Preface

To function properly, neurons cannot tolerate fluctuations of their local environmental variables. This mainly results from their high degree of specialization in synaptic integration and action potential conduction. Even small changes of certain extracellular ion concentrations, as well as in the dimensions of the extracellular space, alter ion channel kinetics in such a way as to distort the information represented by the nerve impulses. Another potential problem is the huge consumption of glucose and oxygen by neurons caused by the heavy compensatory ion pumping used for counteracting passive ion flux. This problem is compounded by the low glucose storage capacity of the neurons. A complicated structure surrounds the neurons to sustain the required level of metabolites and to remove waste products.

The Neuronal Environment: Brain Homeostasis in Health and Disease examines the function of all the components involved, including their perturbation during major disease states, and relates them to neuronal demands. The two introductory chapters focus on neuronal requirements. The dependence of their excitability on external factors that accumulate in the extracellular space, as well as their varying demands for energy metabolites, are described. Following that, the close interaction of neurons with elements of their microenvironment is illustrated. The extracellular space is no longer seen as a passive constituent of the CNS, but as a separate compartment in its own right, as a communication channel, and an entity that reacts with plastic changes in its size that will affect the concentrations of all its contents. Astrocytes participate in many neuronal processes, particularly in the removal of excess waste and signal substances, the supply of energy metabolites, and the modulation of synaptic transmission. In addition to their homeostatic role, astrocytes are now seen as an active partner involved in synaptic transmission between neurons. The classical example of a close relationship of neurons with a component of their environment is, of course, their relationship with the surrounding myelin sheath. This speeds up action potential conduction, but is itself a potential source of problems in various disease states. In the last few years new imaging techniques have demonstrated a close coupling between local blood flow and neuronal activity, and several theories have been put forward to explain these interactions. The special status of the brain in having its own insulated circulation system—the cerebrospinal fluid contained in the ventricles and ducts—is also underlined. The brain is the only organ that is protected from fluctuations of blood-borne chemicals by the existence of the blood–brain barrier. However, windows exist in this barrier in the form of the circumventricular organs that allow direct two-way communication between neurons and blood constituents. Finally, despite their protection and insulation, the neurons are accessible to the immune system. Resident macrophages and invasion by blood-borne immune cells that cross the endothelial cell barrier enable

an immune reaction to take place. This complex interaction of neurons with their immediate environment is integral to the tasks that the neurons must perform to ensure that the organism can cope with its environmental challenges. Most diseases originating in the brain start in these accessory systems of the neuronal microenvironment and affect neurons only second hand. Therefore, understanding the elements of the neuronal environment and the interactions with neurons, and with each other, is crucial in understanding the development and impact of most brain diseases.

All the authors contributing to *The Neuronal Environment: Brain Homeostasis in Health and Disease* have made an attempt not only to explain the normal functioning of these accessory elements, but also their involvement in major diseases. Therefore, this book not only addresses researchers, graduate students, and educators who want to understand the complex environment of neurons, but also health professionals who need to know more about the normal homeostatic role of the neuronal environment to follow disease patterns.

Wolfgang Walz

2
Neuronal Energy Requirements

Avital Schurr

1. NEURONAL ENERGY DEMANDS, SUBSTRATES, AND ENERGY GENERATION

Descriptions of cerebral energy requirements found in the literature may confuse many readers. On one hand, Hawkins *(1)* states that, "Although nervous tissue does not participate in processes that require large amounts of energy, such as mechanical work, osmotic work, or extensive biosynthesis, it has almost as high a rate of oxidative metabolism as some tissues that do." On the other, Clarke and Sokoloff *(2)* assert that, "Although it is sometimes stated that the brain is unique among tissues in its high rate of oxidative metabolism, the overall cerebral metabolic rate for O_2 ($CMRO_2$) is of the same order as the unstressed heart and renal cortex." These two contrasting views are not necessarily contradictory. Whether or not the brain has higher energy requirements than other tissues, the brain is unique, both in its energy-demanding functions and the limitations on the types of fuels it uses and their routes of delivery. The above statements are also indicative of the reason brain-energy metabolism has developed into a separate specialty, in which the energy supply and demand of the brain are studied as the basis for many brain dysfunctions and disorders. The past 15 years witnessed several discoveries and new developments in the field of cerebral energy metabolism, which could explain some of the brain's unique energy requirements, and provide a better understanding of various brain disorders.

1.1. Neuronal Energy Requirements, Energy-Demanding Functions, and Energy Substrates

1.1.1. Neuronal Energy Requirements and Energy-Demanding Functions

The majority of the energy-demanding reactions in the brain belong to two categories: biosynthesis and transport. The biosynthesis of macromolecules, such as proteins, polypeptides, and lipids, occurs mostly in cell bodies; that of smaller molecules, such as neurotransmitters, occurs in nerve terminals. Of the multiple transport processes that take place in the brain, ion transport is believed to demand the most energy (as high as 50–60% of all brain-energy-consuming processes) *(1)*. Of these, the maintenance of sodium ions (Na^+) and potassium ions (K^+) gradients is the most demanding. Unlike other tissues, the central nervous system (CNS) stores only minute amounts of endogenous fuel. Brain tissue glucose levels at any given time are much lower than

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blood glucose levels (1–2 μmol/g brain wet wt, compared with 5–6 μmol/mL blood) *(1)*. Glycogen stores are not much higher than the glucose stores (2–3 μmol/g wet wt). Cahill and Aoki *(3)* suggested that the required large ratio of water:glycogen (3–4 mL water/1 g glycogen) could cause great fluctuations in volume, which are restricted because of the rigidity of the cranium. Astrocytes are the main source of brain glycogen, a fact that led many *(1)* to suggest that glycogen is not a readily available energy source to neurons. As will be discussed later in this chapter, this view is now changing: New data indicate that shuttle systems exist between astrocytes and neurons for different metabolites. Estimates are that the total brain supplies of both glucose and glycogen are sufficient for no more than 5 min of normal oxygen (O_2) consumption, a period that could get even shorter when excessive energy utilization and blood glucose supplies cannot keep up with the demand. With no O_2 reserves, the brain depends on the blood and its flow, for all its O_2 needs, extracting almost one-third of the total blood O_2 under normal conditions. Consequently, blockade or reduction in blood flow would diminish cerebral energy metabolism, because of O_2 deprivation before any diminution of glucose supply is apparent.

1.1.2. Energy Substrates

As mentioned, the bulk of the energy manufactured by the brain is devoted to nerve excitation and conduction. These two functions are dependent on sustained membrane potential, and, hence, most of the brain's energy is consumed by ion transport. Since the brain's energy stores are limited, an unhindered flow of glucose and O_2 is a prerequisite for continuous, uninterrupted production of adenosine triphosphate (ATP). This concept, i.e., that the normal substrates of cerebral energy metabolism are glucose and O_2 and the products are carbon dioxide (CO_2) and water *(2)*, has not changed in decades, and generally is correct, although, as is explained below, it is an oversimplified one. Consequently, glucose utilization in the brain is regarded as obligatory *(2)*. Thus, the brain is considered to be different from other tissues, which are much more flexible in their ability to utilize alternative fuels to glucose. This conclusion is based on measurements of positive arteriovenous differences only, for glucose and O_2, and consistent negative values only, for CO_2. Normally, neither positive nor negative arteriovenous differences can be demonstrated for lactate or pyruvate. Although the lack of positive differences indicates that the brain does not utilize bloodborne lactate or pyruvate as aerobic energy substrates, the lack of negative differences for at least one of these two products, even during a moderate O_2 shortage, is intriguing, and could bear potentially important implications.

Table 1 encapsulates the stoichiometric relationship between glucose utilization and O_2 consumption. The values in Table 1 are calculated medians of measurements reported in the literature, and glucose equivalent of O_2 consumption is the theoretical one (6 mol O_2/mol glucose) *(2)*.

The normal conscious human brain consumes 156 μmol O_2/100 g tissue/min. A similar CO_2 production yields a respiratory quotient of 1.0. As indicated in Table 1, 156 μmol of O_2 (or CO_2) are equivalent to 26 μmol glucose (based on a ratio of 6 μmol O_2 consumed for 1 μmol glucose utilized). Not withstanding, the measured rate of glucose utilization is 31 μmol/100 g tissue/min, a surplus of 5 μmol glucose, which brings down the O_2:glucose ratio from the theoretical 6.0, to 5.5. The discrepancy between the

Table 1
Stoichiometric Relationship Between Glucose Utilization and O_2 Consumption in Normal Young Adult Man[a]

Function	Rate (μmol/100 g brain tissue/min)
Glucose utilization	31
O_2 consumption	156
CO_2 production	156
Glucose equivalent of O_2 consumption (6 mol O_2/mol glucose for complete oxidation)	26

[a]Adapted from ref. *2*.

calculated and the measured O_2:glucose has remained unexplained, although it has been suggested that the extra, nonoxidized glucose is converted to lactate, pyruvate, and other intermediates of carbohydrate metabolism *(2)*. Moreover, it has been postulated that these intermediates are released from the brain into the blood in such minute amounts that they are not detectable as a significant arteriovenous difference *(2)*.

There are two "facts" supporting the claim that glucose is the only energy substrate that the normal brain utilizes. The first is a respiratory quotient of 1.0 (6 mol O_2 consumed and 6 mol CO_2 formed, a quotient that requires a stoichiometric consumption of 6 mol O_2 for every mol glucose utilized, and thus a ratio of O_2/glucose of 6.0). The second is the inability to detect a positive arteriovenous difference for any other potential energy substrate.

Clarke and Sokoloff *(7)* warn their readers about the discrepancies between in vivo and in vitro results concerning brain tissue, and the great hazard of extrapolating from in vitro data to conclusions about in vivo metabolic function. In vitro systems bypass functions, such as blood flow, but the uniqueness of the brain in vivo stems from the blood–brain barrier (BBB). The assumption that BBB-devoid cerebral tissue, as with in vitro systems, behaves differently from the same tissue in vivo has triggered great skepticism toward many in vitro findings, especially among investigators who use only in vivo systems. Do in vitro systems deserve such skepticism? Are warnings about their pitfalls warranted? The answer to both questions should be affirmative, when crushed tissue preparations, or mitochondrial, synaptosomal, or any other organelle-enriched preparations are concerned. However, many in vitro preparations today maintain whole cells, partial or complete brain regions, and even a whole, perfused brain for hours and days, intact and functional. These in vitro preparations deserve attention, and should be given the chance to prove themselves before anyone blindly criticizes data that have been originated by them. Therefore, this chapter describes data on energy metabolism generated using in vivo systems, along with data produced by in vitro preparations, such as cell cultures, excised CNS nuclei, and brain slices.

