CHAPTER

11

Energy Metabolism of the Brain

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OUTLINE

INTRODUCTION

Processes related to signaling require a larger proportion of energy than do 'basic' cellular functions

The human brain is an 'expensive' organ that represents ≈2% of body weight but accounts for ~20% of the body's resting metabolic rate (Attwell & Laughlin, 2001; Clarke & Sokoloff, 1999) [and references therein]; most of this energy is used to maintain or re-establish membrane potentials. About 75% of the brain's calculated energy consumption is related to signaling, with the remainder ascribed to essential cellular activities including turnover of proteins, nucleotides, phospholipids, and other compounds, and axoplasmic transport (Attwell & Laughlin, 2001) (Table 11-1). For comparison, ATP consumption per gram brain per minute used for signaling equals the energy used by a human leg muscle running a marathon or the osmotic work in the kidney (Attwell & Laughlin, 2001; Clarke & Sokoloff, 1999). The overall energy use by human brain is estimated to be 21 µmol ATP/g/min. Local metabolic rates in gray matter are considerably higher and vary over a larger range than in white matter (Clarke & Sokoloff, 1999) (Table 11-2). Even during sleep there is only a relatively small decrease in cerebral metabolic rate; indeed, it may even be increased in rapid eye movement (REM) sleep (Clarke & Sokoloff, 1999). Regional metabolic rates are higher in rodents compared to primates, but the rank order of structures according to glucose utilization rate is similar in all species.

Glucose is the main obligatory energy substrate for adult brain and is also very important for developing brain. The equation for the complete oxidation of glucose is $\mathbf{C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O}$. This equation indicates that the theoretical maximum molar ratio of oxygen to glucose utilization is 6:1. Nearly all of the glucose taken up into normal resting brain is oxidized, but during brain activation glucose utilization usually increases more than oxygen consumption (Linde et al., 1999; Madsen et al., 1995). Oxygen is utilized in the brain almost entirely for the oxidation of carbohydrates (Sokoloff, 1960), consistent with the respiratory quotient (CO_2

TABLE 11-1 Calculated Energy Use by Brain

Use	Energy expense (% of total energy)
SIGNALING	**75**
Action potentials	35.3
Postsynaptic receptors	25.5
Resting potentials	9.8
Glutamate recycling	2.3
Postsynaptic Ca^{2+}	2.3
BASIC CELLULAR ACTIVITIES	**25**
Phospholipid turnover & membrane distribution	≈5
Turnover of proteins & oligonucleotides	≈2
Axoplasmic transport	*
Mitochondrial proton leak	≈20†

*Energy cost unknown.
†Value for brain is comparable to other tissues (Martin Brand, personal communication 2010).
Adapted from Attwell and Laughlin (2001).

production/O_2 consumption) of 0.97 for brain (Table 11-2). Oxidative metabolism provides most of the energy required for the brain's activities and oxygen is also required for catecholamine neurotransmitter synthesis and degradation by the enzymes, tyrosine hydroxylase, dopamine-β-hydroxylase, tryptophan hydroxylase, and monoamine oxidase. In fact, synthesis of serotonin and acetylcholine is extremely sensitive to hypoxia (low oxygen level), and synthesis rates fall with graded hypoxia even before lactate levels rise (Davis et al., 1973; Gibson & Duffy, 1981).

Function-derived signals arising from metabolism are used for brain imaging

Brain activity, metabolic demand, and blood flow rate (to deliver fuel and remove by-products) are very closely linked at a local level (see section below and Figure 11-7). Increased cellular activity related to a specific brain function (e.g., processing information due to sensory stimulation or mental computations) raises local demand for ATP, and stimulates metabolic pathway fluxes, ATP production, and the rate of blood flow to activated tissue; conversely, reduced activity downregulates glucose and oxygen consumption and blood

TABLE 11-2 Cerebral Metabolic Rates (CMR) Vary Regionally and Are Activity Dependent

	Rat	Human
RESTING CMR (WHOLE BRAIN)		
Oxygen consumption (CMR_{O2}, μmol(g min)$^{-1}$)	3.67 (Linde et al., 1999)	1.43 (Madsen et al., 1995)
Glucose utilization (CMR_{glc}, μmol(g min)$^{-1}$)	0.65 (Linde et al., 1999)	0.23 (Madsen et al., 1995)
CMR_{O2}/CMR_{glc}	5.65 (Linde et al., 1999)	6.1 (Madsen et al., 1995)
Respiratory quotient†		0.97 (Clarke & Sokoloff, 1999; Sokoloff, 1960)
REGIONAL DIFFERENCES IN CMR_{glc} (μmol(g min)$^{-1}$)		
Cortex	1–1.6 (Sokoloff et al., 1977; Nakao et al., 2001; Ueki et al., 1992)	0.3–0.4[b]
White matter	0.3–0.4 (Sokoloff et al., 1977)	0.2[b]
Focal stimulation (% increase)*	55–130 % (Nakao et al., 2001; Ueki et al., 1992)	10–60 %[c] (Fox et al., 1988)
Anesthesia‡	0.35–0.65 (Nakao et al., 2001; Ueki et al., 1992)	
Coma§	0.27[a]	0.13–0.15 (Clarke & Sokoloff, 1999; Sokoloff, 1960)

*Metabolic response to a stimulus varies with the pathway, structure in pathway, and intensity and complexity of the stimulus.
†Ratio between CO_2 produced and oxygen consumed; values of approximately one indicate that carbohydrate, not lipid, is the primary fuel for brain.
‡Anesthesia with light barbiturate, halothane or α-chloralose.
§Coma induced by barbiturate, diabetes or hypoglycemia; human values for CMR_{glc} are estimated by calculating as $CMR_{O2}/6$.
[a]Ghajar, J.B., Plum, F., Duffy, T.E. (1982). Cerebral oxidative metabolism and blood flow during acute hypoglycemia and recovery in unanesthetized rats. *Journal of Neurochemistry, 38*, 397–409.
[b]Heiss, W.D., Pawlik, G., Herholz, K., Wagner, R., Goldner, H., Wienhard, K. (1984). Regional kinetic constants and cerebral metabolic rate for glucose in normal human volunteers determined by dynamic positron emission tomography of [^{18}F]-2-fluoro-2-deoxy-D-glucose. *Journal of Cerebral Blood Flow and Metabolism, 4*, 212–223.
[c]Phelps, M.E., Kuhl, D.E., Mazziota, J.C. (1981). Metabolic mapping of the brain's response to visual stimulation: studies in humans. *Science, 211*, 1445–1448.

flow (Clarke & Sokoloff, 1999) (Table 11-2). Signals derived from metabolic pathways or from metabolism of various endogenous or exogenous compounds have, therefore, been used as representative of functional activity under a wide variety of conditions, including normal 'rest' and stimulation, pathophysiological studies of various diseases and their progression, and *in vivo* and *in vitro* pharmacological treatments. Interpretation of brain images requires a detailed understanding of the biochemical, cellular, and physiological basis for the signals (Dienel & Cruz, 2008).

Major cell types and their subcellular structures have different energetic requirements and metabolic capabilities

The brain contains two major classes of cells: neurons and glia. Neurons have many phenotypes that reflect different neurotransmitters and structural and functional properties; glia include astrocytes, oligodendroglia and microglia, each of which has specific and quite different functions. The proportion of energy used by the major cell types is unclear, but major white matter tracts that contain oligodendrocytes have much lower metabolic rates than neuropil that contains astrocytes and neurons; also, neuronal cell bodies have lower rates of glucose utilization than the synapse-rich regions that contain pre- and post-synaptic neuronal processes and astrocytes (Clarke & Sokoloff, 1999). Estimates for glia range from about 17.5% (recalculated from Attwell & Laughlin, 2001) to 40% of oxidative metabolism (Öz et al., 2004; Hertz et al., 2007; Hyder et al., 2006). Some processes with high energy costs, such as phospholipid turnover and maintenance of phospholipid polarization in membranes, are likely to be higher in glia than in neurons due to the large surface area of fine filopodial processes of astrocytes and of the myelin of oligodendrocytes. Most pathways of energy metabolism in brain are similar to those in other tissues, but compartmentation of metabolism in brain due to the highly specialized cellular and subcellular functions and the corresponding localization of transporters, enzymes, and metabolic pathways makes brain cells uniquely interdependent. Metabolic compartmentation is defined as the presence in cells or tissue of more than one distinct pool of a given metabolite. These separate pools of a metabolite are not in rapid equilibrium with each other but maintain their own integrity and turnover rates (Berl et al., 1975).

SUBSTRATES FOR CEREBRAL ENERGY METABOLISM

Energy-yielding substrates enter the brain from the blood through the blood–brain barrier

The brain requires a continuous supply of oxygen and glucose, both of which are essential for developing (see development section) and adult brain. Substrates used by the brain are taken up from the circulation via transporters in the endothelial cells, the tight junctions of which form the blood–brain barrier.

Endothelial cells of the blood–brain barrier and brain cells have specific transporters for the uptake of glucose and monocarboxylic acids

The main energy nutrient transporters in the blood–brain barrier are the highly glycosylated 55 kDa isoform of the glucose transporter (GLUT) GLUT1 and the **M**ono**c**arboxylic acid **t**ransporter (MCT) isoform MCT1 (Figure 11-1). The uptake of energy substrates by brain cells is influenced by the type and distribution of transporters unique to each cell type. Transport rate is dependent on the number of transporters and the catalytic activity of each transporter, i.e., the number of transport turnover cycles (K_{cat}) per second per transporter (Simpson et al., 2007). The selective distribution of the individual isoforms of transporter molecules influences the overall substrate availability in individual types of brain cells and in brain. The 45 kDa form of glucose transporter GLUT1 is found on astrocytes and the choroid plexus (Simpson et al., 2007). The high-capacity glucose transporter GLUT3 is found on neurons and, more recently, to some extent on endothelium (Simpson et al., 2007; Vannucci & Simpson, 2003), and GLUT5 is localized primarily on microglia. Although astrocyte end feet cover ≈96% of the blood–brain barrier, metabolic modeling predicts that most of the glucose entering brain is distributed to brain cells via interstitial fluid and that most of that glucose is taken up into neurons due to similar numbers of glucose transporters in neurons (GLUT3) and astrocytes (GLUT1) but a much higher calculated glucose transport rate into neurons compared to astrocytes (i.e., about ninefold at 5 mmol/L glucose) due to the much greater catalytic capacity of GLUT3 compared to GLUT1 (Simpson et al., 2007; Simpson et al., 2008; Mangia et al., 2009b; DiNuzzo et al., 2010). Approximately equal proportions of deoxyglucose are recovered as deoxyglucose-6-phosphate in cell bodies of neurons and astrocytes in brain *in vivo* (Nehlig et al., 2004), but interpretation of these results is limited by high washout (~50%) of label during immunoassays. The contributions of the metabolically active perisynaptic structures (pre- and post-synaptic neuronal elements and astrocytic filopodia) to glucose utilization are unknown.

