Saunders Company.

Pfeiffer B, Elmer K, Roggendorf W, Reinhart PH, Hamprecht B (1990) Immunohistochemical demonstration of glycogen phosphorylase in rat brain slices. Histochemistry 94:73-80.

Phelps CH (1972) Barbiturate-induced glycogen accumulation in brain. An electron microscopic study. Brain Res 39:225-34.

Phelps CH (1975) An ultrastructural study of methionine sulphoximine-induced glycogen accumulation in astrocytes of the mouse cerebral cortex. J Neurocytol 4:479-90.

Poitry-Yamate CL, Poitry S, Tsacopoulos M (1995) Lactate released by Muller glial cells is metabolized by photoreceptors from mammalian retina. J Neurosci 15:5179-5191.

Poole RC, Halestrap AP (1993) Transport of lactate and other monocarboxylates across mammalian plasma membranes. Am J Physiol. 264:C761-C782.

Poole RC, Sansom CE, Halestrap AP (1996) Studies of the membrane topology of the rat erythrocyte H^+/lactate cotransporter (MCT1). Biochem J 320:817-824.

Prasannan KG, Subrahmanyam K (1966) Effect of insulin on the synthesis of glycogen in cerebral cortical slices of alloxan diabetic rats. Endocrinology 82:1-6.

Quach TT, Rose C, Schwartz JC (1978) [^{3}H]Glycogen hydrolysis in brain slices: responses to neurotransmitters and modulation of noradrenaline receptors. J Neurochem 30:1335-1341.

Ransom BR, Fern R (1996) Anoxic-ischemic glial cell injury: mechanisms and consequences. In: Tissue Oxygen Deprivation (Haddad G, Lister G, eds), pp 617-652. New York: Marcel Dekker, Inc.

Ransom BR, Fern R (1997) Does astrocytic glycogen benefit axon function and survival in CNS white matter during glucose deprivation? Glia 21:134-141.

Ransom BR, Sontheimer H (1992) The neurophysiology of glial cells. J Clin Neurophysiol 9:224-51.

Ransom BR, Stys PK, Waxman SG (1990) The pathophysiology of anoxic injury in central nervous system white matter. Stroke. 21:52-57.

Ransom BR, Walz W, Davis PK, Carlini WG (1992) Anoxia-induced changes in extracellular K^+ and pH in mammalian central white matter. J Cereb Blood Flow Metab 12:593-602.

Ransom BR, Waxman SG, Davis PK (1990) Anoxic injury of CNS white matter: protective effect of ketamine. Neurology 40:1399-1403.

Ransom BR, Waxman SG, Stys PK (1993) Anoxic injury of central myelinated axons: ionic mechanisms and pharmacology. In: Molecular and Cellular Approaches to the Treatment of Neurological Disease (Waxman SG, eds), pp 121-151. New York: Raven Press Ltd.

Reinhart PH, Pfeiffer B, Spengler S, Hamprecht B (1990) Purification of glycogen phosphorylase from bovine brain and immunocytochemical examination of rat glial primary cultures using monoclonal antibodies raised against this enzyme. J Neurochem 54:1474-83.

Revel JP (1964) Electron microscopy of glycogen. J Histochem Cytochem 12:104-114.

Schmoll D, Houston MP, Watkins SL, Burchell A (1997) Expression of constructs between the glucose-6-phosphatase promoter and a reporter gene in an insulinoma cell line: regulation by glucose, dibutyryl cAMP and dexamethasone. Biochem Soc Trans 25:180S.

Schneider U, Poole RC, Halestrap AP, Grafe P (1993) Lactate-proton co-transport and its contribution to interstitial acidification during hypoxia in isolated rat spinal roots. Neuroscience. 53:1153-1162.

Schousboe A, Westergaard N (1995) Transport of neuroactive amino acids in astrocytes. In: Neuroglia (Kettenmann H, Ransom BR, eds), pp 246-272. New York: Oxford University Press.

Schousboe A, Westergaard N, Waagepetersen HS, Larsson OM, Bakken IJ, Sonnewald U (1997) Trafficking between glia and neurons of TCA cycle intermediates and related metabolites. Glia 21:99-105.

Schurr A, Miller JJ, Payne RS, Rigor BM (1999) An increase in lactate output by brain tissue serves to meet the energy needs of glutamate-activated neurons. J Neurosci 19:34-9.

Schurr A, Payne RS, Miller JJ, Rigor BM (1997) Brain lactate is an obligatory aerobic energy substrate for functional recovery after hypoxia: further in vitro validation. J Neurochem 69:423-426.

Schurr A, West CA, Rigor BM (1988) Lactate-supported synaptic function in the rat hippocampal slice preparation. Science 240:1326-1328.