If one is to adhere to the premise that the brain is restricted in its choice of energy substrates, because of the BBB, one need measure only glucose and O_2 consumption and CO_2 production to calculate the brain's energy needs. In vivo studies, at least until recently, did just that: measuring what went into the "box" and what came out of the box. Although, ironically, the intricate processes within the box, which are responsible

for the consumption of glucose and O_2, and the production of CO_2, were elucidated using in vitro systems, one could ignore them entirely when measurement of energy metabolism in vivo is concerned. It is only in the last 15 yr that in vivo measurements, both in humans and animals, have strongly suggested that brain energy metabolism is not simply $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$. As the methods of measuring brain function improve, insights are gained that provide a more complex and elaborate picture.

Recent studies indicate that, under conditions of cerebral stimulation, intracerebral lactate increases to significantly higher levels than those under resting conditions *(4–9)*. These results confirm what was suspected for many years, i.e., that phasic changes in neural activity are supported by glycolysis *(10)*. As early as 1937 *(11,12)*, it was noted that local tissue O_2 levels increase, rather than decrease, during spontaneous focal seizures in human cerebral cortex. Rapid eye movement sleep caused large increases in blood flow *(13)* and glucose utilization *(14)*, but a decrease in O_2 extraction *(15)*. Figure 1 shows the cerebral metabolic rate (CMR) data in humans *(5)* under resting conditions and upon stimulation. In whole brain under resting conditions, $CMRO_2$ and $CMR_{glucose}$ values were 1.50 and 0.37 μmol/min/100 g, respectively: a molar ratio of 4.1:1. In the visual cortex, under resting conditions, $CMRO_2$ was 1.71 μmol/min/100 g and $CMR_{glucose}$ was 0.42 μmol/min/100 g, or, again, a molar ratio of 4.1:1. Upon visual stimulation the $CMR_{glucose}$ value rose a mean of 0.21 μmol/min/100 g (51%), and $CMRO_2$ value increased by a mean of only 0.08 μmol/min/100 g (5%), to produce a molar ratio, for the increases, of only 0.4:1. These results indicate that the brain, under working (stimulated) conditions, is capable of producing large amounts of lactate (via pyruvate), up to 250% of control. This accumulated lactate remains in the brain, and could later be used aerobically for energy metabolism under resting conditions. Other recent studies show significant increases in lactate levels in human visual cortex *(7,8)* and in rat hippocampus and striatum, on physiological stimulation *(9)*.

Thus, although the impermeability of the BBB to lactate is irrelevant, since the bulk of brain lactate is produced in the brain itself, the claim that the BBB is impermeable to lactate is inaccurate, at best. Lactate entry to the brain via the arterial blood is considered to be negligible, but Rowe et al. *(16)* showed that, immediately after a carbohydrate-rich meal, the higher-than-average lactate blood levels diminished on passing the brain. A relatively high brain uptake index value for lactate has been reported when a low lactate concentration (0.011 m*M*) was injected into the carotid artery of adult rats *(17–19)*. If the normal plasma level of lactate is 1.0 m*M (20)*, one could expect brain uptake index for lactate to be even higher. Several studies clearly demonstrate the ability of lactate to quickly permeate the adult BBB *(18,21–24)* via a stereospecific transporter *(18,22)*. A recent study used [3-^{13}C]lactate to demonstrate that the average uptake of lactate during the first 5 min after its injection (iv) to mice was almost 7× higher than the previously calculated V_{max} for uptake of lactate across the BBB *(25a)*.

Since normal lactate concentration in cerebrospinal fluid exceeds (1.6–2.0 m*M*) the plasma lactate level (1.0 m*M*) *(20)*, and the $CMRO_2$:$CMR_{glucose}$ under resting conditions is higher (4.1) than that during cerebral stimulation (2.8), it is obvious that the normal brain produces large amounts of lactate. Moreover, $CMRO_2$:$CMR_{glucose}$ of 4.1 *(5)* is not the expected ratio of 6.0, or even 5.0. $CMRO_2$:$CMR_{glucose}$ values of 5.0 or 4.0 indicate that 17 or 33%, respectively, of the glucose are consumed via nonoxidative metabolism and converted to lactate, and that, under cerebral stimulation, when these

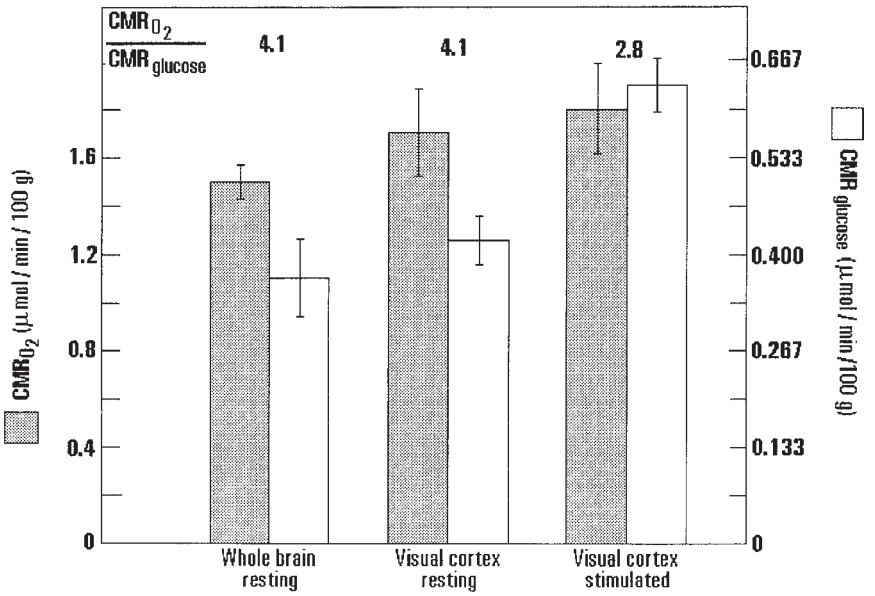

Fig. 1. The ratio of human cerebral metabolic values of oxygen ($CMRO_2$) and glucose ($CMR_{glucose}$) in whole brain and in visual cortex, under resting conditions and during stimulation with a reversing checkerboard stimulus. Under resting conditions, the $CMRO_2$:$CMR_{glucose}$ for the whole brain and the visual cortex were identical (4.1.). However, during stimulation, a significant decrease in this ratio (to 2.8) was observed; the $CMR_{glucose}$ rose by a mean of 0.21 µmol/min/100 g (51%); the $CMRO_2$ rose by a mean of only 0.08 µmol/mi/100 g (5%). This is a molar ratio for the increase in metabolic rate of only 0.4:1. (Modified with permission from ref. *5*.)

values are falling to approx 0.4 *(5)*, 93% of the metabolized glucose is being converted to lactate. Such high production of lactate by the normal brain would explain the higher concentration of lactate in CSF than in plasma *(20)*. Hence, the normal brain continuously produces large amounts of lactate.

If lactate is being produced in the brain regularly as an end product, it should either accumulate there or appear in the venous blood that exits the brain. But in fact neither occurs. A simple explanation for this would be an immediate aerobic cerebral utilization of lactate under resting conditions. Alternatively, lactate could be converted to glycogen, either via gluconeogenesis *(25)* or by direct conversion *(25,26)*. Pyruvate, the precursor of lactate, is as efficient an energy substrate as lactate, it is transported via the same stereospecific transporters that transport lactate, and it is the product that lactate has to be converted to before it can be utilized aerobically. Nevertheless, pyruvate does not accumulate in the brain, and thus the pyruvate:lactate ratio is usually very small.

Before examining in vitro data on lactate as a cerebral energy substrate, two arguments should be reconsidered, that blunt somewhat the warning by Clarke and Sokoloff *(2)* on the limited usefulness of information extracted from in vitro systems resulting from their lack of an intact BBB. First, as has already been pointed out, most, if not all, of the cerebral lactate is produced by the brain itself, and thus the presence or absence of the BBB is irrelevant. Second, as has been shown by the studies mentioned above

(16,18,21–24), the BBB is permeable to lactate, although not to the same degree as glucose. Hence, the relevance and the importance of the in vitro findings described below should not be overlooked.

As early as 1953, McIlwain *(27,28)* was able to demonstrate the ability of guinea pig cortical slices to respire when lactate was the substrate. When such slices, prepared either from guinea pig or rabbit brain, were respiring in glucose-containing media, lactate accumulated in the media *(29)*. This lactate accumulation was initially rapid (50 µmol/g/h), and fell after some minutes, to about 25% of the initial rate of accumulation. This decline results from lactate being served as an oxidizable substrate, when its levels are greater than 3 m*M* *(30)*. Other in vitro results show 2 m*M* to be the minimal lactate concentration that rat hippocampal slices can utilize for energy production *(31)*. The increase in brain lactate levels that accompanies increased cerebral activity, both in humans *(4,5)* and animals *(9)*, has been demonstrated in vitro as a 2–10-fold increase in glycolysis induced by electrical stimulation or incubation with high potassium *(30)*. Thus, rates of lactate formation in vitro, of 100 µmol/g/h during electrical stimulation, are typical, and can be sustained for hours. When the tissue was superfused (3.5 mL/min), lactate formation values rose to 300 µmol/g/h *(30)*. The normal rate of lactate formation by human cerebral tissue in vitro is about 15–20% of the consumed glucose *(30)*.

Evidence published in 1988 *(31)* supports the idea that adult brain tissue is capable of substituting lactate for glucose to fuel its normal function. Later studies *(32–34)* reproduced that finding, demonstrating the ability of adult rat hippocampal slices to utilize lactate (and pyruvate) as the sole energy substrate, upon complete depletion of glucose from the incubation medium. Moreover, in those studies, the inhibition of glycolysis with iodoacetate was ineffective in abolishing synaptic function in lactate-supplemented slices, and completely diminishing such function in glucose-supplemented slices *(31)*.

Cerebellar slices from adult rats exhibited an increase of approx 220% in their ability to oxidize lactate to CO_2, compared to cerebellar slices prepared from early neonates *(34)*. Moreover, at any age, the rate of CO_2 production from lactate is over 300% higher (when slices were supplemented with 10 m*M* lactate) than the rate measured from glucose (when slices were supplemented with 5 m*M* glucose) (Fig. 2). These findings are in agreement with the proposal that, thermodynamically, lactate is a preferred aerobic energy fuel, compared to glucose, since lactate conversion to pyruvate requires no ATP investment; 2 mol ATP are consumed in the conversion of a mole of glucose to pyruvate *(31,35)*. Two recent studies *(25,26)* offer evidence that cultured neonatal mouse astroglial cells are capable of using lactate for gluconeogenesis, and that astroglia-rich primary cultures from neonatal rats are capable of supplying glycogen-derived lactate to neighboring cells.