The levels of both GLUT1 and GLUT3 increase dramatically with cerebral maturation and synaptogenesis. GLUT3 is relatively low in nonvascular portions of rat brain at 7 days of age, then increases almost linearly about 10-fold by 28 days of age (Vannucci & Simpson, 2003). The increase in GLUT1 occurs at a slightly later age; levels remain low in nonvascular portions of rat brain until 14 days of age, then increase several fold until 28–35 days of age (Vannucci & Simpson, 2003). Although the distribution and properties of different transporters give insight into the potential capabilities of brain cells to take up specific substrates, there is still uncertainty about the proportions of glucose actually taken up and metabolized by specific domains. However, metabolic labeling by infused or injected [^{14}C- or ^{13}C-] glucose very rapidly leads to faster and greater labeling of glutamate via α-ketoglutarate, originating primarily in the neuronal TCA cycle compared to glutamine, which is synthesized in astrocytes (van den Berg & Garfinkel, 1971; Fitzpatrick et al., 1990) (see later section for information about labeling studies). Thus, glucose (or the acetyl CoA or other compounds produced from glucose metabolism) must be metabolized in neurons to a considerable extent

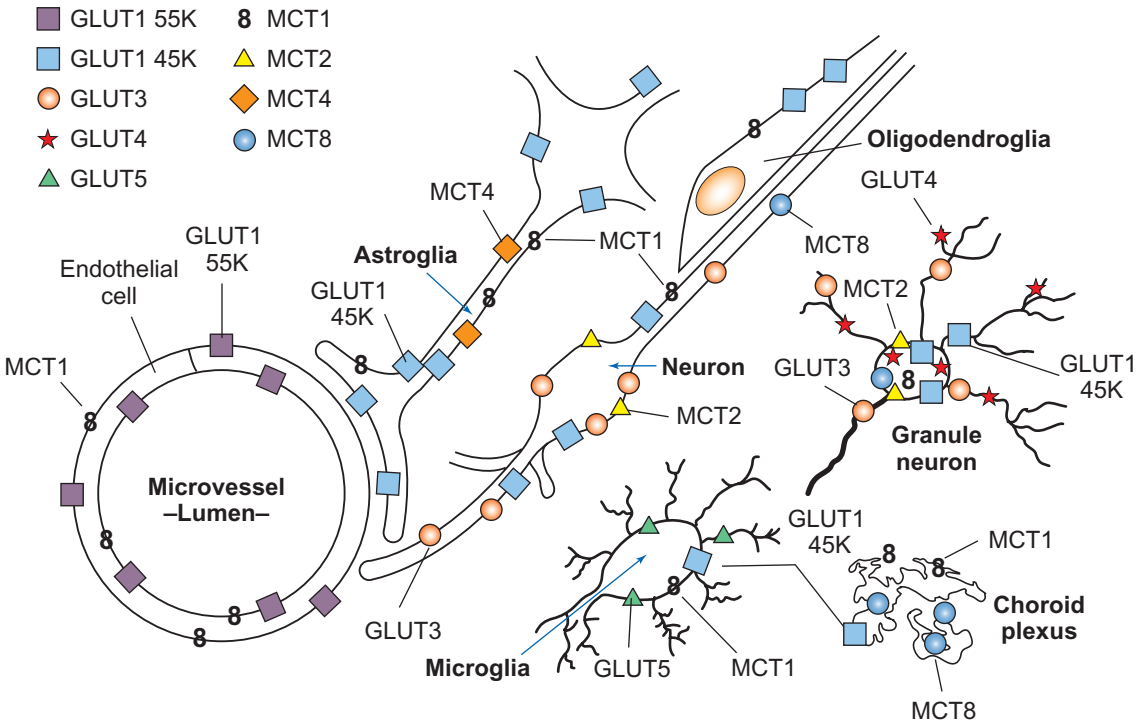


FIGURE 11-1 **Cellular localization of specific isoforms of the glucose and monocarboxylic acid transporters in brain**. Note that specific transporters are localized on different types of brain cells. *(Courtesy of Ian Simpson and Susan Vannucci.)*

(Berl et al., 1975; van den Berg & Garfinkel, 1971; Fitzpatrick et al., 1990).

Eight different members of the proton-coupled MCT transporter family have been identified, but only the MCT1–4 transporters have been characterized in detail in brain. Reports on the distribution of MCT transporters in brain cells are conflicting due to differences between studies *in vivo* and in cultured cells, differences in mRNA expression compared to transporter protein levels, and species differences (Vannucci & Simpson, 2003; McKenna et al., 2006c)[and references therein]. In immature brain, MCT1 and MCT2 have been found in all cell types, which is consistent with the importance of monocarboxylic acids as substrates at early ages (Vannucci & Simpson, 2003). In adult brain, MCT1 is localized on the vasculature and most, but not all, astrocytes *in vivo* (Vannucci & Simpson, 2003; McKenna et al., 2006c)[and references therein]. Kinetic studies identified a lower-affinity astrocytic MCT transporter (Tildon et al., 1993), consistent with MCT4 on some astrocytes (Simpson et al., 2007) (Figure 11-1). The higher-affinity monocarboxylate transporter MCT2 is localized primarily on neurons in mature brain (Vannucci & Simpson, 2003; McKenna et al., 2006c; McKenna et al., 1998) [and references therein]. However, kinetic properties for lactate transport into synaptosomes isolated from adult brain are not fully consistent with properties of MCT2, suggesting the presence of another neuronal MCT transporter (McKenna et al., 1998; Mac & Nalecz, 2003). Although the staining for MCT in brain slices appears more diffuse with maturation, the level of MCT1 in nonvascular tissue remains fairly constant, whereas MCT2 transporter protein level in neurons increases with development (Vannucci et al., 2003).

Blood–brain barrier transport can be altered under pathological conditions

Prolonged hypoglycemia increases the amount of GLUT1 on the vasculature and glucose uptake into brain (Simpson et al., 1999; Kumagai et al., 1995). However, the effects of chronic hyperglycemia and diabetes mellitus on glucose transport are conflicting; some studies report decreased glucose uptake while others find no difference (Pelligrino et al., 1992). A rat model of diabetes mellitus showed no alterations in glucose transport or blood–brain barrier GLUT1 protein content compared to controls, in spite of increased endothelial GLUT1 mRNA level (Simpson et al., 1999), indicating that changes in gene expression need not be reflected by changes in physiological function. MCT1 on the blood–brain barrier may be upregulated by a ketogenic diet and in other conditions with high levels of circulating monocarboxylic acids, such as in diabetes mellitus and cancer (Leino et al., 2001).

AGE AND DEVELOPMENT INFLUENCE CEREBRAL ENERGY METABOLISM

The transporters and pathways of metabolism change during development

The energy metabolism of the brain and the blood flow that sustains the metabolic rate vary considerably from birth to old age. Cerebral oxygen consumption is low at birth, rises rapidly during the period of cerebral growth and development

and reaches a maximal level at about the time maturation is completed. The newborn infant tends to be hypoglycemic but becomes ketotic when it begins to nurse because of the high fat content of the mother's milk (Vannucci & Simpson, 2003; McKenna et al., 2006c; Cremer, 1982; Nehlig, 2004) [and references therein]. The ketone bodies have high rates of transport from circulating blood through the blood–brain barrier and into brain until weaning, when transporter number and transport rate falls quickly, as glucose utilization rises markedly (Vannucci & Simpson, 2003; Cremer, 1982). High amounts of MCT1 and GLUT1 and low levels of MCT2 are present in the microvasculature during development (Vannucci & Simpson, 2003), and as GLUT1 level in the vasculature rises and glucose becomes the predominant fuel entering brain, and MCT1 declines, reflecting changes in the availability of circulating substrates and the developmental progression towards glucose dependence (Vannucci & Simpson, 2003; Cremer, 1982) [and references therein].

During the suckling period the brain is highly dependent on the use of ketone bodies for energy and as carbon source for synthesis of lipids, amino acids and protein (Cremer, 1982; Nehlig, 2004) and hence, the activities of the enzymes for utilization of these substrates are high, whereas activities of pyruvate dehydrogenase and of some oxidative enzymes are lower (Patel et al., 1975). The first two enzymes in the pathway of ketone utilization are D-β-hydroxybutyrate dehydrogenase and aceto-acetyl-succinyl-CoA transferase. These exhibit a postnatal pattern of development that is well adapted to the nutritional demands of the brain (Patel et al., 1975; Settergren et al., 1976). At birth, the activities of these enzymes in brain are low; activities rise rapidly with the ketosis that develops at the onset of suckling. The activities reach their peaks just before weaning and then gradually decline after weaning to normal adult rates of approximately one-third to one-fourth the maximal rates attained at the earlier stages (Patel et al., 1975). When weaned onto the normal, relatively high-carbohydrate diet, high circulating levels of ketones disappear and cerebral ketone utilization decreases considerably. The reports of ketoacidosis and developmental delay in infants with impaired ability to utilize ketone bodies underscore the importance of these substrates during the suckling period (Tildon & Cornblath, 1972). After birth, the activities of enzymes for glycolytic and oxidative use of glucose progressively increase with maturation to reach adult levels shortly after weaning (McKenna et al., 2006c; Cremer, 1982; Patel et al., 1975)[and references therein].

Cerebral metabolic rate increases during early development

Glucose is an essential substrate for developing brain (Vannucci & Simpson, 2003; McKenna et al., 2006c; Cremer, 1982)[and references therein]. When ketone bodies and lactate are utilized primarily for energy, the requirement for glucose may reflect metabolism via the pentose phosphate shunt for formation of ribose-5-phosphate as well as production of the NADPH required for lipid biosynthesis. In general, whole-brain local glucose utilization is very significantly correlated with postconceptional age (Kinnala et al., 1996; Takahashi et al., 1999). In the brain of suckling rat, even during intense use of ketone bodies, the postnatal increases in local glucose use appear to be critically linked to the acquisition of new functions and neurological competence (Cremer, 1982; Nehlig, 2004). This may also be true for neonatal human brain since local increases in the glucose metabolic rate reflect the functional maturation of specific brain regions (Takahashi et al., 1999). The overall rise in oxidative glucose use is associated with the developmental switch in transporters on the blood–brain barrier (Vannucci et al., 2003). These changes are also consistent with the progressive increases in the levels of a number of enzymes of oxidative metabolism in the brain, and with the increase in the activity of enzymes associated with the malate–aspartate shuttle (Ramos et al., 2003) (see below). The increased use of glucose for energy as the brain matures is accompanied by increased oxidation of glycolytically derived pyruvate via the TCA cycle. The rate of blood flow in different structures of the brain reaches peak levels at different developmental stages, depending on the maturation rate of the particular structure. In brain structures that consist predominantly of white matter, the peaks coincide roughly with maximal rates of myelination. From these peaks, blood flow and, probably, cerebral metabolic rate decline to the levels characteristic of adulthood (Clarke & Sokoloff, 1999; Kinnala et al., 1996; Takahashi et al., 1999).

Cerebral metabolic rate declines from developmental levels and plateaus after maturation

Reliable quantitative data on the changes in cerebral circulation and metabolism in humans from the middle of the first decade of life to old age have been reported (Clarke & Sokoloff, 1999; Kinnala et al., 1996; Takahashi et al., 1999). By 6 years of age, cerebral blood flow and oxygen consumption already have attained high rates, and they decline thereafter to the rates of normal young adulthood (Kennedy & Sokoloff, 1957). Cerebral oxygen consumption of 5.2 ml/100 g brain tissue/min in a 5–6-year-old child corresponds to total oxygen consumption by the brain of ≈60 ml/min, or more than 50% of the total body basal oxygen consumption, a proportion markedly greater than that occurring in adulthood. The reasons for the extraordinarily high cerebral metabolic rates in children are unknown, but presumably they reflect the extra energy requirements for the biosynthetic processes associated with growth and development. By early adulthood the rate of cerebral oxygen consumption has decreased to 3.5 ml/100 g brain tissue/min, a rate that is subject to minimal decline in normal elderly brain (Clarke & Sokoloff, 1999).

FUELING BRAIN: SUPPLY–DEMAND RELATIONSHIPS AND CEREBRAL METABOLIC RATE

Both excitatory and inhibitory neuronal signals utilize energy derived from metabolism

The greatest proportion of energy utilization by brain serves signaling-related processes (Table 11-1), which include

the balance between excitatory and inhibitory neuronal signaling and neuronal interactions with astrocytes, all of which are integrated with respect to time and location within the brain. Both excitatory and inhibitory signaling depend on transmembrane electrical potentials, which, in turn, are maintained or restored by energy-dependent ion transport. The continual signaling is reflected in the persistent electrochemical activity of the brain, as well as movement of signaling molecules (neurotransmitters and second messengers) between and within cells. Thus, brain energy metabolism and the flow of energy-yielding substrates via the cerebral blood flow are linked to or regulated by brain function and, in fact, are used as measures of regional brain function (see Figure 11-7 at end of chapter). Integrative regulation of blood flow with local neuronal and astrocytic activity is a very complex process involving many chemical signals (e.g., CO_2, NO, various neurotransmitters, oxygen level, possibly lactate release, and neuron–astrocyte interactions). Blood flow regulation is a current research topic, and emerging results demonstrate that astrocytes respond to neurotransmitter signaling and have an important, active role in governing vascular diameter (Iadecola & Nedergaard, 2007; Gordon et al., 2008).