Sieber FE, Traystman RJ (1992) Special issues: glucose and the brain. Crit Care Med 20:104-114.

Siesjö BK (1978) Brain Energy Metabolism, New York: John Wiley & Son.

Siesjö BK (1988) Hypoglycemia, brain metabolism, and brain damage. Diabetes Metab Rev 4:113-144.

Silver IA, Erecinska M (1994) Extracellular glucose concentration in mammalian brain: continuous monitoring of changes during increased neuronal activity and upon limitation in oxygen supply in normo-, hypo-, and hyperglycemic animals. J Neurosci 14:5068-5076.

Sloviter HA, Kamimoto T (1970) The isolated, perfused rat brain preparation metabolizes mannose but not maltose. J Neurochem 17:1109-1111.

Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M (1977) The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem 28:897-916.

Somjen GG (1988) Nervenkitt: notes on the history of the concept of neuroglia. Glia 1:2-9.

Sonnewald U, White LR, Odegard E, Westergaard N, Bakken IJ, Aasly J, Unsgard G, Schousboe A (1996) MRS study of glutamate metabolism in cultured neurons/glia. Neurochem Res 21:987-993.

Stryer L (1995) Biochemistry, New York: W.H. Freeman & Co.

Stys PK (1998) Anoxic and ischemic injury of myelinated axons in CNS white matter: from mechanistic concepts to therapeutics. J Cereb Blood Flow Metab 18:2-25.

Stys PK, Ransom BR, Waxman SG (1991) Compound action potential of nerve recorded by suction electrode: a theoretical and experimental analysis. Brain Res 546:18-32.

Stys PK, Ransom BR, Waxman SG, Davis PK (1990) Role of extracellular calcium in anoxic injury of mammalian central white matter. Proc Natl Acad Sci USA 87:4212-4216.

Stys PK, Waxman SG, Ransom BR (1991) Na^+-Ca^{2+} exchanger mediates Ca^{2+} influx during anoxia in mammalian central nervous system white matter. Annal Neurol 30:375-380.

Swanson RA (1992) Physiologic coupling of glial glycogen metabolism to neuronal activity in brain. Can J Physiol Pharmacol 70:S138-44.

Swanson RA, Choi DW (1993) Glial glycogen stores affect neuronal survival during glucose deprivation *in vitro*. J Cereb Blood Flow Metab 13:162-169.

Swanson RA, Morton MM, Sagar SM, Sharp FR (1992) Sensory stimulation induces local cerebral glycogenolysis: demonstration by autoradiography. Neuroscience 51:451-461.

Swanson RA, Sagar SM, Sharp FR (1989) Regional brain glycogen stores and metabolism during complete global ischaemia. Neurol Res 11:24-28.

Swanson RA, Shiraishi K, Morton MT, Sharp FR (1990) Methionine sulfoximine reduces cortical infarct size in rats after middle cerebral artery occlusion. Stroke 21:322-7.

Swanson RA, Yu AC, Chan PH, Sharp FR (1990) Glutamate increases glycogen content and reduces glucose utilization in primary astrocyte culture. J Neurochem 54:490-496.

Swanson RA, Yu AC, Sharp FR, Chan PH (1989) Regulation of glycogen content in primary astrocyte culture: effects of glucose analogues, phenobarbital, and methionine sulfoximine. J Neurochem 52:1359-1365.

Tamarappoo BK, Raizada MK, Kilberg MS (1997) Identification of a system N-like Na^+-dependent glutamine transport activity in rat brain neurons. J Neurochem 68:954-960.

Tholey G, Roth-Schechter BF, Mandel P (1981) Activity and isoenzyme pattern of lactate dehydrogenase in neurons and astroblasts cultured from brains of chick embryos. J Neurochem 36:77-81.

Tsacopoulos M, Magistretti PJ (1996) Metabolic coupling between glia and neurons. J Neurosci 16:877-885.

Tsacopoulos M, Veuthey AL, Saravelos SG, Perrottet P, Tsoupras G (1994) Glial cells transform glucose to alanine, which fuels the neurons in the honeybee retina. J Neurosci 14:1339-1351.

Vannucci SJ, Maher F, Simpson IA (1997) Glucose transporter proteins in brain: delivery of glucose to neurons and glia. Glia 21:2-21.

Vega C, Poitry-Yamate CL, Jirounek P, Tsacopoulos M, Coles JA (1998) Lactate is released and taken up by isolated rabbit vagus nerve during aerobic metabolism. J Neurochem 71:330-337.

Villar-Palasi C, Larner J (1960) Levels of activity of the enzymes of the glycogen cycle in rat tissues. Arch Biochem Biophys 86:270-273.