These in vitro studies strongly suggest a major role for lactate (and pyruvate) as a substrate for cerebral energy metabolism. Based on studies with astrocytic and neuronal cultures, Magistretti et al. *(36–39)* hypothesized that, when the presynaptically released excitatory neurotransmitter, glutamate (Glu) is taken up by astrocytes, it stimulates the production of glycolytic lactate and, consequently, the aerobic utilization of lactate by neurons. The importance of lactate as an aerobic cerebral energy substrate could become even greater under certain conditions, such as hypoxia/ischemia (*see* Sub-

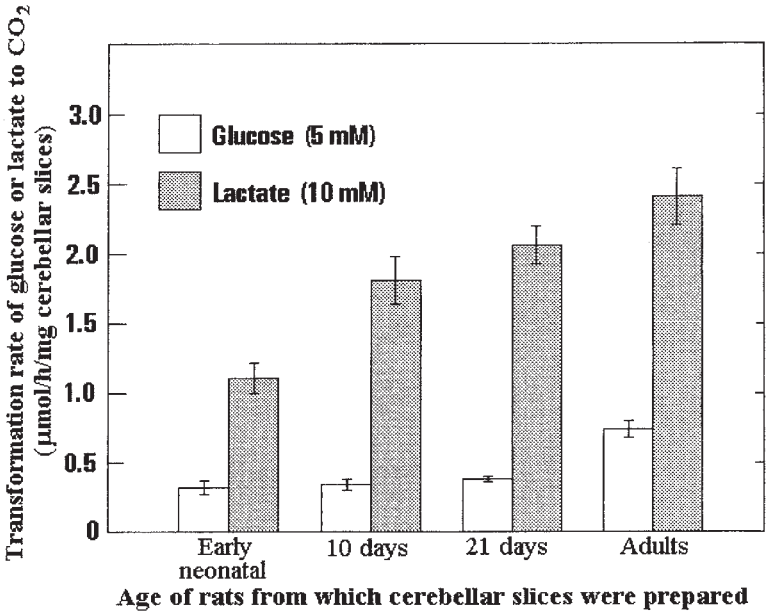

Fig. 2. Conversion of glucose (5 m*M*) or lactate (10 m*M*) to CO_2 by cerebellar slices prepared from rats of different ages. (Modified with permission from ref. *34*.)

heading 4.), as mentioned earlier. Although pyruvate is as useful as lactate for the oxidative production of ATP, it does not accumulate in large quantities, as lactate does. Thus, although pyruvate can support neuronal function in vitro, as a sole energy substrate *(32)*, its levels are too low to account for such support in vivo. Larrabee has shown, using excised chick ganglia, that lactate metabolism competes with glucose metabolism, and vice versa *(40–42)*.

Other substances, such as the ketones, β-hydroxybutyrate and acetoacetate, could, under certain circumstances, serve as energy substrates. Neonates have a great ability to utilize these alternative substrates *(2,43)*. In cases of diabetes or starvation, in which blood levels of ketones are elevated, because of an increase in lipid catabolism, brain tissue shows an adaptive ability to metabolize ketones as energy substrates, using the same monocarboxylate transporters that transport lactate and pyruvate. Ketones are usually converted to acetyl CoA, which directly enters the tricarboxylic acid cycle (TAC).

Although glycogen stores in the brain are low, this polycarbohydrate is the main energy reserve of the brain, and may be utilized during periods of high glucose demands, when glucose supplies cannot keep up with the glycolytic flux. Such high demands, as indicated in the previous subheading, occur regularly, upon increased neuronal activity. It is a long-held dogma that the BBB limits the entry of glucose into astrocytes and neurons during periods of high demands. We have shown, however, using brain slices, in which the BBB is absent, that the rate of neuronal glucose entry is too slow to keep up with its glycolytic consumption *(44)*. If that is true, the role of glycogen, found mainly in astrocytes in the adult brain, could be even more important, especially if some or all of it is converted first to lactate *(26)* before being transported

into neurons as an aerobic energy substrate *(40–42)*. Calculations indicate that the glycogen stored in brain tissue (3.3 μmol/g rat brain) could last for less than 5 min as the sole energy source *(2)*. However, in vitro results indicate that astrocytes are capable of synthesizing glycogen from lactate *(25)*, implying that the breakdown and synthesis of glycogen could take place concomitantly.

As the rate of brain glycolysis increases, the level of glycogen's first precursor, glucose-6-phosphate (glucose-6-P) decreases, as the result of an enhancement in the rate of fructose-6-P phosphorylation to fructose-1,6-diP, by the enzyme, phosphofructokinase. This enzyme is the main site of glycolytic regulation. Hence, with the reduction in glucose-6-P levels during periods of energy demands, glycogen synthesis also declines. The uridine diphosphoglucose, formed from glucose-1-P, is the unit transferred and linked, via a α-1,4-glycosidic bond, to the terminal glucose on a nonreducing side of an amylose chain. This reaction, catalyzed by glycogen synthetase, is the rate limiting reaction of glycogen synthesis *(2)*. On the degradation side of glycogen, most of the phosphorylase is in its phosphorylated form, "a," the active form of the enzyme. Nevertheless, it is still unknown if and how glycogenolysis in the brain is regulated. The hydrolysis of glycogen at the α-1,4-glycoside bond leaves a chain of α-1,6-glycoside linkages, upon which glycogen can be resynthesized. The hydrolysis of the α-1,6-glycosidic chain by the debranching enzyme is slower than the hydrolysis of α-1,4-glycosidic bonds, and its product is glucose. This reaction may be the rate-limiting step in glycogenolysis. Approximately 1 mol free glucose is formed for each 11 mol glucose-6-P released, when 1 mol glycogen is completely hydrolyzed. As is discussed in Subheading 2., glycogen metabolism is tightly coupled to neuronal activity. It is rapidly hydrolyzed during shortage in energy supply, and synthesized during adequate supplies of glucose and O_2.

2. COUPLING OF FUNCTION AND ENERGY METABOLISM IN THE BRAIN

The assumption that activation of brain tissue is dependent on energy supplied by oxidative metabolism of glucose was challenged over a decade ago *(4,5)*. As has been seen from the first subheading, magnetic resonance imaging measurements of glucose consumption and concomitant calculations of O_2 consumption from blood flow measurements indicated a possible mismatch between the two, upon activation of the brain. More recent studies *(45,46)* claim that no such mismatch exists, and that the initial increase in glucose consumption, measured upon brain activation, is accompanied by a similar increase in O_2 uptake.

For years, elevated lactate levels have been considered to signal the existence of hypoxia and anaerobic energy metabolism in tissues *(47,48)*. Substantial evidence has been accumulated *(48,49)* to indicate that large amounts of lactate can be produced in many tissues under fully aerobic conditions, but brain tissue has been presumed to be an exception. Lactate production has been promoted as an exclusively anaerobic process, and its accumulation was thought to be a major detrimental factor in ischemic brain damage *(50)*.

Now, however, many studies *(4–9,51)* suggest that the brain is not necessarily different from other tissues, in that it does produce lactate under aerobic conditions. Upon cerebral stimulation, intracerebral lactate increases to significantly higher levels than

those under resting conditions, both in humans *(4,5,7,8,37,38)* and rats *(9)*. These results confirmed what had been suspected for some time: Phasic changes in neural activity are supported by glycolysis, a much less efficient ATP biosynthetic pathway than the TCA and oxidative phosphorylation. Moreover, a recent study *(52)* has suggested that, upon activation, a significant pool of lactate is available to the brain as a source of energy. Nevertheless, as has been already mentioned, other studies demonstrate a concomitant increase in O_2 consumption upon activation, to match the increase in glucose utilization. These studies disagree with the idea that increase in energy demands by activated brain is met by glycolysis alone *(45,46)*.

2.1. Glutamate, Neuro–Glial Interaction, and Coupling of Function and Energy Production

Magistretti et al. *(39)*, using primary cultures of mouse cerebral cortical astrocytes, demonstrated the ability of Glu, the main excitatory neurotransmitter in the brain, to stimulate glycolysis, i.e., glucose consumption and lactate production. They hypothesized that Glu, released from active presynaptic neurons and taken up by astrocytes, is the signal that couples neuronal activity to glucose utilization. According to this hypothesis, astrocytic Glu uptake, via Na^+ co-transport, activates the Na^+/K^+-ATPase pump. Glu uptake from the synaptic cleft occurs through specific astroglial Glu transporters, namely, Glu transporter 1 and astrocyte-specific Glu transporter *(52)*. These transporters show a stoichiometry of $3Na^+$ contransported with each Glu molecule. The Na^+-pumping activity, fueled with ATP formed by membrane-bound glycolytic enzymes, increases glycolytic flux and, thus, glucose consumption and lactate production. An additional ATP-requiring reaction takes place, upon Glu entry into astroglia, i.e., Glu conversion to glutamine ($GluNH_2$) by the astroglial enzyme, glutamine synthase *(52)*. Lactate, once released from astrocytes, is taken up by neurons to be utilized aerobically as an energy substrate. Such a mechanism could explain the observed increases in glucose utilization and lactate production, without a concomitant increase in O_2 consumption upon brain stimulation *(4–7,48,49)*. Since neurons consume lactate aerobically *(31–34,53)*, any elevation in its levels should be short-lived.

The coupling of neuronal activity and energy metabolism has also been studied in rat hippocampal slices *(53)*. Although activation of these slices with Glu could result in a significant elevation in the production of lactate, no such increase was detected. Does this lack of increase in lactate tissue level indicate lack of lactate production, or that lactate is consumed as quickly as it is produced? To test whether or not such elevation occurs, lactate utilization must be prevented, or at least slowed down. One way to block lactate use is to inhibit its transport from its site of production to its site of utilization, i.e., from astroglia to neurons. As has been mentioned in Subheading 1., lactate transport occurs via a specific MCT, located in the membrane of both astroglia and neurons *(54,55)*. The use of a specific inhibitor of this transporter, α-cyano-4-hydroxycinnamate (4-CIN), has proved to be an efficient way to block lactate transport into neurons *(53,56)*. Upon activation of neurons with Glu in the presence of 4-CIN, tissue lactate accumulation could be observed (Fig. 3). 4-CIN induced lactate accumulation in brain slices, but it also prevented the recovery of normal neuronal function after the removal of Glu (Fig. 3).