Continuous cerebral circulation is required to sustain brain function

Since the amount of O_2 and glucose stored in brain is extremely small compared with rates of utilization, the brain requires a continuous fuel supply from the circulation, and disruption of glucose or O_2 delivery quickly leads to decrements in brain function and consciousness. Not only does the brain utilize O_2 at a very rapid rate, but the brain is also absolutely dependent on uninterrupted oxidative metabolism for maintenance of its functional and structural integrity. The glycolytic pathway produces only 2 ATP per molecule of glucose metabolized, compared to about 32 ATP generated via the oxidative pathways. This is lower than the theoretical maximum of 38 ATP due to proton leaks across the mitochondrial membrane. (See Table 11-1.) Even at its maximal rate, glycolysis can provide only a small portion of the required energy, and lack of O_2 causes functional failure.

Cerebral **m**etabolic **r**ate (**CMR**) for any compound is equal to the product of **c**erebral **b**lood **f**low (**CBF**) and **a**rterial–**v**enous (**A–V**) concentration difference of that compound across the brain at steady state according to the following equation: $CMR = CBF(A-V)$. The O_2 concentration in cerebral venous blood is substantially lower than in the cerebral arterial blood due to high extraction of oxygen, which is about 50% compared to about 10% for glucose (Linde et al., 1999; Paulson, 2002). Thus, glucose delivery to the neural tissue can be elevated by increasing the extraction ratio without change in the blood flow, but for oxygen there is less extraction reserve and an increase in blood flow is needed (Paulson, 2002). During brain activation, venous oxygen content increases due to disproportionately greater increases in cerebral blood flow compared to oxygen metabolism (Linde et al., 1999; Madsen et al., 1995; Fox & Raichle, 1986). Reduced venous *deoxy*hemoglobin content generates a **b**lood **o**xygen **l**evel–**d**ependent (**BOLD**) signal that is detectable with **m**agnetic **r**esonance **i**maging (**MRI**) (Ogawa et al., 1990), and functional MRI (**fMRI**) is widely used to map changes in neural activity in humans and experimental animals. Because O_2 reaches the brain cells purely by diffusion and because there are no intracellular O_2 stores in brain (in contrast to myoglobin, which stores O_2 in muscle), the net consumption of O_2 (i.e., cerebral metabolic rate of oxygen, CMR_{O2}) is reflected in the O_2 concentration gradient across the blood–brain barrier. The precise mechanism(s) by which increased electrical activity, and thus increased metabolism, triggers these increases in cerebral blood flow are not completely understood. The brain can, however, adapt to prolonged hypoxic conditions by the action of the signaling molecule **h**ypoxia-**i**nducible **f**actor (**HIF**). In brief, oxygen-dependent hydroxylation of HIF-1α leads to continuous degradation of the protein, but under hypoxic conditions, protein levels accumulate, leading to heterodimer formation with HIF-1β; this complex is a transcription factor that combines with the **h**ypoxia **r**esponse **e**lement (**HRE**) and upregulates production of erythropoietin, glycolytic enzymes, angiogenic factors, and glucose transporters (LaManna et al., 2007).

In contrast to O_2, which freely diffuses across cell membranes, glucose is transported across the blood–brain barrier by facilitated diffusion (see above). The product of net uptake of glucose by brain and blood flow rate (i.e., cerebral metabolic rate of glucose, CMR_{glc}), is largely independent of the plasma glucose concentration (Gruetter et al., 1993). The brain, like the liver but in contrast to muscle, has a significant tissue glucose concentration ($\approx$1–3 mmol/l) that is about 25% that of plasma (depending on the species and plasma concentration), and the brain has a high physical distribution space for glucose (Holden et al., 1991; Choi et al., 2001). Maximal glucose transport capacity across the blood–brain barrier is about three times maximal phosphorylation capacity (Holden et al., 1991), and glucose delivery is not likely to play a major role in regulating cerebral glucose utilization under physiologic conditions, since at normoglycemia the average intracellular concentration of glucose is well above the K_m of hexokinase for glucose, 0.05 mmol/l (see below).

Glucose is the main obligatory substrate for energy metabolism in adult brain

Brain glucose metabolism exhibits large regional variations at rest and is profoundly affected by local brain activation (Sokoloff et al., 1977; Nakao et al., 2001; Ueki et al., 1992), demonstrating a strong link between energy metabolism and brain function (Table 11-2). Brain glucose concentration is constant across brain regions despite large metabolic differences between white and gray matter and among different brain nuclei, indicating close linkage of glucose supply and demand. Cerebral blood flow differs with respect to the brain region, consistent with differences in regional resting metabolic activity (Clarke & Sokoloff, 1999). However, brain glucose concentrations can change acutely, consistent with the facilitative nature of glucose transport, and are higher when metabolism is reduced (e.g., under barbiturate anesthesia). Brain glucose can become rate limiting during metabolic extremes, e.g., during the earliest stages of profound activation or during hypoglycemia. Nevertheless, even when the intracellular concentration of

glucose approaches the Km of hexokinase (e.g., during hypoglycemia), significant facilitated transport of glucose into brain still occurs *in vivo* because CBF increases (Choi et al., 2001), possibly regulated by adenosine and K^+-ATP channels (Horinaka et al., 1997). Glycogen is the only endogenous fuel store that can provide significant amounts of energy (in the form of glucosyl equivalents) in periods during which the local supply of glucose does not meet metabolic demand (see below).

The brain can make limited use of other substrates for metabolism, e.g., ketone bodies during prolonged fasting when endothelial MCT transporters are upregulated (see above). Under normal conditions, the venous lactate concentration is generally slightly higher than the arterial concentration, indicating a small net lactate output from the brain. Net uptake of lactate into brain during strenuous exercise indicates that the adult human brain can use lactate as a supplementary fuel when its plasma levels are elevated markedly (Quistorff et al., 2008). Despite the ability of the brain to use other fuels, glucose is the main obligatory substrate for energy metabolism.

METABOLISM IN THE BRAIN IS HIGHLY COMPARTMENTALIZED

Over the past few decades it has become quite clear that metabolism of neurons and glial cells is interrelated and that these cells function in an integrated fashion. Several important enzymes, e.g., pyruvate carboxylase and glutamine synthetase, are selectively localized in astrocytes, and many transporters (e.g., for glucose, monocarboxylic acids, glutamate and glutamine) are differentially distributed on neurons and glial cells (McKenna et al., 2006c; Yu et al., 1983; Martinez-Hernandez et al., 1977) (Table 11-3) (see above). Neurons contain a specific isoform of enolase called **n**euronal **s**pecific **e**nolase (**NSE**), and immunocytochemical detection of the enolases makes them useful in determining neuron:glia ratios in tissue samples. The compartmentation of enzymes and transporters leads to metabolic specialization of brain cells, which in turn requires intercellular trafficking of metabolites (Hertz et al., 2007; McKenna, 2007). One result of the selective localization of brain enzymes is that astrocytes must provide certain substrates (e.g., glutamine) to neurons for replenishment of the neuronal TCA cycle and for neurotransmitter synthesis (Hertz et al., 2007; McKenna, 2007). Thus, astrocytes and neurons are essential partners in brain function, and the continuous interactions of neurons and glial cells are essential for brain function.

Glucose has numerous metabolic fates in brain

Brain glucose does not serve solely as an energy substrate (Figure 11-2). Glucose fulfills many critical roles for brain function, including that of a substrate for inositol biosynthesis and glycogen formation. Moreover, its carbon skeleton is incorporated into acetylcholine, lactate, glutamate, glutamine, aspartate, GABA, alanine, serine, glycine, lipids, one-carbon compounds used in methylation reactions and complex carbohydrates involved in protein glycosylation. In developing brain, metabolism via the pentose phosphate shunt (PPS) is particularly important to provide ribose-5-phosphate for nucleotide synthesis and the NADPH required for lipid biosynthesis and glutathione reduction.

TABLE 11-3 Selective Distribution of Brain Enzymes in Neurons and Astrocytes

Neurons	Astrocytes
CYTOSOLIC ENZYMES	
Choline acetyltransferase	Glutamine synthetase‡
Neuronal enolase‡	Malic enzyme*
Lactate dehydrogenase-1*	Lactate dehydrogenase-5*
Glutamic acid decarboxylase†	
Branched-chain aminotransferase (cBCAT)[a]	
MITOCHONDRIAL ENZYMES	
Phosphate-activated glutaminase†	Pyruvate carboxylase‡
Mitochondrial malic enzyme†	Neurosteroid synthesis†
Ubiquitous mitochondrial creatine kinase	Phosphate-activated glutaminase (low)
	Branched chain aminotransferase (mBCAT)[a]

See McKenna, 2007 for more details on distribution of enzymes and transporters (McKenna, 2007).
Extent of enrichment:
*moderately enriched;
†highly enriched;
‡very highly enriched
[a]Sweatt, A.J., Garcia-Espinosa, M.A., Wallin, R., Hutson, S.M. (2004) Branched chain amino acids and neurotransmitter metabolism: expression of cytosolic branched-chain aminotransferase (BCATc) in the cerebellum and hippocampus. *Journal of Comparative Neurology 477*, 360–70.

GLYCOLYSIS: CONVERSION OF GLUCOSE TO PYRUVATE

Regulation of brain hexokinase

Glycolytic flux is the rate at which glucose is converted to pyruvate in the cytosol (Clarke & Sokoloff, 1999; McKenna et al., 2006c)[and references therein]. The first step of glucose metabolism, phosphorylation of glucose by hexokinase, is essentially an irreversible reaction and is the first key point in regulation of glucose metabolic rate in brain; the second major regulatory enzyme is phosphofructokinase (Lowry & Passonneau, 1964) (Figure 11-3). Hexokinase converts glucose (Glc) plus Mg-ATP to Glc-6-P, which represents a branch point in glucose metabolism because it is a common substrate for enzymes involved in glycolysis, pentose phosphate shunt (PPS) and glycogen-forming pathways (Figure 11-2). Glc-6-P regulates hexokinase activity in two ways; its accumulation (1) inhibits hexokinase and (2) solubilizes hexokinase from its

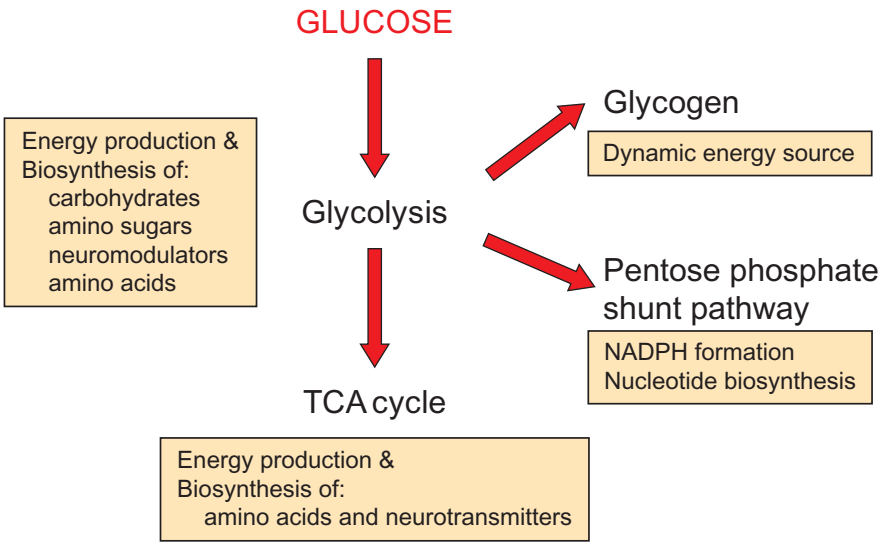


FIGURE 11-2 **Glucose has multiple metabolic fates in brain**. Glucose is the main substrate for energy production via glycolysis and TCA cycle metabolism. Furthermore, glucose metabolism is closely connected to carbohydrate, amino sugar, neuromodulators (D-serine and glycine), amino acids, and neurotransmitter biosynthesis via glycolytic and TCA cycle intermediates. Glucose is the main precursor for glycogen synthesis, which is localized mainly in astrocytes. Metabolism of glucose via the pentose phosphate shunt provides ribose-5-phosphate for synthesis of nucleotides and NADPH for lipid biosynthesis and maintenance of reduced glutathione.

binding to mitochondria, thereby decreasing activity (Clarke & Sokoloff, 1999; McKenna et al., 2006c). The brain isoenzyme of hexokinase can be soluble in the cytosol or attached firmly to mitochondria; the extent of binding is inversely related to the ATP:ADP ratio, i.e., conditions in which energy utilization exceeds supply shift hexokinase towards the bound form and produce a greater potential capacity for initiating glycolysis to meet the energy demand (Clarke & Sokoloff, 1999) [and references therein]. This mechanism allows ATP to function both as an enzyme substrate and as a regulator to decrease ATP production through its influence on enzyme binding. Mitochondrial hexokinase binding also facilitates preferential utilization of ATP generated by mitochondrial oxidative phosphorylation for glucose phosphorylation. The total sum of these mechanisms is a fine-tuning of the activity of the initial enzyme in glycolysis in response to changes in the cellular environment.