Volk C, Kempski B, Kempski OS (1997) Inhibition of lactate export by quercetin acidifies rat glial cells in vitro. Neurosci Lett 223:121-124.

Walz W, Mukerji S (1988) Lactate release from cultured astrocytes and neurons: a comparison. Glia 1:366-70.

Walz W, Mukerji S (1990) Simulation of aspects of ischemia in cell culture: changes in lactate compartmentation. Glia 3:522-528.

Waxman SG, Ritchie JM (1993) Molecular dissection of the myelinated axon. Annal Neurol 33:121-136.

Wender R, Brown AM, Ransom BR (1999) Manipulating glycogen stores in adult white matter influences nerve injury during hypoglycemia. Soc Neurosci Abstr 29:737.3.

Wiesinger H, Hamprecht B, Dringen R (1997) Metabolic pathways for glucose in astrocytes. Glia 21:22-34.

Wijesinghe RS, Gielen FL, Wikswo JP (1991) A model for compound action potentials and currents in a nerve bundle. I: The forward calculation. Ann Biomed Eng 19:43-72.

Vita

Regina Wender

EDUCATION

University of Washington School of Medicine, 1994-present
Medical Scientist Training Program
Departments of Physiology/Biophysics and Neurology
United States Medical Licensing Exam Step I passed June 1996
expected graduation: M.D./Ph.D., June 2002

Western Washington University, BA *magna cum laude* (History), 1994

TEACHING ASSISTANTSHIPS

Academic year 1998-1999
Signaling Mechanisms in Excitable Cells (for first-year Graduate Class)
Supervisor: Bertil Hille, Ph.D.
Academic year 1997-1998
Cell Physiology (for first-year Medical Class)
Supervisor: Wayne E. Crill, M.D.

PUBLICATIONS

1. R. Wender, A. M. Brown, R. Fern, R. A. Swanson, and B. R. Ransom, "Astrocytic glycogen influences axon function and survival during glucose deprivation in central white matter," submitted to *J. Neurosci.* (May 2000).

2. R. Wender, R. Fern, A. M. Brown, and B. R. Ransom, "Metabolic substrates other than glucose support axon function and improve recovery from hypoglycemia in central white matter," submitted to *J. Neurochem.* (May 2000).

3. A. Barth, D. Newell, L. Nguyen, H. R. Winn, R. Wender, J. Meno, and D. Janigro, "Neurotoxicity in organotypic hippocampal slices mediated by adenosine analogues and nitric oxide," *Brain Res.* 762, 79-88 (1997).

4. D. Janigro, R. Wender, G. Ransom, D. Tinklepaugh, and H. R. Winn, "Adenosine-induced release of nitric oxide from cortical astrocytes," *Neuroreport* 7(10), 1640-1644 (1996).

ABSTRACTS

1. R. Wender, A. M. Brown, and B. R. Ransom, "Manipulating glycogen stores in adult white matter influences nerve injury during hypoglycemia," Soc. for Neuroscience 29th Annual Meeting (Miami Beach, Fla., 1999).

2. R. Wender, R. Fern, and B. R. Ransom, "Metabolic substrates other than glucose support axonal conduction in CNS white matter," Soc. for Neuroscience 28th Annual Meeting (Los Angeles, Calif., 1998).

3. R. Wender, B. J. Snider, R. Fern, and B. R. Ransom, "Effect of glial heat-shock protein on sensitivity of CNS white matter axons to anoxia," Soc. for Neuroscience 27th Annual Meeting (New Orleans, La., 1997).

4. R. Wender, A. Barth, D. Newell, H. R. Winn, and D. Janigro, "Chloroadenosine-mediated neurotoxicity," Soc. for Neuroscience 26th Annual Meeting (Washington, DC, 1996).

5. R. Wender, D. Janigro, and H. R. Winn, "Adenosine induces NO release from cultured cortical astrocytes," 24th Annual Western Student Medical Research Forum (Carmel, Calif., 1996).

6. D. Janigro, R. Wender, A. Barth, and H. R. Winn, "Chloroadenosine- and nitric-oxide-mediated hippocampal neurotoxicity *in vitro*," International Adenosine Symposium '96: Role of Adenosine in the Nervous System (Kobe, Japan, 1996).

7. R. Wender, D. Janigro, G. Ransom, and H. R. Winn, "Adenosine-induced release of nitric oxide from cortical astrocytes," Soc. for Neuroscience 25th Annual Meeting (San Diego, Calif., 1995).

8. D. Janigro, R. Wender, and H. R. Winn, "Adenosine induces release of nitric oxide from cultured cortical astrocytes," Am Heart Assoc. 68th Scientific Sessions (Anaheim, Calif., 1995).