These results lead one to conclude that, under control conditions, any glycolytic lactate formed during exposure to Glu is immediately consumed by neurons as an aero-

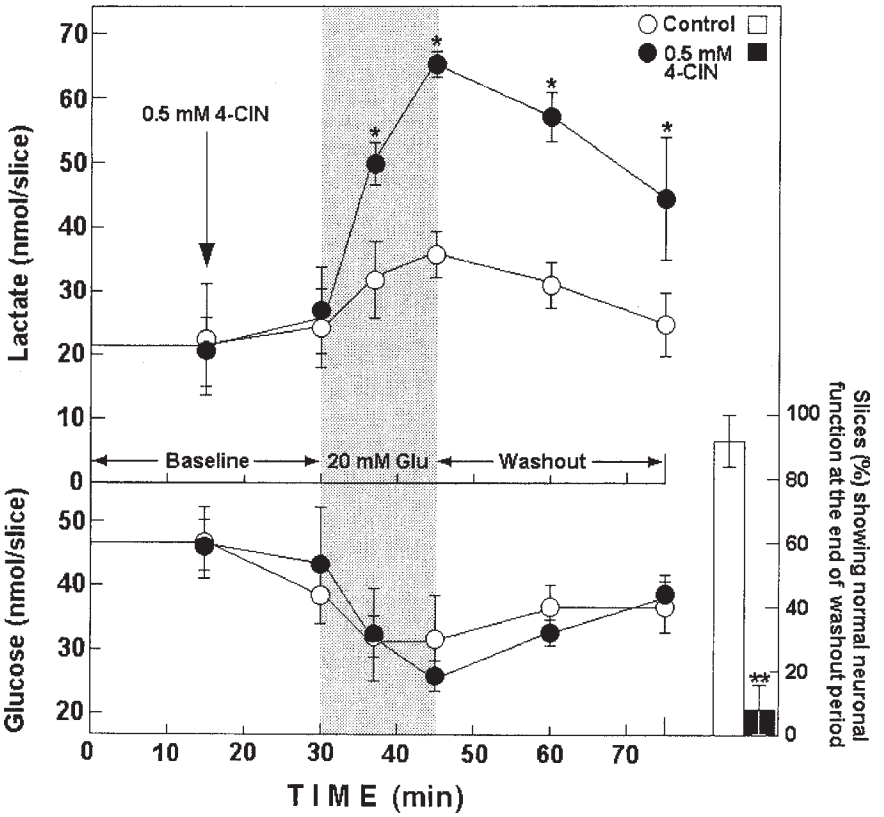

Fig. 3. The content of glucose (**bottom panel**) and lactate (**top panel**), before (baseline) and during exposure to Glu and during Glu washout, in control (open symbols) and 4-CIN-treated (filled symbols) rat hippocampal tissue slices. Slices were supplied with artificial cerebrospinal fluid containing 10 m*M* glucose. The recovery rates of neuronal function at the end of 30 min washout in control (open bar) and 4-CIN-treated slices (filled bar) are shown at the right of the bottom panel. Values are means ± SD. Significantly different from control (*$p < 0.03$; **$p < 0.0001$).

bic energy substrate, and that lactate formation takes place mostly in astroglia. Moreover, lactate appears to be an energy substrate of utmost importance for neuronal recovery after activation. These conclusions are based on three facts: First, in the absence of 4-CIN, no lactate accumulation was observed; second, when 4-CIN was present, inhibition of lactate transport into neurons prevented its utilization, indicating that lactate is produced outside the neuronal compartment; third, 4-CIN prevented the recovery of neuronal function after activation, indicating that lactate could be an obligatory energy substrate during neuronal activation. The blockade by 4-CIN of the neuronal recovery after exposure to Glu ensued, despite the ample supply of glucose (4–10 m*M*) that was present throughout the experimental period.

The importance of glycolysis's role, in the mechanism that couples brain activity with energy metabolism, could be tested by glycolytic inhibition. Such inhibition should weaken the ability of hippocampal slices to maintain neuronal function during exposure to Glu, since glucose utilization would be blocked and lactate would not be

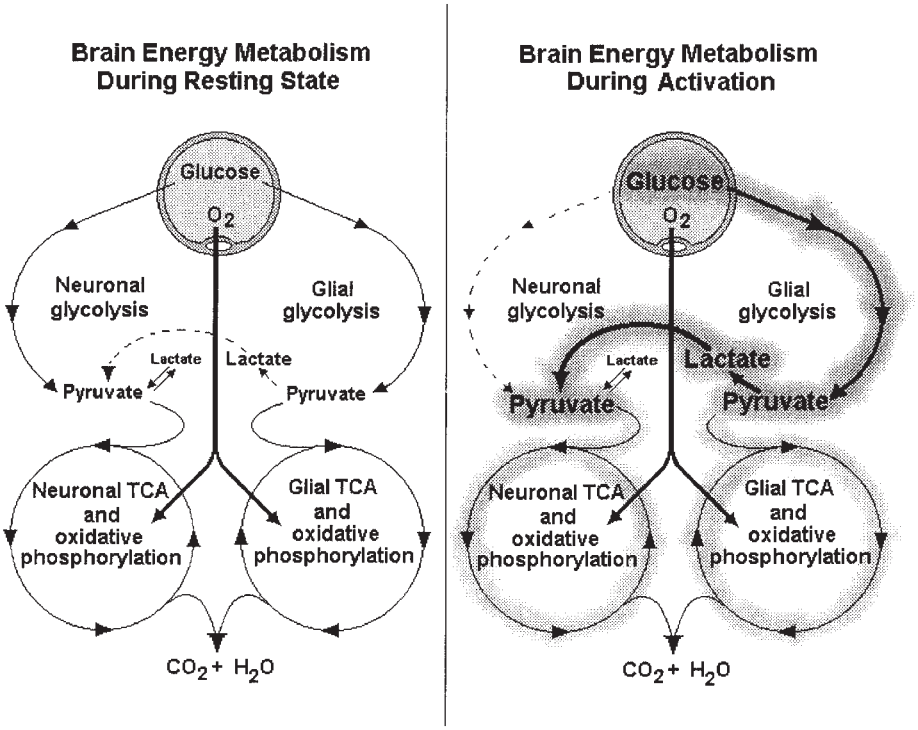

Fig. 4. A schematic diagram of the two main pathways of energy metabolism (glycolysis and oxidative phosphorylation), in neuronal and astroglial compartments, during rest (**left**) and during activation (**right**). For more details, *see* text.

produced. When 2-deoxy-D-glucose (2DG), a nonmetabolizable analog of glucose, was supplied to slices, instead of glucose during exposure to Glu, neuronal function did not recover upon Glu washout. However, when lactate was supplemented along with 2DG, more than 50% of the slices showed neuronal function after Glu washout. This outcome indicates that lactate plays a crucial role in assuring the preservation of neuronal viability during periods of brain tissue activation and/or overactivation. Notwithstanding, a recent study *(57)* claims that lactate is capable of inhibiting glial oxidative glucose metabolism, and consequently O_2 consumption, by up to 40%.

The following scenario (Fig. 4) can be drawn for brain energy metabolism during resting state and during activation (exposure to Glu). During the brain's resting state, most of the glucose taken up from blood supply is metabolized aerobically, both in the astroglial and the neuronal compartments. Under resting conditions, the astroglial production of lactate usually exceeds that of neurons, mostly because of the higher activity of ion pumping via the Na^+,K^+-ATPase pump *(36–39,43,52)*. The majority of this lactate is being transported into neurons, and directly enters the neuronal TCA. Upon activation (with Glu), astroglial Glu uptake immediately ensues, accompanied by Na^+ transport *(36–39,43,52)*. The need to pump out the extra Na^+ brings about a dramatic increase in glial Na^+, K^+-ATPase activity, and thus an equally dramatic increase in glucose consumption, most of which is glycolytic. This increased glycolytic activity is not accompanied by an increase in O_2 consumption, since lactate is its main product.

The large amount of astroglial lactate produced under conditions of activation is handled by two MCT systems. First, lactate is released from glia via the glial MCT system. Second, it is transported into neurons via the neuronal MCT system, where it becomes the main aerobic energy substrate. Thus, the increase in lactate utilization causes a decrease in neuronal glucose utilization *(40–42)*. This scenario could explain the observation made by many investigators, that the stimulation of brain tissue increases glucose uptake and consumption, without a concomitant increase in O_2 consumption.

Are neurons intrinsically programmed to utilize lactate during activation, or is it simply the result of inadequate supply of glucose? To answer this important question, one has to estimate the relative contribution of neurons to the total glucose consumption of the brain. To determine this contribution, the basal (resting) and activation-driven glucose consumption of both neurons and astroglia should be calculated, and the ratio between these two populations of cells in the brain region of study must be taken into account.

Studies *(58–60)* have shown that neurons occupy no more than 50% of the volume of cerebral cortex. In most brain regions, astrocytes outnumber neurons 10:1 *(52,59)*. Taking into account that basal astrocytic glucose consumption is over 3× higher than that of neurons *(61)*, and that, during activation, this consumption increases significantly *(37,52)*, a picture emerges in which neuronal contribution to glucose consumption during activation appears to be minor, if not negligible. Moreover, because of the ratio of astrocytes to neurons (10:1) and the fact that, upon activation, most of the consumed glucose is by astrocytic glycolysis, neurons appear to have no choice but to use lactate as their oxidative energy substrate during activation: This is the only one available to them. The following equations attempt to demonstrate this point.

Astrocytic Energy Substrate Consumption and Metabolite Production

$$\text{glucose} + 5O_2 \longrightarrow 5CO_2 + 5H_2O + 0.33\ \text{lactate} + 32\text{ATP (Basal)}$$

$$\text{glucose} \longrightarrow 2\ \text{lactate} + 2\text{ATP (Activation)}$$

$$2\ \text{glucose} + 5O_2 \longrightarrow 5CO_2 + 5H_2O + 2.33\ \text{lactate} + 34\text{ATP (Basal + activation)}$$

Neuronal Energy Substrate Consumption and Metabolite Production

$$\text{glucose} + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 38\text{ATP (Basal)}$$

$$2\ \text{lactate} + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 34\text{ATP (Activation)}$$

These equations are based on several postulates. First, that $CMR_{glucose}$:$CMRO_2$ is not the theoretical 6, but the practical 5, meaning that, under resting (basal) conditions, astrocytes use approx 17% of their glucose to support the Na^+,K^+-ATPase pumping activity, through glycolysis, while converting it to lactate. During activation, most if not all of the consumed extra glucose, here assumed to be equal to its basal level, is converted to lactate. Neurons, in contrast, may be capable of a $CMR_{glucose}$:$CMRO_2$ of 6, whether they utilize glucose or its astrocytic product, lactate, for oxidative

energy metabolism. If one relies on Begnami's *(59)* estimate of astrocyte:neuron ratio (10:1), the sum of $CMR_{glucose}$ and $CMRO_2$ for the two cellular compartments during activation is:

$$10 \text{ Astrocytes} \times (2 \text{ glucose} + 5O_2 \longrightarrow 5CO_2 + 5H_2O + 2.33 \text{ lactate} + 34ATP)$$

$$1 \text{ Neuron} \times (2 \text{ lactate} + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 34ATP)$$

$$20 \text{ glucose} + 56O_2 \longrightarrow 56CO_2 + 56H_2O + 21.30 \text{ lactate} + 374ATP)$$

The $CMRO_2$:$CMR_{glucose}$ for the above sum is 2.8, the very value found by Fox et al. *(5)* with activated human visual cortex. It is evident from these equations that 10 astrocytes produce enough lactate to provide for all the oxidative energy needs of one neuron, with enough left over to create a pool of lactate *(62)*.