Phosphofructokinase is the major regulator of brain glycolysis

Fructose-6-phosphate and Mg-ATP are substrates of phosphofructokinase-1, the key and most complex regulatory enzyme controlling glycolysis (Lowry & Passonneau, 1966). Like other regulatory reactions, it is essentially irreversible. It is modulated by a large number of metabolites and cofactors whose concentrations under different metabolic conditions have a great effect on glycolytic flux. Prominent among these are the availability of high energy phosphate (~P) and citrate. Brain phosphofructokinase-1 is inhibited by ATP, Mg^{2+} and citrate and stimulated by NH_4^+, K^+, PO_4^{3-}, 5′-AMP, 3′,5′-cAMP, ADP and fructose-2,6-bisphosphate. In the steady state, the concentrations of ATP and citrate in brain are apparently sufficient to keep phosphofructokinase-1 relatively inhibited as long as the concentration of positive modulators, or disinhibitors, is low. When the steady state is disturbed, activation of this enzyme produces an increase in glycolytic flux, which takes place almost as fast as events changing the internal milieu. Note that the allosteric regulators are components of different pathways and can serve to integrate fluxes in the glycolytic and oxidative pathways.

Glycolysis produces ATP, pyruvate for mitochondrial metabolism, and precursors for amino acids and complex carbohydrates

Overall, the glycolytic pathway consumes two and produces four molecules of ATP, thus providing a net of two ATP molecules per glucose molecule. Two reactions are responsible for ATP production in glycolysis, namely phosphoglycerate kinase and pyruvate kinase. Pyruvate kinase requires K^+ and may be regulatory, since the enzyme can be phosphorylated and dephosphorylated, and it is inactive in its phosphorylated form. Formation of pyruvate, which can enter mitochondria and undergo oxidative metabolism via the TCA cycle, is a major role of glycolysis. Branch points from the glycolytic pathway (see Figure 11-2) divert some carbon for other uses, such as for the synthesis of amino acids, one-carbon units for methylation reactions, neuromodulators, and precursors for complex carbohydrates, glycoproteins, glycolipids, ethanolamine and choline. Fluxes into these pathways are highest during development, but synthesis of these compounds is also important in adult brain. Reduction of glucose to sorbitol by NADPH-dependent aldose reductase generates an osmolyte that contributes to damage of nerves, the eye and other tissues in diabetics. Due to the high K_m of the aldose reductase for glucose, the

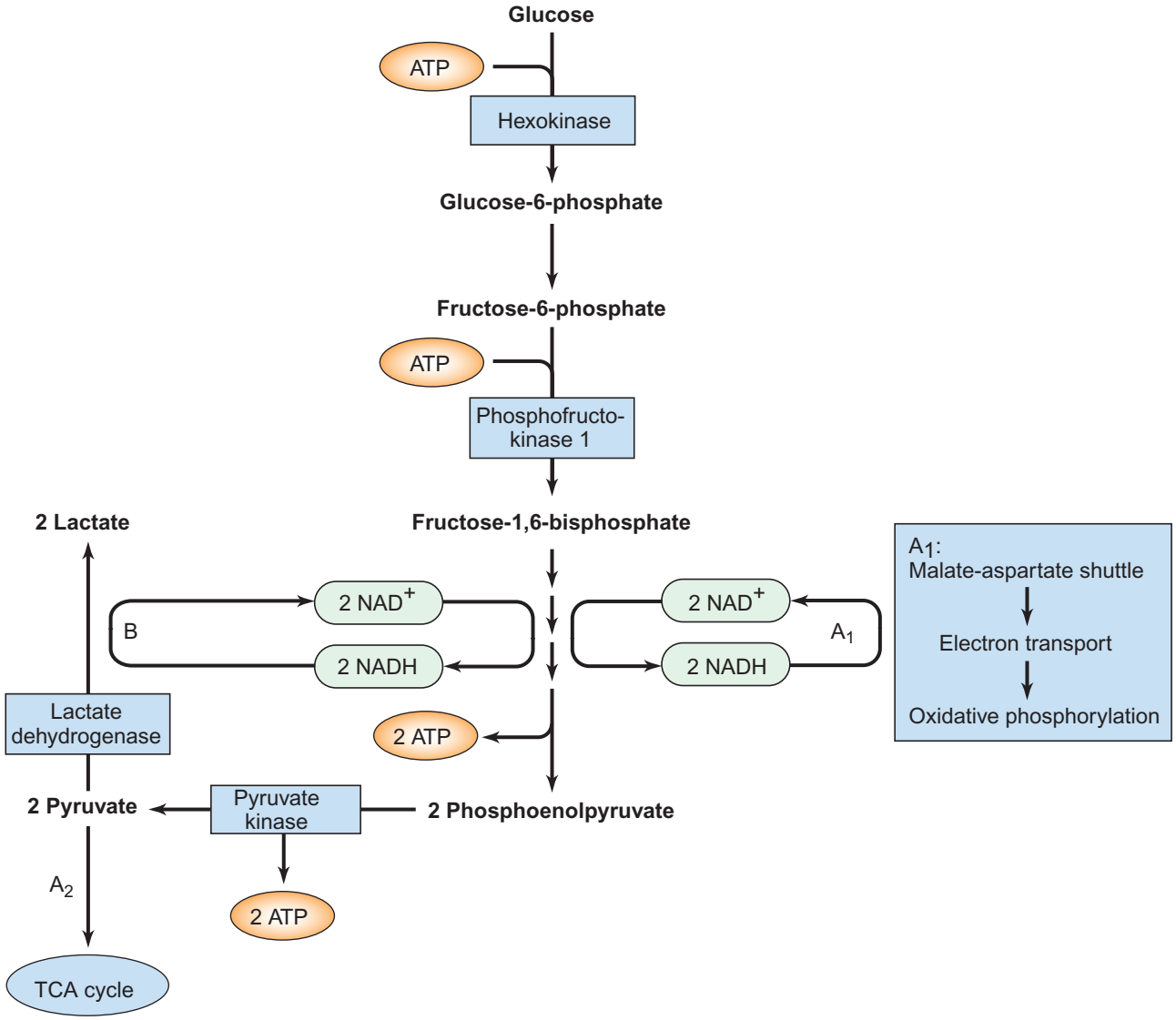


FIGURE 11-3 **Glycolysis produces pyruvate and requires regeneration of NAD^+.** A schematic representation of aerobic (A_1 and A_2) and anaerobic (B) glycolysis. Glucose is phosphorylated to glucose-6-phosphate and subsequently to fructose-1,6-bisphosphate via fructose-6-phosphate and phosphofructokinase 1, the main regulatory enzymes in brain glycolysis. NADH is produced in the conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate. Either the NADH produced is oxidized in the lactate dehydrogenase reaction by reduction of pyruvate to lactate (B), or the reducing equivalent from NADH is transferred to the mitochondria via the malate aspartate shuttle to be oxidized in the electron transport chain for oxidative phosphorylation (A_1). Pyruvate from aerobic glycolysis is subsequently metabolized via the tricarboxylic acid (*TCA*) cycle (A_2). Note that lactate can also be produced under aerobic conditions, e.g., when glycolytic flux exceeds that of the TCA cycle.

polyol pathway is only significant when blood and tissue glucose levels are greatly elevated.

GLYCOGEN IS ACTIVELY SYNTHESIZED AND DEGRADED IN ASTROCYTES

Brain glycogen concentration (6–12 µmol glucosyl units g wet weight) (Cruz & Dienel, 2002) normally exceeds that of free brain glucose (1.5-3 µmol/g wet weight), and glycogen is the only energy reserve in brain. Glycogen and its enzymes are compartmentalized in adult animals, where glycogen is located predominantly, but not exclusively, in astrocytes; in immature animals, glycogen is found in both astrocytes and some neurons (Clarke & Sokoloff, 1999; McKenna et al., 2006c). The role of glycogen as a significant, endogenous store of fuel had been discounted because of its low concentration compared to the total brain glucose utilization rate (Table 11-2), and if glycogen were the only fuel source, it would be consumed in minutes. However, emerging evidence derived from *in vivo* and *in vitro* studies indicates that a portion of the glycogen is rapidly degraded and re-synthesized during stimulation of normal physiological activity, indicating an important role in astrocytic energetics. In fact, recent studies have shown that inhibition

of glycogen phosphorylase increases the rate of utilization of glucose supplied by blood or culture medium, supporting the significance of glycogen metabolism to normal brain function (Walls et al., 2009; Sickmann et al., 2009; Dienel et al., 2007). Reports showing linkage of glycogen turnover with brain functional activity are consistent with its rapid mobilization by many neurotransmitters, increased extracellular K^+ concentration, and oxidative stress (Hertz et al., 2007). Glycogen level also fluctuates during the sleep–wake cycle and sleep deprivation. Under abnormal conditions, such as energy failure or inadequate fuel delivery to brain (due to ischemia, hypoxia, anoxia, aglycemia, hypoglycemia or extremely intense electrical stimulation), glycogen is rapidly mobilized; in contrast, depression of brain function and metabolism by anesthetics leads to increased levels of glycogen (Brown, 2004).

The steady-state concentration of glycogen is regulated by coordination of separate degradative and synthetic enzymatic processes

Glycogen is a large, complex glucose polymer with a heterogeneous, branched structure comprised of glucose molecules linked in 1,4- and 1,6-linkages. This structure gives the glycogen phosphorylase, the degrading enzyme, multiple sites for attack and rapid degradation. Glycogen mobilization has the advantage that each glucosyl unit is converted to glucose-1-phosphate (Glc-1-P), then to Glc-6-P, which can enter the glycolytic and pentose phosphate shunt pathways. Because no ATP is required for this process, 3 ATP are produced by glycolytic metabolism of 1 glucosyl unit from glycogen, a 50% gain over glycolytic metabolism of glucose, which requires 1 ATP to form glucose-6-phosphate, with a net glycolytic yield of 2 ATP. The 'price' for this energetic advantage is paid during the glycogen synthesis that occurs when energy and glucose levels exceed demand.

Two isoforms of glycogen phosphorylase exist in brain: muscle (M) and brain (B) isoforms. In particular, the M isoform is hormonally regulated via phosphorylation, but the unphosphorylated form is also activated allosterically by AMP (Clarke & Sokoloff, 1999; McKenna et al., 2006c)[and references therein]. The B isoform is poorly activated by phosphorylation and regulator AMP is required for full activation of both the phosphorylated and unphosphorylated forms of the enzyme. Thus, the activation of glycogen breakdown is particularly sensitive to the local energetic state of the intracellular astrocytic microenvironment. In cultured astrocytes, glycogen is degraded to lactate and released to the medium (Dringen et al., 1993). The final metabolic and cellular fates of glycogen carbon in brain *in vivo* remain to be established. Negligible Glc-6-phosphatase activity in brain prevents conversion of glycogen to free glucose *in vitro* and *in vivo* (Dienel et al., 1988; Gotoh et al., 2000).

The initial substrate for glycogen synthesis, Glc-6-P, usually varies inversely with the rate of brain glycolysis, and a decline in Glc-6-P level during high energy demand slows glycogen formation (Clarke & Sokoloff, 1999; McKenna et al., 2006c). The high-energy substrate for the rate-controlling enzyme, glycogen synthase, is UDP-glucose (Clarke & Sokoloff, 1999; McKenna et al., 2006c). Glycogen synthase occurs in both a phosphorylated form, synthase-b, which depends on Glc-6-P as a positive modulator, and a dephosphorylated, independent synthase-a form, which is much more active.