Until an in vivo quantitative determination can be made of the contribution of each of the two cellular compartments to the total glucose consumption, the question of whether the main neuronal energy substrate is glucose or lactate will remain unanswered. Nevertheless, there is one additional point that must be considered here. With all the lactate that astrocytes produce, does the relatively small number of neurons account for the consumption of this lactate? Considering the above equations, it is clear that neurons would consume only a fraction of the astrocytic lactate produced during activation. However, since no change in the concentration of lactate can be detected in the venous blood exiting the brain during activation (*see also* Subheading 3.), one must assume that all the lactate produced in the brain is consumed by the brain. Hence, if not the activated neurons, then other consumers, such as neurons of surrounding areas, and even white matter and axons, could consume the rest of the lactate.

There is one additional observation that supports Magistretti's hypothesis. Bittar et al. *(63)* studied the distribution of lactate dehydrogenase (LDH) isozymes among neurons (lactate consumers) and astrocytes (lactate producers). They used specific polyclonal antibodies against LDH1, the isozyme found mostly in heart muscle, and LDH5, the one found mainly in skeletal muscle. The antibody raised against LDH1, the isozyme that converts lactate to pyruvate, stained human hippocampal and cortical neurons; hippocampal and cortical astrocytes were immunoreactive with an antibody raised against LDH5. Such selectivity in these two isozymes' distribution indicates that astrocytes in certain brain nuclei, such as the hippocampus and the cortex, contain exclusively the LDH activity that converts pyruvate to lactate, and, as such, are probably very active glycolytically, producing large amounts of lactate. In contrast, the neurons in these regions are enriched with LDH1, the isozyme that converts lactate to pyruvate, typical of tissues that utilize lactate (and pyruvate) for manufacturing energy via the TCA.

Although the greatest emphasis throughout this chapter has been on glucose and lactate (and pyruvate) as the substrates of cerebral energy metabolism, both in rest and during activation, one should not ignore the role played by glycogen. The turnover of this polysaccharide in the brain is very rapid *(43)*, and finely tuned to the energy needs of the activated tissue *(64,65)*. Astrocytic glycogen levels rapidly fall during brain stimulation, but the opposite occurs when brain activity is depressed *(43)*.

3. NEURONAL ENERGY DEPRIVATION

There are three major conditions, or encephalopathies, that could lead to neuronal energy deprivation: Hypoglycemia, or glucose deficiency in the blood; anoxia (hypoxia), or O_2 deficiency in the inspired air; and ischemia, or deficiency in blood supply, which, in essence, combines the first two deficiencies. All three encephalopathies are characterized by an inadequate supply of one of the two major energy substrates, or both. Since glucose and O_2 are the two major energy substrates of the brain, it is easy to understand how their deficiencies will affect the production of cerebral ATP and all the energy-demanding processes. Nevertheless, there are many other encephalopathies of intrinsic origin, such as deficiencies in one enzyme or another of the energy metabolic pathways, and seizures. Others may arise from extrinsic factors, such as vitamin deficiencies, toxicity of heavy metals, and viral infections. This chapter covers only the three major encephalopathies, i.e., hypoglycemia, anoxia (hypoxia), and ischemia.

There are two groups of researchers who study degenerative diseases of the brain. Basic scientists, who attempt to better understand the workings of cerebral energy metabolism and its regulation, and clinical scientists, who delve into the mysteries of these disorders, in an attempt to alleviate their terrible toll. Both groups depend on each other's discoveries to bring them closer to their goals. The basic scientist's approach is the one taken here, keeping the clinical aspects of the major encephalopathies to a necessary minimum. For scientists to study any disorder and its consequences, an experimental model which either mimics or closely resembles this disorder, is a must. An in vivo model usually employs an experimental animal; an in vitro one employs any number of subcellular, cellular, tissue, or organ preparations.

Once established, a good experimental model affords a better understanding of the sequence of events that lead to the final outcome of the disorder under study. The model also opens an opportunity for experimentation with potential treatments or preventive measures that could minimize or eliminate the damaging outcome of the disorder. This is when basic scientists and clinicians closely collaborate, namely, in the discovery, development, and later, the clinical test of these treatments for their potential neuroprotective value.

3.1. Hypoglycemia and Ischemia/Hypoxia

Although the phenomena of hypoglycemia, ischemia, and hypoxia have been dealt with separately in many publications, the decision to combine the three under one heading here is based chiefly on information published over the past two decades, which indicated many overlaps and parallels between these three encephalopathies. The main reason they have been traditionally separated has to do more with the distinguished metabolic vehicles that lead to the observed outcome, rather than with the outcome itself.

Hypoglycemia may occur in diabetes through insulin overdose, liver disease, and in many enzyme deficiencies, such as glucose-6-phosphatase, fructose-1,6-biphosphatase, phosphoenol-pyruvate carboxykinase, pyruvate carboxylase, or glycogen synthetase. Each of these deficiencies could affect normal function of the brain, and lead, if not corrected, to neurological deficits.

The most common outcome of the interruption of glucose supply to the brain is hypoglycemia-induced coma, which ensues when glucose blood levels drop to 8–9 mg/100 mL, or about 10% of the normal isoglycemic glucose level of 90 mg/100 mL

(66). In contrast, arterial O_2 content, mean blood pressure, cerebral respiratory quotient, and cerebral blood flow, all remain unchanged. However, cerebral O_2 consumption declines in hypoglycemic coma (56% of normal), along with a dramatic decline in cerebral glucose consumption *(66)*. These declines can be produced by an overdose of insulin, hepatic insufficiency, or by the inhibition of glucose metabolism with sufficiently high dose of 2DG *(67–69)*. Nevertheless, one should be aware that symptomatic hypoglycemia may occur at blood glucose levels that are significantly different from those mentioned above. Thus, in the diabetic, a decrease in blood glucose level from 300 to 100 mg/100 mL, upon insulin administration, may cause symptomatic hypoglycemia *(70)*. Yet, in a better-controlled diabetes, symptomatic hypoglycemia may not occur until the blood glucose levels reach 50 mg/100 mL. In normal, young humans after glucose tolerance test, blood glucose levels may fall to 30–40 mg/100 mL, without the appearance of any symptoms *(70)*.

Intraperitoneal injection of insulin is the most common method employed by investigators for the induction of hypoglycemia. With the right dose of insulin (50–100 U/kg), hypoglycemic coma ensues within 1–2 h following insulin injection in rats or mice *(71)*. In larger animals, such as the dog, higher doses of insulin (200–300 U/kg) are required to achieve coma. For metabolic studies, a small animal is the model of choice. For studies in which cerebral blood flow is involved, a larger animal model is required.

As mentioned earlier, hypoglycemic symptoms can be induced with a pharmacologic dose (400 mg/kg) of 2DG *(67–69)*. When hypoglycemia is induced with 2DG, blood glucose levels actually increase, rather than decrease, which is an indication that symptomatic hypoglycemia is not the result of low levels of blood glucose, but rather the result of a reduction in glucose metabolism or inadequate supply of glucose to its transporters, because of their occupation by 2DG.

Thus, although both methods abolish glucose metabolism, the consequences of these deprivations are markedly different. Insulin enhances glucose metabolism to the point at which the monosaccharide levels are low enough to trigger the utilization of alternative energy substrates, such as glycogen and ketones. In contrast, 2DG competes with glucose for both the glucose transporter and the enzyme, hexokinase, which neither reduces the glucose levels nor triggers the utilization of alternative substrates. Apparently, the effect of 2DG would be more swift and severe than that of insulin injection. Nevertheless, many of the gross consequences of hypoglycemia, such as histologic, electroencephalographic, and behavioral changes induced by both methods, should be similar.

Histologically, cerebral cortex neurons of rats exposed to insulin-induced hypoglycemia exhibit changes dependent on the duration of the hypoglycemic period beyond the onset of isoelectric EEG. A short, 30-min hypoglycemia produces mostly shrinkage and condensation of both cytoplasm and nuclei, but rats that are exposed to a longer, 60-min hypoglycemia exhibit significantly greater number of damaged cells *(71)*. Moreover, hypoglycemia produces cell swelling and the appearance of empty vacuoles. Swollen neurons are more abundant after the longer hypoglycemic period than after the shorter one. Although cell damage observed in other encephlopathies is frequently irreversible, the histologic changes that are recorded in hypoglycemic rats can be reversed by glucose supplement. Within 4 h after the onset of glucose supplementation by iv injection, more than 95% of the neurons have normal appearance *(71)*. The

phenomenon of swollen neurons could be explained by the derailment of the energy-dependent ion homeostasis. Shrinkage of cells, as acutely as it occurs in hypoglycemia, remains an unclear phenomenon, especially its reversibility with glucose supplementation.

Slow-wave EEG occurs when glucose blood levels fall to 2 m*M*. When glucose levels fall to 1 m*M* or lower, EEG becomes isoelectric *(71)*. Brain tissue in vitro responds in a similar way when glucose levels in the incubation medium are reduced. Evoked population spikes in the CA1 region of hippocampal slices cannot be sustained when glucose concentration falls below 1–2 m*M (31)*. In vivo, cerebral metabolic rate of glucose does not decrease until plasma glucose levels fall below 2.5 m*M (72)*. At these levels, the rate of glucose transport into the brain decreases to values 2–3× slower than normal, so that glucose concentration is insufficient to saturate hexokinase *(71,73)*. Rats, like humans, exhibit hypoglycemia-related behavioral changes that range from normal to comatose, in close correlation with the blood glucose levels *(71)*. Coma usually ensues when blood glucose levels fall to 1 m*M* or less. In some cases, however, hypoglycemia may induce seizures and seizure-like EEG, in which event, some of the changes, including the rate of decline in the blood glucose levels, and hence the onset of coma, may occur faster than expected.

Despite all these changes, reports on variations in cerebral blood flow range from an increase to no change during hypoglycemia-induced coma *(71)*. Similarly, depending on the depth of hypoglycemia and accompanying events, such as seizures, O_2 consumption can increase, decrease, or stay unchanged *(71)*.

One of the typical events that accompany hypoglycemia is hypothermia. By all accounts, this phenomenon is directly dependent on the reduction in glucose availability, rather than any of the possible causes of hypoglycemia. The fact that 2DG-induced hypoglycemia is accompanied by hypothermia *(67,69)* is a strong indication that 2DG, although increasing blood glucose levels, also produces functional hypoglycemia *(71)*. Thus, the postulate has been advanced that, when glucose becomes unavailable somehow, through specific temperature-regulating receptors, it induces the observed hypoglycemic hypothermia. The hypothermic effect of hypoglycemia can be reversed by high doses of fructose (5× the dose of 2DG), when administered intraventricularly, along with 2DG *(71,74)*. High doses of glucose should have similar effect to that of fructose, and, based on several recent in vitro studies, lactate, and possibly pyruvate, should also be able to overcome 2DG-induced hypothermia.