Glycogen synthase kinase (GSK3) is a serine/theronine kinase involved in the regulation of glycogen metabolism in astrocytes, but it also has important roles in the regulation of many signaling pathways in various cell types. GSK3 is a multifunctional enzyme; thus its presence in other brain cell types does not mean that these cells synthesize glycogen. GSK3 is necessary for neuronal development (Hur & Zhou, 2010), regulation of molecular motors involved in axonal transport (Morfini et al., 2004), and is thought to have roles in brain diseases, e.g., Alzheimer's disease and mood disorders (Phukan et al., 2010).

THE PENTOSE PHOSPHATE SHUNT HAS ESSENTIAL ROLES IN BRAIN

The **p**entose **p**hosphate **s**hunt (**PPS**), also termed the hexose monophosphate pathway, involves oxidative decarboxylation of carbon one of Glc-6-P in two consecutive reactions leading to production of CO_2 + ribulose-5-P and 2 NADPH + $2H^+$. Next, a series of non-oxidative reactions involving isomerization, epimerization, and transketolase and transaldolase reactions ultimately generate glyceraldehyde-3-P and fructose-6-P; the latter hexose can be converted to Glc-6-P and be reutilized in the PPS pathway. Alternatively, these two intermediates can re-enter the glycolytic pathway. The PPS has relatively high activity in developing brain, reaching a peak during myelination, when its main contribution is probably to produce the NADPH needed for reductive reactions in lipid synthesis. The shunt also provides pentose for nucleotide synthesis; however, only a small fraction of the activity of this pathway would be required. NADPH is also necessary for maintenance of reduced glutathione, which is required for the inactivation of reactive oxygen/nitrogen species. Under basal conditions ~5% of glucose in adult brain is metabolized via the PPS (Ben-Yoseph et al., 1995), which is more active in cultured neurons than cultured astrocytes (Herrero-Mendez et al., 2009). The activity of the PPS increases during oxidative stress, and it is regulated by the concentrations of Glc-6-P, $NADP^+$, glyceraldehyde-3-P and fructose-6-P. Transketolase, an enzyme in this shunt, requires thiamine pyrophosphate as a cofactor, and poor myelin maintenance in thiamine deficiency may reflect failure of this pathway to provide sufficient NADPH for lipid synthesis (Clarke & Sokoloff, 1999; Dringen et al., 2007; Matthews & van Holde, 1996).

THE MALATE–ASPARTATE SHUTTLE HAS A KEY ROLE IN BRAIN METABOLISM

The malate–aspartate shuttle is the most important pathway for transferring reducing equivalents from the cytosol to the mitochondria in brain

NADH cannot penetrate mitochondrial membranes, and regeneration of NAD^+ in the cytoplasm depends on the activities of the LDH reaction and a shuttle system that transfers

reducing equivalents to mitochondria. The malate–aspartate shuttle involves both the cytosolic and mitochondrial forms of aspartate aminotransferase and malate dehydrogenase, the mitochondrial aspartate–glutamate carrier, and the dicarboxylic acid carrier in brain (Figure 11-4) (McKenna et al., 2006b). The electrogenic exchange of glutamate and a proton for aspartate via the aspartate–glutamate carrier is irreversible (LaNoue & Tischler, 1974); the exchange favors entry of glutamate into and efflux of aspartate from mitochondria. Exchange via this carrier, which is the overall rate-limiting step of the shuttle, is stimulated by Ca^{2+} binding to a domain on the outer side of the inner mitochondrial membrane; the concentration of Ca^{2+} inducing half maximal activation is approximately 320 nM (Satrustegui et al., 2007). Two Ca^{2+}-sensitive aspartate–glutamate carriers have been identified: citrin (AGC2), which is found primarily in liver and kidney, and aralar (AGC1), which is found in skeletal muscle and brain. Aralar is highly enriched in neuronal mitochondria and is often found in areas with high levels of cytochrome oxidase (Ramos et al., 2003). A considerably lower level of aralar and malate–aspartate shuttle activity is present in astrocytes (Ramos et al., 2003). However, all necessary components of the malate–aspartate shuttle have been identified in a study of the transcriptome of acutely isolated astrocytes from adult rat brain, and these astrocytes can oxidize glucose, indicating that they do have a redox shuttle system (Lovatt et al., 2007).

The malate–aspartate shuttle has a role in linking metabolic pathways in brain

Maintaining a low NADH/NAD^+ ratio is essential for continuation of glycolysis (McKenna et al., 2006b). Without the shuttle system to regenerate cytoplasmic NAD^+, glucose-derived carbon is converted to lactate and released from the cell in order to maintain a high glycolytic rate. Pyruvate only becomes available for mitochondrial metabolism by means of the malate–aspartate shuttle, not LDH activity. Thus, the malate–aspartate shuttle links glycolysis and the TCA cycle in brain cells, as well as neurotransmitter labeling from glucose and the formation of neurotransmitter glutamate from glutamine (McKenna et al., 2006b). This shuttle is also essential for transferring the reducing equivalents from glycolysis to the mitochondrial electron transport chain and subsequent oxidative phosphorylation.

The activity of the malate–aspartate shuttle increases during development in parallel with synaptogenesis, which is consistent with the high activity and its importance in neurons and synaptic terminals. The high activity of this shuttle in neurons is in line with the involvement of the shuttle in the synthesis of neurotransmitter glutamate from glutamine. The activity of the malate–aspartate shuttle is impaired in pathological conditions including hypoxic/ischemic brain damage (McKenna et al., 2006b) and this damage limits the ability of neurons to oxidize lactate for energy (McKenna et al., 1998). The presence of aralar and activity of the malate–aspartate shuttle are essential for the formation of N–acetylaspartate (NAA) in neurons (Satrustegui et al., 2007; Jalil et al., 2005). Mutations in aralar lead to global hypomyelination in animal models and human brain since NAA synthesized in neurons is released, taken up by oligodendroglia, and used to provide acetyl groups for synthesis of myelin lipids (Satrustegui et al., 2007; Jalil et al., 2005; Wibom et al., 2009) (**see Clinical Correlation Box**). Another mechanism to shuttle redox equivalents into mitochondria is the glycerol phosphate shuttle, but it is thought to have a minor role in brain compared to other tissues (McKenna et al., 2006b; McKenna et al., 2006c) [and references therein]. Although the glycerol phosphate shunt can serve to maintain the NAD^+ required for glycolysis, the question of the cellular localization of this shuttle in brain is unresolved (McKenna et al., 2006b). Glycerol phosphate dehydrogenase (GPDH) is indirectly associated with glycolysis and reduces dihydroxyacetone phosphate to glycerol-3-phosphate, oxidizing NADH in the process.

THERE IS ACTIVE METABOLISM OF LACTATE IN BRAIN

Lactate–pyruvate interconversion

The bidirectional enzyme lactate dehydrogenase (LDH) converts pyruvate to lactate, thereby oxidizing the cytoplasmic NADH produced by the glyceraldehyde-3-P dehydrogenase reaction in the glycolytic pathway to regenerate the cytosolic NAD^+ required for glycolysis to continue. This process reduces pyruvate and prevents its oxidation via the TCA cycle. On the other hand, use of lactate as an oxidative fuel requires its conversion to pyruvate plus NADH + H^+ (McKenna et al., 2006c), and continuation of this process is dependent on the activity of the malate–aspartate shuttle (Fig. 11-4) (McKenna et al., 1993). LDH exists in different isoforms (named LDH1–5), consisting of tetramers formed from two distinct subunits. The A (m-type) and B (h-type) subunits of LDH are the products of separate genes and are differentially and developmentally regulated (McKenna et al., 2006c)[and references therein]. These isoforms have slightly different kinetic properties regarding rates of catalysis and affinities for pyruvate. The A subunit in LDH5 and LDH4 (corresponding to A4 and A3B) is the predominant isoform in astrocytes, and the B subunit in LDH1 and LDH2 (corresponding to B4 and B3A) is enriched in synaptic terminals. All isoforms are in both astrocytes and neurons (O'Brien et al., 2007), and the direction of the reaction is driven by concentration gradients, not isoform composition.

Lactate is formed in brain under many conditions

LDH functions under aerobic, hypoxic (reduced O_2 level), anaerobic (zero O_2) and ischemic (no blood flow) conditions, enabling glycolytic ATP production to continue and accelerate as O_2 becomes limiting (Siesjö, 1978). The terms aerobic and anaerobic glycolysis have historically been related to the extent of conversion of glucose to pyruvate or lactate under conditions of 'adequate' O_2 and no O_2, respectively. However, some lactate is generated during aerobic glycolysis when the glycolytic flux exceeds the oxidation of pyruvate or shuttling of NADH equivalents to mitochondria. In normal brain, the ratio of lactate/pyruvate concentration is ~10–15 (Siesjö, 1978) and the fluctuations in pyruvate level associated with variations in metabolic

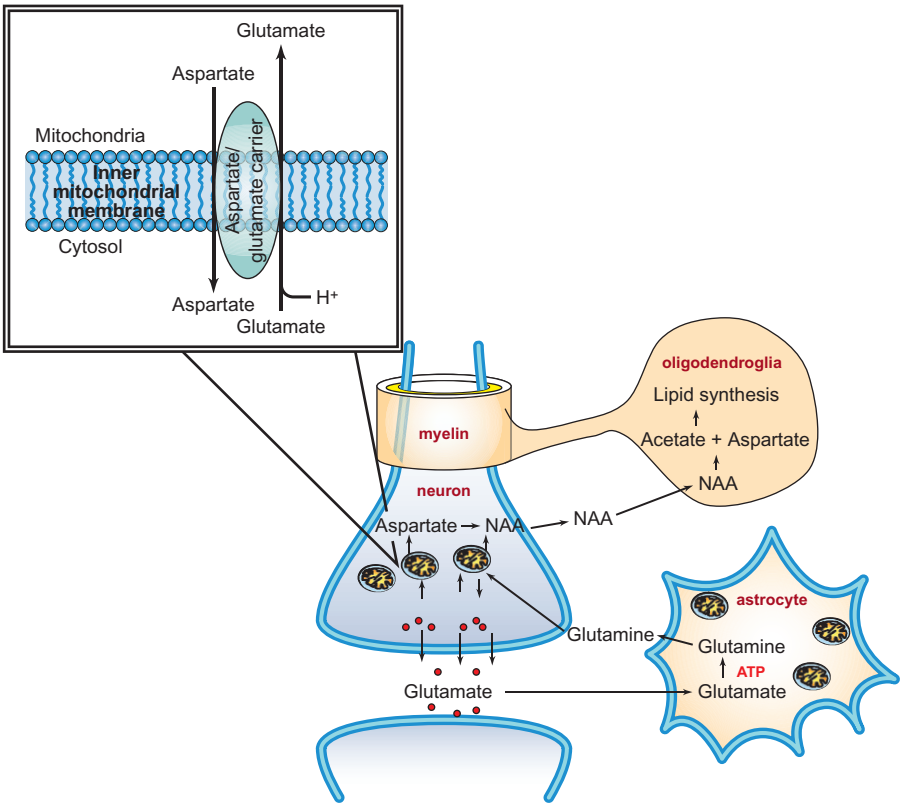
A MUTATION IN THE *SLC25A12* GENE REVEALS ITS CRITICAL ROLES IN N-ACETYLASPARTATE FORMATION, MYELINATION AND BRAIN FUNCTION

Mary C. McKenna, Gerald A. Dienel, Ursula Sonnewald, Helle S. Waagepetersen, Arne Schousboe

The *SLC25A12* gene encodes the mitochondrial aspartate–glutamate carrier (denoted as aralar or AGC1), an essential component of the malate–aspartate shuttle that facilitates the exchange between intramitochondrial aspartate and cytosolic glutamate plus a proton (see Figure 11-4 in chapter) (see chapter text and references therein). A mutation in AGC1 has recently been identified in humans, and data from the first patient and from studies in aralar knockout mice link this carrier to neuronal synthesis of N-acetylaspartate (NAA), synthesis of myelin lipids in oligodendroglia in developing brain, and overall brain function (see Box Figure 11.1).