Since glucose is the main energy substrate in the brain, it is obvious that any deprivation of this fuel can lead to a shutdown of the very pathways of its metabolism. Thus, both the glycolytic pathway and the mitochondrial TCA are first to be affected by hypoglycemia. The levels of pyruvate, lactate, and glycogen fall significantly, once glucose blood levels fall to 2.5 m*M* or lower *(71)*. Similarly, the levels of the TCA intermediates also decrease in response to hypoglycemia *(71)*. Of the different metabolic effects of hypoglycemia, none is more important than the decline in brain ATP levels and, to a lesser degree, phosphocreatine levels. Notwithstanding, of the different brain regions, the cerebellum does not exhibit such falls in ATP and phosphocreatine levels, even late into the isoelectric period *(71)*. Although this unique ability of the cerebellum to withstand metabolic strain has been documented *(71)* using other models of metabolic stress, the mechanism governing this ability is unknown.

All the hypoglycemic symptoms, functional and behavioral, which are induced by an overdose of insulin, can be completely reversed by an administration of glucose.

Hypoxia is defined as low O_2 content or tension or O_2 deficiency in the inspired air. The extreme case of hypoxia is anoxia, the complete absence of O_2. Ischemia is defined as deficiency in blood supply caused by functional constriction or actual obstruction of a blood vessel *(75)*. Clearly, an organ that suffers from a deficiency in blood supply is also afflicted with hypoxia. Hence, many of the symptoms associated with hypoxia and ischemia are identical. Furthermore, the cellular processes and mechanisms that lead to posthypoxic and postischemic damage are identical. Thus, the term "cerebral ischemia" is being used throughout this subheading, with the understanding that it also refers to cerebral hypoxia.

There are probably more books, review articles, and research papers written about cerebral ischemia than any other encephalopathy. Focal cerebral ischemia (stroke) is the third leading cause of death in the United States and other industrialized countries. Stroke survivors usually suffer permanent and debilitating brain damage. The huge social and economic cost of this encephalopathy, on one hand, and the potential benefits from a future protective modality, on the other, continue to stimulate the research in this field.

The complete dependency of the CNS on glucose and O_2 for the generation of sufficient ATP supplies makes the brain the most vulnerable of all tissues to ischemia. Pure hypoxia is rarely encountered in humans. Pure hypoxia is experienced only in cases of pulmonary insufficiency, such as in pulmonary emphysema, in anemic patients, or in normal individuals who are acutely exposed to altitudes above 2400 m. Obviously, cerebral ischemia of identical duration should be more devastating than hypoxia, or even anoxia, because the ischemic brain suffers not only from oxygen deficiency, but also from shortage of glucose supplies. It is the compounded effect that makes cerebral ischemia so devastating. As is demonstrated below, and has been the case with hypoglycemia, both the brain in vivo, and any of its in vitro preparations, can tolerate either hypoxia or hypoglycemia alone longer than when the two are combined. In other words, the presence of one deficiency sensitizes the brain to the effect of the other. Thus, ischemia of a given duration is always more damaging than either hypoxia or hypoglycemia of a similar duration.

The most common events causing cerebral ischemia are stroke, cardiac arrest, and head injury or trauma. Stroke or focal ischemia occurs when blood supply is interrupted to a specific region of the brain; global ischemia occurs when blood supply to the entire brain is interrupted, as in cardiac arrest. Furthermore, both focal and global ischemia, depending on their severity and duration, may be complete (a total absence of blood flow) or incomplete (a drastic reduction in blood flow). The severity and the duration of these insults determine whether a reversible or irreversible cell injury occurs. An irreversible injury eventually leads to cell death (infarction).

Since there are brain regions that are more sensitive to ischemic injury than others, they are referred to as "selectively vulnerable" regions of neurons. In many of these regions, the neuronal death does not occur during or immediately after the ischemic insult. Frequently, neuronal death is delayed for 3–7 d. In focal ischemia, the vasculature surrounding the ischemic umbra, known as the penumbra, may still provide some collateral, although compromised, circulation, which, if treated early enough, could

rescue the penumbric neurons. Of the sensitive brain regions, the most vulnerable neurons are located in the CA1 sector of the hippocampus. Neurons in certain portions of the caudate nucleus, and in layers 3, 5, and 6 of the neocortex, are also selectively vulnerable. Understanding the mechanism that leads to this vulnerability is a major goal of many research laboratories around the world.

Several recently published monographs on cerebral ischemia *(76–78)* describe both in vivo and in vitro models of this encephalopathy. Many of the animal models of yesteryear, such as monkey, dog, cat, rabbit, and pig, are only rarely used today, mostly because of their very high cost. Most of the animal models of the past two decades make use of rodents. These models proved to be both cost-effective and reliable. Moreover, many of the recent advances in this field of research appear to be directly applicable to humans.

The focus of this chapter is on the effect of energy deprivation on neurons, but much can be learned from the enormous number of studies conducted over the last three decades on the cellular mechanisms leading to ischemic neuronal death. The putative roles of lactic acid, Glu, Ca^{2+} influx, and many other factors, in causing the delayed demise of ischemic neurons, have been discussed exhaustively elsewhere and are not the focus of this chapter. However, the hypotheses that were erected to explain this neuronal demise led to many elegant studies that reveal important details about cerebral energy metabolism and the energy-dependent cellular processes that could go awry when ATP is diminished. Of these processes, only those that are directly affected by failure of energy metabolism upon ischemia, hypoxia, and hypoglycemia are of interest here.

Of the many developments characterizing the past two decades, with which cerebral ischemic research is concerned, the employment of in vitro systems, such as the hippocampal slice preparation and cell cultures, has had the greatest impact. These systems can be manipulated, controlled, and analyzed in ways that in vivo systems cannot tolerate. Because most recent knowledge on the effects of energy deprivation on neuronal tissue has emerged from in vitro studies, the last portion of this chapter deals mainly with their findings.

A great advantage of the hippocampal slice preparation, compared to other in vitro systems, such as cell cultures, is the ability of the investigator to use electrophysiologic means to record neuronal function indistinguishable from in vivo records. Moreover, the recording can be made from the most vulnerable sector to ischemia/hypoxia in the hippocampus, the CA1 region. Within 2–3 min of changing the atmosphere that slices are exposed to, from 95% O_2/5% CO_2 to 95% N_2/5% CO_2, the evoked population spike (neuronal function) amplitude falls to 0 mV. The ability of hippocampal slices to recover neuronal function, upon return to normal reoxygenation, depends on the duration of the hypoxic period, and on the concentration of glucose in the artificial cerebrospinal fluid. The higher the glucose concentration, the longer the hypoxic period from which neuronal function can recover (Fig. 5).

Reduction in glucose concentration does not visibly affect neuronal function in hippocampal slices, until it falls below 1.5 m*M*. However, when glucose deprivation is combined with hypoxia (in vitro ischemia), a reduction in glucose concentration, from 10 to 5 m*M*, significantly decreases the ability of hippocampal neuronal function to recover posthypoxia (Fig. 5). In contrast, increasing glucose concentration, from 10 to

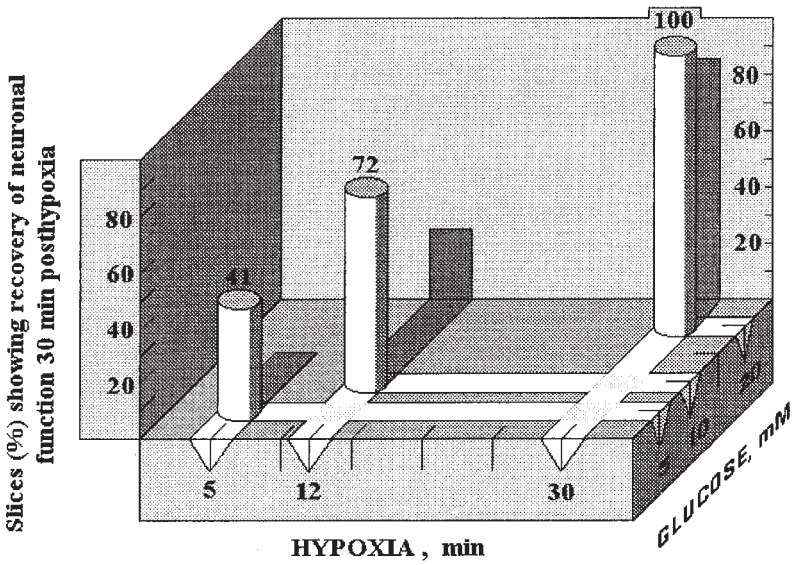

Fig. 5. Rates of recovery of neuronal function (orthodromically evoked CA1 population spike) in rat hippocampal slices after 5, 12, or 30 min of hypoxia and 30 min reoxygenation in the presence of 5, 10, or 20 m*M* glucose, respectively. The higher was the glucose concentration in the perfusion medium, the longer was the duration of hypoxia slices could tolerate.

20 m*M*, significantly prolongs the hypoxic period from which hippocampal neuronal function could recover (Fig. 5). Thus, the higher the concentration of glucose during O_2 deprivation in vitro, the better the recovery of neuronal function posthypoxia. This relationship is in disagreement with in vivo findings, in which hyperglycemia has been shown to aggravate neuronal ischemic damage, a phenomenon known as the "glucose paradox" *(79,80)*. These findings led to the formulation of the "lactic acidosis" hypothesis of cerebral ischemic damage *(50,81)*. The glucose paradox, and other apparent contradictions between results obtained in vitro and in vivo, mostly in the early and mid-1980s, impeded the acceptance of the hippocampal slice preparation as a valuable tool in the study of brain energy metabolism and brain encephalopathies.

A hallmark of ischemic/hypoxic conditions in all tissues, including brain, is the increase in lactate production above the baseline levels under normoxia. When hippocampal slices are exposed to O_2 deprivation, lactate production quickly increased 5–6-fold *(44)*. As mentioned earlier, lactate can support normal neuronal function in hippocampal slices as the sole energy substrate. Moreover, lactate also has been shown to be a preferred energy substrate over glucose in excised sympathetic chick ganglia *(40–42)* and in rat cerebellar slices *(34)*. Lactate is preferable to glucose when recovery from hypoxia is concerned *(44)*. Moreover, lactate has been shown to be a mandatory aerobic energy substrate for recovery of neuronal function posthypoxia in hippocampal slices *(44,56,82)*.