Brain energetics requires a redox shuttle system to regenerate cytoplasmic NAD^+ for continuation of glycolysis and production of pyruvate as substrate for oxidative metabolism. In brief, the oxidation–reduction (shown in orange in Figure 11-4) and transamination (shown in turquoise in Figure 11-4) reactions of the malate–aspartate shuttle transfer reducing equivalents from cytoplasm into mitochondria for ATP production. Aspartate formed within neuronal mitochondria is also a precursor for synthesis of NAA, and oligodendroglia are known to hydrolyze NAA to provide acetate for synthesis of specific myelin lipids (Burri et al., 1991; Chakraborty et al., 2001). Indeed, a deficiency of NAA-derived acetate may contribute to the pathology of Canavan's disease (Madhavarao et al., 2005; Moffett et al., 2007; Arun et al., 2010), in which a lack of the aspartoacylase enzyme prevents the hydrolysis of NAA.

Cloning of aralar led to the development of knockout mice, identification of human mutations, and a better understanding of the essential roles of aspartate formation and release from neuronal mitochondria in brain function. Mice with a complete knockout of aralar ($Aralar^{-/-}$) are developmentally delayed and die by postnatal day 22. $Aralar^{-/-}$ mice have a profound decrease in the concentrations of aspartate and NAA without apparent neuronal loss, and the patient with a mutation in the *SLC25A12* gene has low brain NAA levels. $Aralar^{-/-}$ mice also have hypomyelination and low concentrations of specific myelin lipids, consistent with cerebral hypomyelination in the aralar-deficient child, who also had severe psychomotor developmental delay and seizures. Mitochondria isolated from muscle of the aralar-deficient child had a non-functional carrier and severely impaired respiration with glutamate. Since brain lactate level



BOX FIG. 11-1 The role of the neuronal aspartate–glutamate carrier (aralar, AGC1) in N-acetylaspartate (NAA) formation and trafficking and providing acetyl groups for synthesis of myelin lipids.

A MUTATION IN THE SLC25A12 GENE REVEALS ITS CRITICAL ROLES IN N-ACETYLASPARTATE FORMATION, MYELINATION AND BRAIN FUNCTION (*cont'd*)

was not elevated in this child, Wibom et al. (2009) questioned the importance of the malate–aspartate shuttle in brain and raised the possibility of a compensatory shuttle system.

Use of neuronally generated NAA by oligodendrocytes and reduced NAA levels without apparent neuronal loss validate the concept proposed by Clark (1998) that NAA is a marker of neuronal mitochondrial function rather than of neurons *per se*. This distinction has important implications for interpretation of ^{1}H-NMR spectra used for clinical diagnosis because a decrease in NAA or ratio of NAA/total creatine is often interpreted as a loss of neurons (Moffett et al., 2007; Clark, 1998; Bates et al., 1996). However, decrements in NAA level can also represent *reversible* neuronal or mitochondrial dysfunction (Moffett et al., 2007).

Mitochondrial respiration is essential for neurons, and the question of whether there is a compensatory redox shuttle system is important. Two alternatives are the glycerol phosphate shuttle and the aspartate–glutamate carrier, citrin (AGC2). Upregulation of citrin, which can substitute for aralar in AGC1 deficiency, could preserve malate–aspartate shuttle function; residual expression of citrin could explain the regional hypomyelination in the patient. Low levels of citrin are found in specific neuronal clusters in mouse brain (Contreras et al., 2010); this finding should stimulate studies to evaluate citrin levels in *Aralar*$^{-/-}$ mice and in human brain.

In summary, studies of aspartate–glutamate carrier deficiency highlight the critical multifunctional roles of a single neuronal mitochondrial protein and a single amino acid (aspartate). This carrier is essential for brain energetics, intra- and intercellular metabolite trafficking for biosynthesis of key lipid components of the myelin sheath, neuron-oligodendroglia interactions and overall brain development and function. Reduced aspartate formation and/or efflux from neuronal mitochondria leads to reduced NAA levels, hypomyelination, and severe neurological defects. These findings raise a number of important issues, including the lack of lactate accumulation and possibility of alternative shuttle systems, the origin of the 4-carbon backbone for neuronal aspartate that is used to synthesize and export NAA, and the fate of the aspartate generated by hydrolysis of NAA in oligodendroglia. Thus, detailed analysis of the phenotypes associated with mutations in critical brain proteins can lead to a better understanding of the interactions among seemingly unrelated pathways and among neurons, oligodendroglia and astrocytes.

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fluxes are reflected by corresponding changes in lactate content (reviewed in Dienel & Cruz, 2008). Under resting conditions, brain tissue lactate concentration is about 0.5–1 µmol/g (Hertz et al., 2007; Dienel et al., 2007; Mangia et al., 2007), although values as high as 2–2.5 mM have also been reported (McKenna et al., 1998; Itoh et al., 2003) [and references therein]. There is generally a small efflux of lactate from brain to blood, consistent with the CMR_{O2}/CMR_{glc} ratio of slightly less than the theoretical maximum of 6.0. Metabolic labeling studies show that brain lactate is rapidly labeled from glucose metabolism and it is continuously turning over during normal conditions in the presence of sufficient O_2 (Dienel & Cruz, 2009; Dienel & Cruz, 2003)[and references therein]. When glycolytic flux is upregulated by sensory stimulation of normoxic subjects, lactate concentration rises by about 0.5–1 µmol/g (Dienel & Cruz, 2008; Mangia et al., 2007). However, this accumulated lactate corresponds to only a very small fraction (~5%) of the glycolytic flux through the pyruvate pool (Dienel & Cruz, 2009; Dienel & Cruz, 2003). A glycogen phosphorylase inhibitor selectively enhances local rates of glucose utilization in brain during sensory stimulation of conscious rats, implying that glycogen turnover contributes significantly to energetics of brain activation and blockade of its utilization causes increased demand from glucose supplied by blood (Hertz et al., 2007; Dienel et al., 2007).

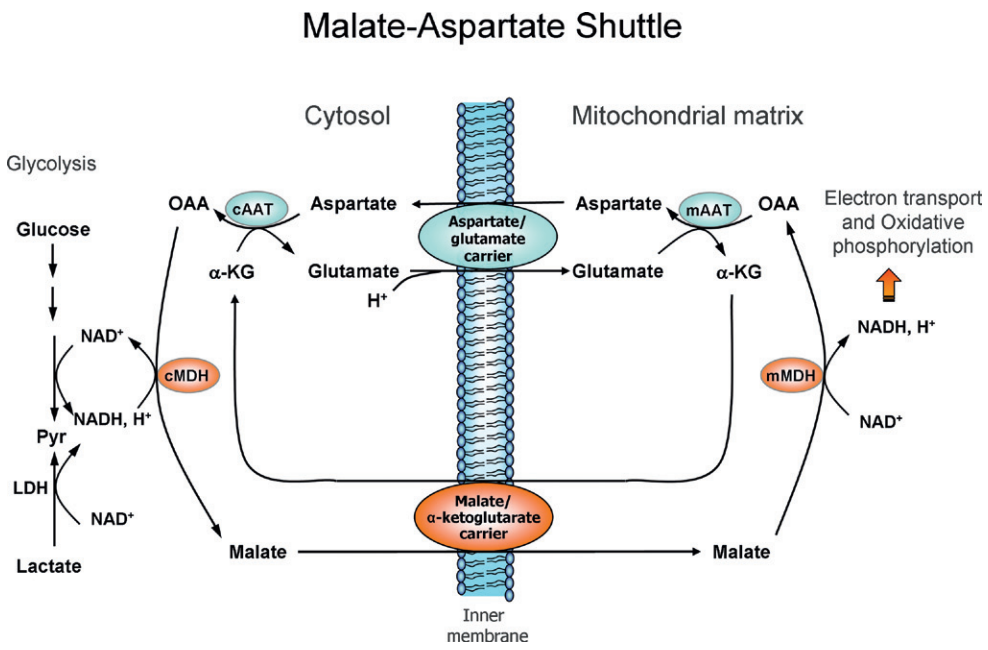

FIGURE 11-4 **The malate–aspartate shuttle transfers reducing equivalents from cytosol to mitochondria.** NADH is produced in glycolysis and in the conversion of lactate (*Lac*) to pyruvate (*Pyr*) via lactate dehydrogenase (*LDH*). The reducing equivalents from NADH are transferred via the malate aspartate shuttle to be oxidized via electron transport to support oxidative phosphorylation. Oxaloacetate (*OAA*) is converted to malate (*Mal*) by cytosolic malate dehydrogenase (*cMDH*) and NADH is oxidized to NAD^+. Malate is transported into the mitochondrial matrix by a carrier and converted into OAA, and NAD^+ is reduced to NADH via mitochondrial malate dehydrogenase (mMDH). Within mitochondria, oxaloacetate is converted to aspartate, and α-ketoglutarate (*α-KG*) is formed from glutamate (*Glu*) via mitochondrial aspartate aminotransferase (*mAAT*). Aspartate leaves the mitochondrial matrix in exchange for a molecule of glutamate + H^+. The irreversible aspartate–glutamate carrier favors glutamate uptake, as the transport is driven in the direction of the mitochondrial membrane potential. In the cytosol, aspartate is converted back to oxaloacetate via cytosolic aspartate aminotransferase (*cAAT*).

Lactate production also rises as O_2 becomes limiting, and both the malate–aspartate shuttle and TCA cycle are inhibited under anaerobic conditions (Siesjö, 1978). Under hypoxic conditions, oxidative metabolism is restricted, and increased amounts of pyruvate are converted to lactate and reoxidize NADH to maintain a high glycolytic rate. The lactate/pyruvate ratio rises sharply, tissue lactate level increases to the pathophysiological range (>4–5 µmol/g), and more lactate is released from brain to blood. Under anaerobic (zero oxygen) conditions, brain lactate levels rise much higher, and if blood flow continues, lactate washout will also take place. However, if cerebral anoxia is due to a heart attack or vascular occlusion (ischemia means no blood flow and no oxygen–glucose delivery), lactate is formed from glucose and glycogen present in the brain prior to cessation of flow. There will be no lactate efflux to blood and the final lactate level will correspond to approximately the sum of glucose plus glycogen in tissue.

Compartmentation of the pyruvate–lactate pool is unexpectedly complex

Carbon-13 labeling and **n**uclear **m**agnetic **r**esonance (**NMR**) spectroscopic studies of glucose metabolism in cultured brain cells and *in vivo* reveal an unexpected complexity of the pyruvate–lactate relationship. Alanine produced from pyruvate has a different labeling pattern depending upon whether the pyruvate is derived from glucose or from exogenously supplied lactate (Qu et al., 2000; Schousboe et al., 2003). In addition, pyruvate and lactate can be formed from the metabolism of the TCA cycle intermediate malate, and possibly oxaloacetate, via a partial pyruvate recycling pathway in brain (McKenna et al., 1996). NMR studies show evidence of lactate compartmentation, since lactate formed from glucose metabolism does not mix with lactate formed via the pyruvate recycling pathway from TCA cycle intermediates derived from metabolism of glutamate (McKenna et al., 1996). Indeed, there is considerable evidence that distinct pools of many metabolites exist within the cytosol of a cell (McKenna et al., 1996; McKenna et al., 1990) suggesting that cytosol should not be considered to be a well-mixed solution, but rather a highly organized gel containing many proteins and surfaces on which directed transfer of metabolites and cofactors can restrict free diffusion and label equilibration. The high protein content and affinity of enzymes for their substrates and cofactors can restrict the diffusion and mixing of small cytosolic molecules (Wheatley, 1998).

Lactate can serve as fuel for brain cells under various conditions

Glucose metabolism is highly regulated, whereas lactate utilization is 'opportunistic' because its oxidation is governed

by availability and delivery via equilibrative reactions. Lactate is readily taken up and oxidized by cultured astrocytes and neurons, and oligodendroglial cells may use some lactate for energy and lipid biosynthesis (Sanchez-Abarca et al., 2001). The carbon skeleton from lactate is incorporated into the neurotransmitters glutamate and GABA in neuronal cultures and in synaptosomes even in the presence of glucose (McKenna et al., 1994; Waagepetersen et al., 1998a; Waagepetersen et al., 1998b). In young animals, lactate and ketone bodies are fuel for brain until weaning. Adult brain will use lactate when blood lactate levels rise markedly, as during strenuous exercise (Quistorff et al., 2008). However, it is important to recognize that glucose is an obligatory fuel and lactate cannot substitute for requirements that are satisfied by the glycolytic and pentose phosphate shunt pathways. Lactate and pyruvate cannot reverse effects of hypoglycemia on cerebral function *in vivo* even though they are readily oxidized by brain slices and brain cells; their lack of complete restorative action compared to glucose is likely due primarily to limited delivery of the monocarboxylic acids across the blood–brain barrier (Clarke & Sokoloff, 1999; Sokoloff, 1960; Vannucci & Simpson, 2003).