To demonstrate this role of lactate in the recovery of neuronal function posthypoxia, two experimental protocols were used. The first manipulated the ability of the glucose analog, 2DG, to block lactate production during hypoxia via inhibition of glycolysis (Fig. 6). Normally, 100% of slices perfused with 20 m*M* glucose recover their neuronal

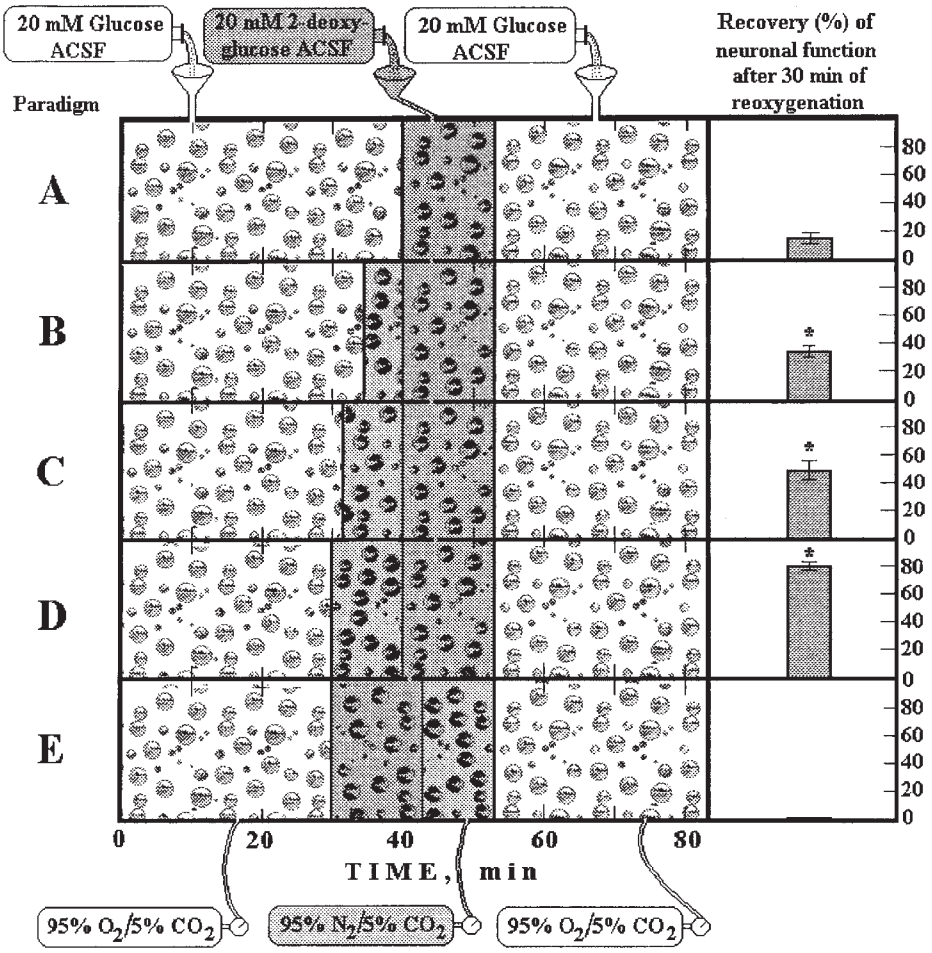

Fig. 6. A schematic illustration of the experimental paradigms used in establishing the role of anaerobically produced lactate in the posthypoxic recovery of neuronal function in rat hippocampal slices. Shown are five different experimental paradigms (**A–E**), the various compositions of artificial cerebrospinal fluid that perfused the slices during each paradigm, and the different gas mixtures (bubbles) that were passed over the slices during these paradigms. In each paradigm, slices were first equilibrated with a medium containing 20 m*M* glucose and an oxygenated atmosphere, for at least 30 min (pretreatment). After a treatment period, slices were allowed to recover for 30 min under pretreatment conditions, before measuring recovery of neuronal function (bars on the right side of each paradigm's panel). In paradigm **A**, slices were exposed to 13-min hypoxia (gray shading), as 20 m*M* glucose-containing medium was replaced with 20 m*M* 2DG-containing medium. In paradigms **B–D**, the hypoxic period started 5, 8, or 10 min prior to the onset of 2DG perfusion, and continued an additional 13 min, for a total of 18, 21, or 23 min of hypoxia, respectively. The longer the hypoxic period prior to the blockade of glycolysis with 2DG, the more lactate produced and the higher the recovery rate of neuronal function posthypoxia. Thus, in what appears as a paradox, slices that were exposed to a total of 23 min hypoxia exhibited a significantly higher rate of neuronal function recovery than slices exposed to only 13 min hypoxia. Paradigm **E** is similar to paradigm **D**, except that, for the first 13 min of hypoxia, slices were perfused with 2DG, and, for the last 10 min, slices were perfused with glucose-containing medium. Slices treated according to paradigm **E** were unable to produce energy and lactate glycolitically during the first 13 min of hypoxia and, hence, the lack of recovery of neuronal function. Histograms on the right are mean values +SD. *Significantly different from paradigm **A** ($p < 0.0005$).

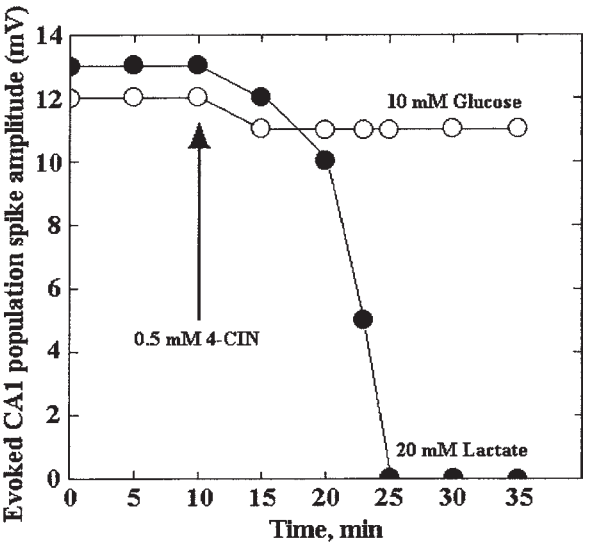

Fig. 7. The effect of 0.5 m*M* 4-CIN, a monocarboxylate transporter inhibitor, on the evoked population spike (neuronal function) amplitude in two slices, over time. One slice (open circles) was perfused with artificial cerebrospinal fluid containing 10 m*M* glucose, while the other was perfused with the same fluid containing 20 m*M* lactate. When 4-CIN was added to the perfusion medium of both slices, only the one that was perfused with the medium containing lactate exhibited diminution of neuronal function, because the inhibitor blocked the entry of lactate into neurons. The inhibitor had no effect on the entry of glucose into neurons.

function after 30-min reoxygenation from 23-min hypoxia. Control slices continued to be perfused with 20 m*M* glucose during the first 10 min of hypoxia, at which time the perfusion was switched to 20 m*M* 2DG for the last 13 min of hypoxia: 80% of these slices exhibited recovery of synaptic function posthypoxia (Fig. 6, paradigm D). Experimental slices were also exposed to 23-min hypoxia; however, 20 m*M* 2DG was perfused during the first 13 min of hypoxia, followed by 20 m*M* glucose for the last 10 min of hypoxia. None of the experimental slices recovered neuronal function posthypoxia (Fig. 6, paradigm E). Although control slices were able to produce lactate during the first 10 min of hypoxia, experimental slices were unable to do so, since glycolysis was inhibited from the onset of hypoxia, thus preventing lactate production. Results shown in Fig. 6 clearly demonstrate the importance of lactate in the posthypoxic recovery of neuronal function. The more time slices were allowed to produce lactate anaerobically before the inhibition of glycolysis with 2DG, the higher the percentage of slices that recovered neuronal function posthypoxia. Hence, more slices that were exposed to a total of 23 min hypoxia recovered their neuronal function than did slices that were exposed to 13-, 18-, or 21-min hypoxia (Fig. 6, paradigms A–D). The most plausible explanation for this phenomenon is the higher levels of lactate found in tissue slices exposed to 23-min hypoxia than the levels in slices exposed to shorter hypoxic periods.

The second experimental protocol employed the specific neuronal lactate transporter inhibitor, 4-CIN *(56,83,84)*. As shown in Fig. 7, this compound is able to block lactate-

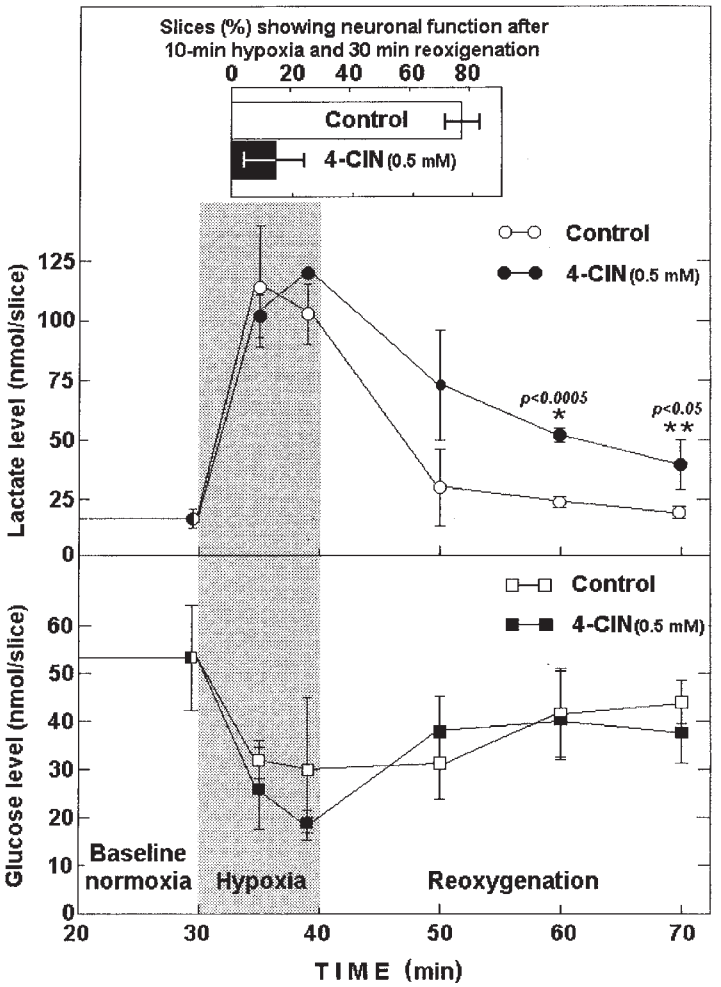

Fig. 8. The effect of 0.5 m*M* 4-CIN on rat hippocampal slices' ability to recover their neuronal function posthypoxia (histogram in **upper panel**), and on their levels of lactate and glucose. A significantly lower percentage of slices recovered neuronal function posthypoxia in the presence of 4-CIN. This poor recovery rate probably resulted from inhibition of neuronal lactate utilization by the monocarboxylate transporter inhibitor. The significantly higher levels of lactate that were present during reoxygenation in 4-CIN-treated slices indicate that neuronal lactate utilization was inhibited. Bars represents means ± SD. *Significantly different from control slices, $p < 0.0005$; **$p < 0.05$.

supported neuronal function in rat hippocampal slices (*see also* ref. *56*). When perfused with 10 m*M* glucose, 78% of control slices recovered neuronal function after 10-min hypoxia; only 15% of experimental slices recovered neuronal function after being exposed to 10-min hypoxia in the presence of 0.5 m*M* 4-CIN (Fig. 8). Both groups of slices produced high levels of lactate during O_2 deprivation, although experimental slices exhibited a significantly slower decline in those levels during reoxygenation. This slower decline could result from blockade of neuronal lactate utilization by 4-CIN (Fig. 8).