The astrocyte–neuron lactate shuttle is controversial

In recent years the possibility that lactate, formed within the brain and released by astrocytes, is an important neuronal substrate both for energy and incorporation into neurotransmitters has been the subject of many studies and considerable controversy. Lactate can be formed by both neurons and astrocytes, and it has been proposed that lactate is formed within the brain by astrocytes during excitatory glutamatergic neurotransmission and is taken up and oxidized by nearby neurons as a major fuel [reviewed in (Hyder et al., 2006; Pellerin et al., 2007) and references cited therein]. The concept of astrocyte to neuron trafficking of lactate *in vivo* is technically difficult to prove, and is the subject of debate because direct *in vivo* evidence for the shuttle is lacking, and most support comes from *in vitro* studies (Hertz et al., 2007; Chih & Roberts, 2003; Mangia et al., 2009a)[and references cited therein]. Large amounts of lactate are produced by astrocytes in tissue culture, and the production is exaggerated due to dilution of lactate into the large culture medium volume compared to intracellular fluid, thereby 'pulling' lactate out of the cell by the equilibrative reactions of LDH and monocarboxylic acid transport (Dienel & Hertz, 2001). Thus, release of lactate by cultured astrocytes cannot be extrapolated to metabolism *in vivo*.

There is no doubt that lactate is produced during brain activation (Dienel & Cruz, 2007; Mangia et al., 2007; Prichard et al., 1991) [and references therein] but the cellular origin of the lactate is unknown. Lactate can be a substrate for brain cells, but there is also strong evidence for release of lactate from activated brain (Dienel & Cruz, 2008). Modeling studies that take into account differences in the much higher transport capacity of the GLUT3 glucose transporter in neurons and compared to GLUT1 in astrocytes predict that lactate is generated in neurons and shuttled to astrocytes (Simpson et al., 2007; Mangia et al., 2009b; DiNuzzo et al., 2010). The extent and specific circumstances in which neurons and astrocytes *in vivo* generate, utilize or release lactate is unknown, and this is an important, active area of research related to interpretation of functional metabolic studies using PET or MRS, particularly in disease states with reduced oxidative metabolism.

Although astrocytes release lactate, they can oxidize lactate for energy and can produce it from TCA cycle intermediates (Tildon et al., 1993; McKenna et al., 1993; Sonnewald et al., 1993a). Astrocytes control the microenvironment of brain and respond to neurotransmitters as well as to changes in the concentrations of substrates in the extracellular milieu (Hertz et al., 2007; Malik et al., 1993). Certain substrates, such as lactate and glutamine (Yudkoff et al., 1988; Auestad et al., 1991), can be produced as well as consumed by astrocytes, depending on the composition of substrates in the extracellular milieu. It is important to recognize the complexity of changes in lactate level, which can be the net result of many processes, including plasma lactate level, altered transport into and release from brain cells, changes in the glycolytic rate, alterations in the rates of the malate–aspartate shuttle activity, activity of the pyruvate dehydrogenase complex, TCA cycle activity, the cytoplasmic pH and redox state, the rate of glycogenolysis in astrocytes and the rate of lactate efflux from brain (Dienel & Cruz, 2003). Astrocytes have a much higher rate and capacity for lactate uptake from extracellular fluid compared to neurons (Tildon et al., 1993; McKenna et al., 1998; McKenna et al., 1993; Sonnewald et al., 1993a), and in slices from adult rat brain astrocytes can disperse lactate from a point source through gap junctions more readily than lactate can be transferred to nearby neurons (Gandhi et al., 2009).

MAJOR FUNCTIONS OF THE TRICARBOXYLIC ACID (TCA) CYCLE: PYRUVATE OXIDATION TO CO_2, NADH/$FADH_2$ FORMATION FOR ATP GENERATION AND SYNTHESIS OF GLUTAMATE AND ASPARTATE

The TCA (citric acid) cycle is multifunctional

Most of the ATP derived from metabolism of glucose is generated from oxidative metabolism in the TCA cycle by transfer of reducing equivalents to NADH and $FADH_2$, which interact with the electron transport chain to produce ATP and regenerate the oxidized redox compounds (Figure 11-5). Oxidative metabolism begins when pyruvate from glycolysis enters the mitochondrion and is converted to acetyl CoA. The first step of the TCA cycle is the formation of citrate by the condensation of acetyl CoA with a molecule of the 4-carbon catalytic intermediate, oxaloacetate. In each turn of the cycle, two carbons are lost as CO_2 and oxaloacetate is regenerated, so there is no net change in carbon content. Various compounds can enter the TCA cycle at several points, leading to formation of TCA cycle intermediates, and TCA cycle intermediates are used for synthesis of the amino acids glutamate, GABA, aspartate, and glutamine. Thus, the TCA cycle is essential for energy production, amino acid biosynthesis and neurotransmission (McKenna 2007).

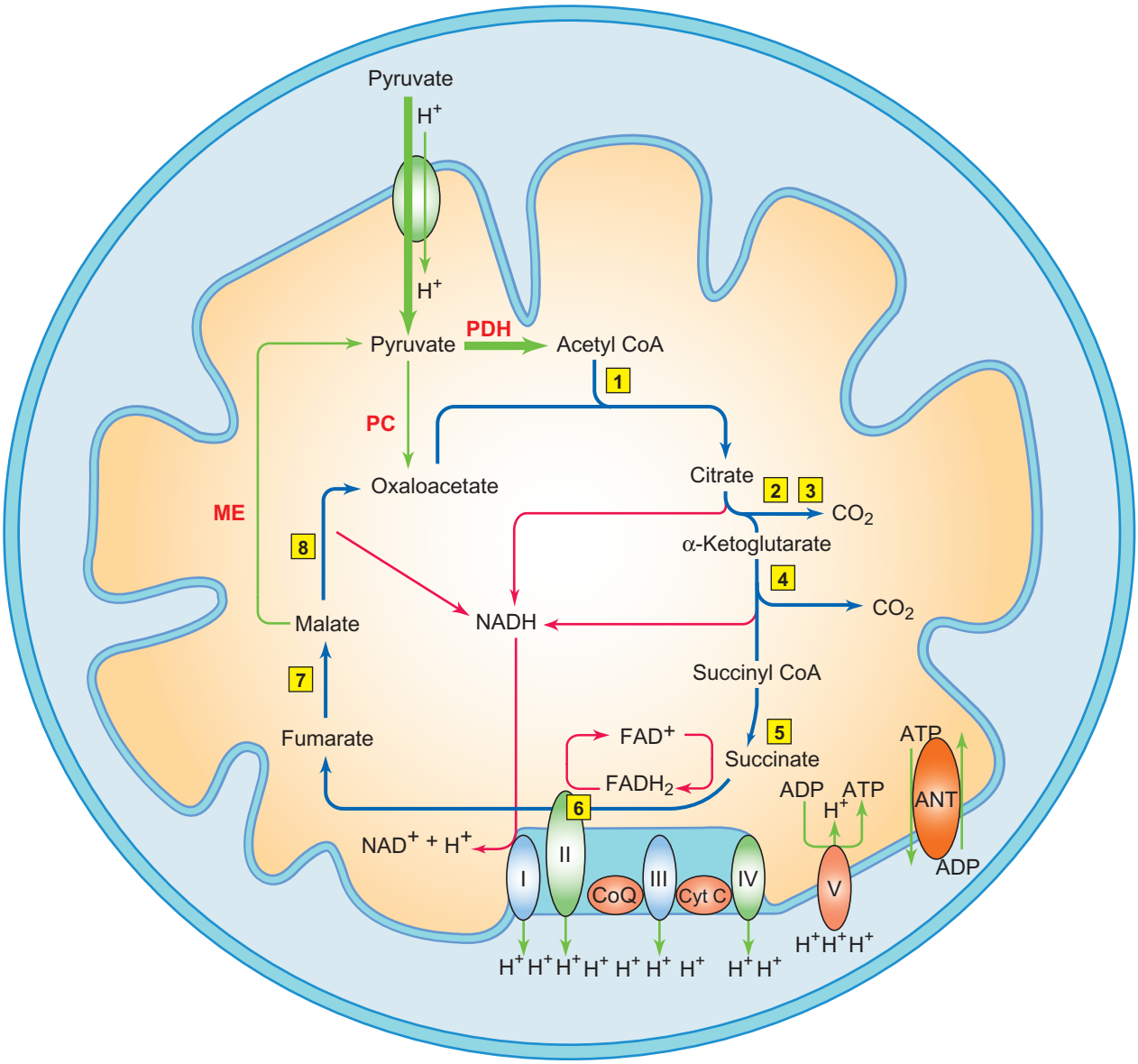

FIGURE 11-5 **Tricarboxylic acid cycle and oxidative phosphorylation**. Pyruvate is carried into the mitochondrial matrix for oxidative decarboxylation to acetyl-CoA via the pyruvate dehydrogenase complex (*PDH*) or for carboxylation to oxaloacetate via pyruvate carboxylase (*PC*). Acetyl-CoA is condensed with oxaloacetate via citrate synthase *(1)* to form citrate, which is converted to α-ketoglutarate via aconitase *(2)* and isocitrate dehydrogenase *(3)*. α-Ketoglutarate is subsequently decarboxylated via the α-ketoglutarate dehydrogenase complex *(4)* to succinyl-CoA. Succinate is formed from succinyl-CoA via succinyl CoA synthase *(5)*. Complex II of the respiratory chain/succinate dehydrogenase *(6)* oxidizes succinate to fumarate, which is converted into malate via fumarase *(7)*. Malate is oxidized to oxaloacetate via malate dehydrogenase *(8)* or it can be converted to pyruvate via malic enzyme (ME). NADH is re-oxidized in complex I, and FADH in complex II of the electron transport chain. The electrons are carried by the complexes, coenzyme Q (*CoQ*), and cytochrome C (*Cyt c*) to O_2, which is reduced to H_2O in complex IV. The electron transport generates a proton gradient over the inner membrane driving the ATP synthesis in complex V. Proton leakage across the membrane back into the matrix reduces the net yield of ATP (Table 11-1).

Turnover of the TCA cycle-derived amino acids involves their biosynthesis and oxidative degradation. De novo biosynthesis requires a 'new' 4-carbon 'backbone' that is produced by the pyruvate carboxylase (PC) reaction in astrocytes (Sonnewald & Rae, 2010), whereas catabolic removal of the 4-carbon backbone from the amino acid pools occurs via a series of reactions or by glutamine release from brain. PC adds CO_2 to pyruvate, forming oxaloacetate, thereby adding net carbon to the glial TCA cycle and allowing the formation of a new molecule of glutamate and, subsequently, glutamine without depleting the TCA cycle intermediate pool. Thus, the 'de novo' synthesis of the most abundant neurotransmitters, glutamate and GABA, as well as replenishing of the neuronal TCA cycle intermediates, require both pyruvate carboxylation in astrocytes and synthesis of glutamine in astrocytes and the trafficking of glutamine from astrocytes to neurons in brain. Note

that there is also shuttling of compounds between astrocytes and neurons (without net de novo synthesis and degradation), and the trafficking of neurotransmitter carbon between astrocytes and neurons is referred to as the glutamate–glutamine cycle and the GABA–glutamate–glutamine cycle (see Figure 11-6 and related text, below).