The results of the above experiments also explain the ability of elevated glucose concentration, if supplemented before hypoxia, to afford neuroprotection against hypoxic damage in hippocampal slices (Figs. 5 and 6). This neuroprotection is probably the result of two separate processes: First, the increase in glucose availability allows the tissue to maintain glycolytic flux for a longer period of time, for the support of ion homeostasis; second, the longer glycolytic flux produces a significant increase during hypoxia in the lactate level, which in turn is available for aerobic utilization during the initial stages of reoxygenation, when both ATP and glucose levels are very low. The ability of the specific neuronal monocarboxylate transporter inhibitor, 4-CIN, to block posthypoxia recovery of neuronal function, provides further evidence that aerobic lactate utilization is crucial for such recovery.

Although these in vitro results befit the general understanding of both the aerobic and anaerobic energy metabolism pathways and their consequences (including the "more glucose, better tolerance to hypoxia" concept), most investigators in the field of cerebral ischemia find them hard to accept fully. The reason for this skepticism has much to do with the heavily heralded in vivo phenomenon, in which preischemic hyperglycemia significantly exacerbates delayed neuronal damage, as measured 4–7 d postischemia. This glucose paradox phenomenon of cerebral ischemia was first reported almost 25 yr *(79)*, and has been reproduced numerous times in different in vivo models of ischemia. It became the cornerstone of the lactic acidosis hypothesis, which attributes the bulk of the delayed neuronal damage observed 4–7 d postischemia to the increase in lactic acid levels in the brain and the resultant acidosis *(50)*.

The glucose paradox of cerebral ischemia also appears to affirm the main premise of the lactic acidosis hypothesis: more glucose = more lactic acid = more delayed neuronal damage. But does it? If acidosis (pH 6.8–6.5) is detrimental to neurons exposed to O_2 deprivation or to O_2–glucose deprivation, the same trend should be seen in vitro. However, experiments, both in brain slices and in neuronal cultures, showed that acidosis not only did not exacerbate hypoxic or ischemic neuronal damage in vitro, but in essence was actually neuroprotective *(85–87)*. Again, this contradiction between the in vitro and in vivo outcomes was used to criticize the in vitro approach to the study of cerebral ischemia. Nevertheless, the agreement of in vitro results on cerebral ischemia with in vivo findings, over the past two decades, significantly surpasses the disagreement.

If one hypothesizes lactate to be a major energy substrate upon reoxygenation after hypoxia, rather than to be a detrimental factor, perhaps one could uncover supportive in vivo data. Of the vast number of in vivo studies over the past three decades, several included measurements of tissue levels of energy substrates and metabolites, such as glucose, lactate, ATP, and adenylate energy charge, before, during, and after an episode of cerebral ischemia. Such data from three key studies *(88–90)* are depicted in Fig. 9. The trend illustrated by these three studies is typical: Normal preischemic brain levels of glucose (1.7–3.0 μmol/g) and lactate (1.0–1.8 μmol/g) were drastically changed following 5–10 min of anoxia or ischemia. Although glucose levels fell (to 0–0.5 μmol/g), those of lactate rose sharply (to 12–20 μmol/g). After only 15 min of reperfusion/reoxygenation, glucose in the brain climbed to levels significantly higher than those existing preischemia *(88–90)*. Even after 90 min of reperfusion, brain glucose level remained over 200% of preischemic level *(88)*. Despite the dramatic increase

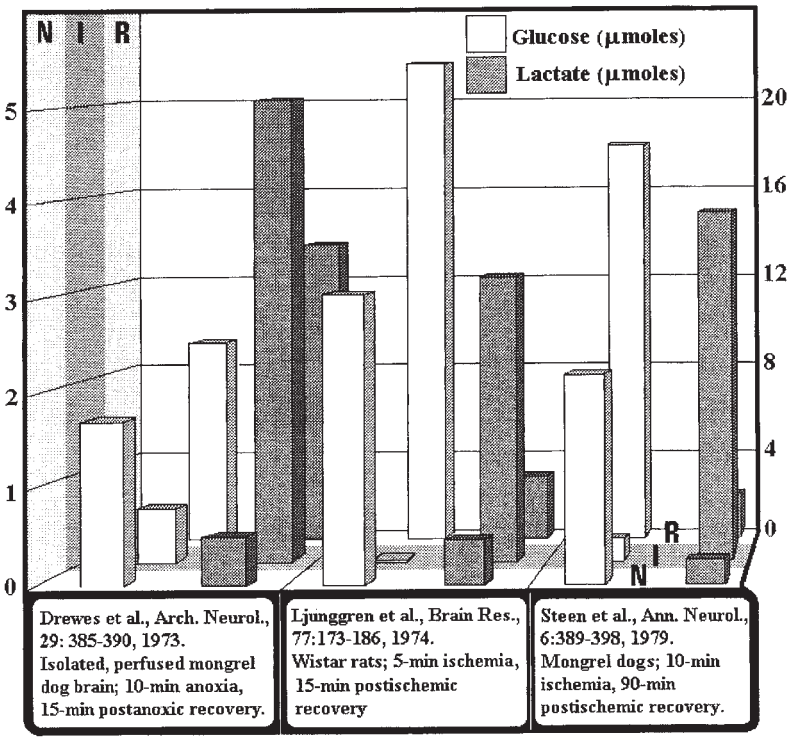

Fig. 9. Brain tissue levels (µmol/g) of glucose (white bars) and lactate (gray bars) at normoxia (N), at the end of ischemia/anoxia (I), and after reperfusion/reoxygenation (R), as reported in three separate and independent studies *(88–90)*.

in lactate concentration after 10 min of anoxia, the rate of its efflux from the brain to the venous blood remained unchanged from the very low control levels (approx 0.2 µmol/g/min) before anoxia *(89)*.

These findings indicate that the brain tightly conserves the lactate it produces, and agree with the results described above for hippocampal slices *(44,56,82)*. The postischemic/postanoxic increase in brain glucose was accompanied by a rapid decrease in lactate levels. The investigators themselves termed this decrease "lactate utilization" *(89)*. Energy charge (EC, [ATP + 1/2ADP/{ATP + ADP + AMP}]) was also measured before anoxia (0.89), after 10 min of anoxia (0.39), and after 15 min of reperfusion/reoxygenation (0.92) *(89)*. Similar results were reported in the other two studies *(88,90)*.

A plausible explanation for a postischemic/postanoxic decline in brain lactate levels, and a concomitant increase in glucose levels, could be as follows. Upon reoxygenation, lactate is utilized, aerobically, as an energy substrate, but glucose remains mostly unused, resulting in its accumulation above control levels. This aerobic lactate utilization is sufficient to rapidly restore preanoxic ATP levels and energy charge values.

Although it would be prohibitive to review the vast body of data here, the most revealing information can be extracted from a study *(81)* that was aimed at investigat-

ing the role of lactic acidosis in ischemic brain damage under hyperglycemic conditions. But it is the outcome during hypoglycemic conditions that deserves closer attention. Rats fasted for 16–24 h, and exposed to 30 min of severe incomplete ischemia, showed more than a 17-fold increase in brain lactate (from 0.88 to 15.5 μmol/g). Both glucose and glycogen levels fell essentially to zero (from 2.78 and 2.84 μmol/g, respectively) during the same period; ATP levels fell by 95% from the control level. Recirculation for 90 min brought about a significant decrease in brain lactate levels, from 15.5 to 3.19 μmol/g (a decrease of 79%). Glucose levels rose concomitantly to 106% of control, although the rats were not supplemented with glucose during recirculation. ATP rose to 81% of its control level, and 54% of the glycogen pools was restored. Even more striking, however, were the results when another group of fasting rats was infused with glucose (2 mL 50% glucose solution) during the first 10 min of recirculation. After 30 min of recirculation, the lactate level dropped 58% (from 15.5 μmol/g at the end of 30 min of ischemia, to 6.44 μmol/g), declining by 83% after 90 min recirculation. In contrast, glucose levels, which rose only to 3.58 μmol/g in the control, nonischemic animals, rose dramatically in the ischemic rats, to 15.7 μmol/g (439% increase, compared to control) at 30 min recirculation. Even after 90 min recirculation, glucose levels were still 380% of the control level. Again, this 20-yr-old study indicates clearly that, during the first hour or so of postischemia, the brain metabolizes lactate aerobically, while glucose remains unused.

In a recent preliminary study, using a rat model of cardiac-arrest-induced transient global cerebral ischemia (TGI), three important results were obtained *(91)*. First, the glucose paradox, the phenomenon that was heralded as the proof for the detrimental role of lactic acidosis in delayed neuronal damage, can be brought about by hyperglycemia (2 g/kg glucose loading), when induced up to 1 h pre-TGI. Rats that were treated in this way exhibited a significant increase in delayed neuronal damage post-TGI. Second, loading glucose 2–4 h pre-TGI, while rendering the rats hyperglycemic at the time of the ischemic insult, did not exacerbate the delayed neuronal damage post-TGI. Rather, these rats showed significantly less neuronal damage than that measured in control, isoglycemic rats. Third, brain lactate levels were equally high in both hyperglycemic groups, thus refuting the argument that increased lactate level during an ischemic insult is the reason for exacerbation of neuronal damage.

4. CONCLUDING REMARKS

Neuronal energy metabolism has emerged in recent years as an active field of study, with great potential of shedding light on newly explored intrinsic mechanisms supporting special neuronal energy needs. The bulk of the research until now has been done using in vitro systems, laying the foundation on which future in vivo studies will undoubtedly appear. The few in vivo studies already available do not necessarily agree with each other, especially regarding the question of anaerobic vs aerobic glucose utilization during neural activation. Nevertheless, the new information challenges some of the old dogmas of brain cellular energy metabolism both under normal conditions and under conditions of energy deprivation. The next few years promise to be exciting in terms of new knowledge, its understanding, and its practical uses.

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