The pyruvate dehydrogenase complex plays a key role in regulating oxidation of glucose

In order for glucose to be oxidized to CO_2, pyruvate formed by glycolysis needs to enter the TCA cycle. The first step in this process is accomplished via the pyruvate dehydrogenase (PDH) complex localized in the mitochondria, which controls the rate of pyruvate entry into the TCA cycle as acetyl coenzyme A (acetyl-CoA). Pyruvate dehydrogenase is a multienzyme complex consisting of pyruvate decarboxylase and two other enzymes, lipoate acetyltransferase and lipoamide dehydrogenase, as well as the coenzymes thiamine pyrophosphate, lipoic acid, CoA, FAD and NAD^+. PDH is inactivated by being phosphorylated at the decarboxylase moiety by a tightly bound Mg^{2+}-ATP-dependent protein kinase called PDH kinase, which is inhibited by ADP, NAD^+, CoA-SH and pyruvate, and stimulated by ATP, NADH and acetyl-CoA. PDH is activated by dephosphorylation by a loosely bound Mg^{2+}- and Ca^{2+}-dependent phosphatase, which is stimulated by insulin, phosphoenolpyruvate (PEP) and AMP, but competitively inhibited by ATP, NADH and acetyl-CoA. About half the brain enzyme is usually active. ADP is a competitive inhibitor of Mg^{2+} for the inactivating kinase. Under conditions of greater metabolic demand, increases in pyruvate and ADP and decreases in acetyl-CoA and ATP make the complex more active. Pyruvate dehydrogenase is inhibited by NADH (which stimulates inactivation by phosphorylation), thereby decreasing formation of acetyl-CoA during hypoxia and allowing more pyruvate to be reduced by lactate dehydrogenase, thus forming the NAD^+ necessary to sustain glycolysis. PDH defects do occur in several mitochondrial enzyme-deficiency states (DiMauro et al., 1985), and oxidative and nitrosative damage and deactivation of PDH can occur during pathological conditions (Vereczki et al., 2006).

TCA cycle rate

The flux through the TCA cycle depends on glycolysis and acetyl coenzyme A production. However, the activity of the TCA cycle is subject to control at several enzymatic steps of the cycle (Clarke & Sokoloff, 1999; Matthews & van Holde, 1996), the dehydrogenases, and by the local ADP concentration, which is a prime activator of the mitochondrial respiration to which the TCA cycle is linked (Figure 11-5). As in other tissues, there are two isoforms of isocitrate dehydrogenase in brain. One is active primarily in the cytoplasm and requires nicotinamide adenine dinucleotide phosphate ($NADP^+$) as cofactor; the second is the mitochondrial isocitrate dehydrogenase, which requires NAD^+ and participates in the TCA cycle. The NAD^+-linked enzyme catalyzes an essentially irreversible reaction and has allosteric properties, i.e., it is inhibited by ATP and NADH and may be stimulated by ADP. The function of cytoplasmic $NADP^+$ isocitrate dehydrogenase is uncertain but it has been postulated that it supplies the NADPH necessary for many reductive synthetic reactions. The relatively high activity of this enzyme in immature brain and white matter is consistent with such a role. α-Ketoglutarate dehydrogenase, which oxidatively decarboxylates α-ketoglutarate, requires the same cofactors as pyruvate dehydrogenase.

Succinate dehydrogenase is the enzyme that catalyzes the oxidation of succinate to fumarate. Succinate dehydrogenase is bound tightly to the inner mitochondrial membrane and is an integral part of the respiratory chain, as it constitutes complex II (Figure 11-5). Isocitrate and succinate concentrations in brain are affected little by changes in the flux of the TCA cycle as long as an adequate glucose supply is available. The highly unfavorable free energy change of the malate dehydrogenase reaction is overcome by the rapid removal of oxaloacetate. The latter is maintained at a very low concentration under steady-state conditions by the condensation reaction with acetyl-CoA catalyzed by citrate synthase.

Malate dehydrogenase is one of several enzymes in the TCA cycle present in both the cytoplasm and mitochondria

Both the mitochondrial and cytoplasmic enzymes are components of the malate–aspartate shuttle. This represents a branch point in metabolism, since mitochondrial malate dehydrogenase (mMDH) can lead to metabolism via the TCA cycle or via the malate–aspartate shuttle. The direction of metabolism depends on the redox state and on the association of mMDH into transient multienzyme complexes called *metabolons* with either citrate synthase plus α-ketoglutarate dehydrogenase, or with aspartate aminotransferase plus glutamate dehydrogenase (Malik et al., 1993; McKenna et al., 2006a) [and references therein]. The association of mMDH into metabolons, thus directing the pathway of metabolism, is influenced by the concentration of key metabolites in the mitochondria (e.g., oxaloacetate, α-ketoglutarate and citrate) (Malik et al., 1993; McKenna et al., 2006a).

The electron transport chain produces ATP

Reducing equivalents are transferred from the carrier molecules (NADH and $FADH_2$) to the electron transport chain in complex I or complex II, and the flow of electrons is coupled to extrusion of H^+ across the inner mitochondrial membrane; this gradient is used to drive ATP synthesis (Figure 11-5). Note that the ATP yield for entry via complex II is less than that for complex I because fewer protons are extruded. ADP is an obligatory substrate for the electron transport chain, and, ultimately, consumption of ATP by biological work generates the ADP that is the substrate for this process, thereby directly linking energy generation to energy utilization.

ATP production in brain is highly regulated

Oxidative steps of carbohydrate metabolism normally contribute 36 of the 38 high-energy phosphate bonds (~P) (assuming

a theoretically maximal P:O ratio of 3:1) generated during aerobic metabolism of a single glucose molecule. However, it is not likely that there is a net production of 38 ATP equivalents per mole of glucose (Table 11-1), since a fraction of the glucose taken up is converted to lactate, and there is a proton leak as well as specific non-ATP synthase uses of protons (e.g., malate–aspartate shuttle) across the mitochondrial membrane. The steady-state concentration of ATP is high and represents the net sum of very rapid synthesis and utilization. On average, half of the terminal phosphate groups turn over in about 3 seconds; the turnover is probably much faster in certain brain regions (Clarke & Sokoloff, 1999). The level of ~P is kept constant by regulation of ADP phosphorylation in relation to ATP hydrolysis. The active adenylyl kinase reaction, which forms equivalent amounts of ATP and AMP from 2 ADP, prevents any great accumulation of ADP. Only a small amount of AMP is present under steady-state conditions; thus, a relatively small decrease in ATP may lead to a relatively large increase in AMP, which is a positive modulator of phosphofructokinase and AMP kinase, which in turn regulate many reactions that lead to increased ATP synthesis (Ronnett et al., 2009). Such an amplification factor provides a sensitive control for maintenance of ATP levels, which are surprisingly constant *in vivo* (Siesjö, 1978).

Phosphocreatine has a role in maintaining ATP levels in brain

The concentration of phosphocreatine (PCr) in brain is even higher than that of ATP, and creatine phosphokinase (CPK) is extremely active. The PCr level is exquisitely sensitive to changes in oxygenation, providing ~P for ADP phosphorylation and thus maintaining ATP levels. The CPK system also may function in regulating mitochondrial activity. In neurons with a very heterogeneous mitochondrial distribution, the creatine/phosphocreatine shuttle may play a critical role in energy transport (Meyer et al., 1984). The precise role of CPK in brain (whether as an energy buffer or as transport of ATP equivalents) is uncertain. However, it should be noted that knocking out the CPK enzymes in mouse brain had an effect on spatial learning but not on motor function (Jost et al., 2002).

Pyruvate carboxylation in astrocytes is the major anaplerotic pathway in brain

De novo synthesis of glutamate and glutamine in brain only occurs in astrocytes via metabolism in the pyruvate carboxylase (PC) pathway (Hertz et al., 2007). This enzyme is primarily localized in astrocytes (Yu et al., 1983) and is responsible for the high carboxylation rates in rat brain (Öz et al., 2004; Patel et al., 2005). PC fulfills an "anaplerotic" or "refilling" function by adding a CO_2 to pyruvate forming oxaloacetate which is then combined with acetyl-CoA to form a 'new' molecule of citrate (Figure 11-5). Although malic enzyme can fix CO_2, it does not perform this function to a significant extent in brain (McKenna et al., 1995; Patel, 1974). From the 'new' citrate molecule, the brain can synthesize a new molecule of glutamate or aspartate by transamination of α-ketoglutarate or oxaloacetate, respectively. Glutamine is synthesized in astrocytes by the ATP-requiring enzyme glutamine synthetase and serves as a precursor for the neuronal neurotransmitter pools of glutamate and GABA. It is important to note that net de novo synthesis of glutamate and glutamine in brain only occurs via metabolism in the pyruvate carboxylase pathway in astrocytes. Synthesis of the neuroactive amino acids (e.g., glutamate and aspartate) from glucose in brain is required because these compounds cannot cross the blood–brain barrier; if these transmitter–modulator amino acids could enter from blood, neurotransmission could be deleteriously affected by plasma levels, particularly after meals.

Citrate is a multifunctional compound predominantly synthesized and released by astrocytes

As a key TCA cycle intermediate, citrate must be formed in catalytic amounts for the small intramitochondrial pool in both neuronal and glial mitochondria by condensation of oxaloacetate with acetyl CoA. However, formation of a releasable pool of citrate requires the action of pyruvate carboxylation (Westergaard et al., 1994; Waagepetersen et al., 2001). Bicarbonate stimulates the release of citrate, which is threefold greater from cerebellar astrocytes than from cortical astrocytes (Westergaard et al., 1994). This difference reflects the functional specialization of astrocytes in different brain regions, possibly in response to the neuronal specialization. One role for extracellular citrate may be as a chelator, since it can attenuate the inhibitory action of Zn^{2+} on NMDA-receptor-mediated glutamate release. The ability of astrocytes to release large amounts of citrate may be related to the ability of these cells to significantly upregulate glycolysis under certain conditions (Waagepetersen et al., 2001), because release of citrate would decrease its intracellular concentration, thereby relieving the inhibitory action of citrate on PFK1 in the glycolytic pathway (Figure 11-3). The steady-state concentration of citrate in brain is relatively high compared to other TCA cycle and glycolytic intermediates, reaching 0.4 mmol/l in the cerebrospinal fluid (Bell et al., 1987). This is compatible with the ability of astrocytes to synthesize and release citrate *in vitro*; however, the extent to which citrate is released into the interstitial space *in vivo* is not known.

Acetyl-Coenzyme A formed from glucose is the precursor for acetylcholine in neurons

Acetylcholine synthesis is normally controlled by the rate of choline uptake and choline acetyltransferase activity. Choline is a dietary requirement for mammals and uptake is independent of the acetyl-CoA concentration. Since acetylcholine synthesis is taking place in the synaptoplasmic compartment, acetyl-CoA is either directly released from mitochondria through Ca^{2+}-dependent high permeability anion channels or formed by cleavage of citrate by ATP citrate lyase (Tomaszewicz et al., 2003). The cytosol of cholinergic nerve endings is rich in citrate lyase, and inhibition of this enzyme leads to decreased stimulated release of acetylcholine (Jankowska-Kulawy et al., 2010). During hypoxia or hypoglycemia, acetylcholine synthesis can be rapidly

inhibited by altered rates of acetyl-CoA supply (Gibson & Duffy, 1981).

MITOCHONDRIAL HETEROGENEITY: DIFFERENTIAL DISTRIBUTION OF MANY TCA CYCLE ENZYMES AND COMPONENTS OF OXIDATIVE PHOSPHORYLATION IN NEURONAL AND GLIAL MITOCHONDRIA

Mitochondria are distributed with varying densities throughout the central nervous system, with the more vascular parts containing most of the mitochondria

Mitochondria are present throughout the perikaryon, cytoplasm, dendrites, axons and nerve endings and also in endfeet, major processes, and fine processes of astrocytes (Lovatt et al., 2007; Pardo et al., 2011). The recent reports that astrocytic fine processes appear to have a higher density of mitochondria than surrounding neuropil (Lovatt et al., 2007) clearly allow these cells to have a pronounced oxidative metabolism. A detailed review on this topic (Hertz et al., 2007) concluded that astrocytes have an oxidative metabolism which per volume unit is similar to that in neurons. Other regions of particular abundance are nerve endings, the axon hillock and nodes of Ranvier. Mitochondrial heterogeneity has been demonstrated in the brain by separation of mitochondria of synaptic and nonsynaptic (neuronal cell bodies and glial) origin, which showed differences in distribution of enzymes catalyzing the TCA cycle reactions, as well as of mitochondrial enzymes not directly involved in the cycle (McKenna et al., 1995; Lai et al., 1977). Another example of mitochondrial heterogeneity is the dramatically different distribution of respiratory chain components in neurons and astrocytes (Bolaños et al., 1995). This may be due in part to the fact that most of the TCA cycle enzymes are encoded by the nuclear DNA in the host cell and imported into the mitochondrial matrix, in contrast to enzymes of the respiratory chain complexes, which are encoded by the mitochondrial DNA.

Mitochondrial heterogeneity leads to multiple simultaneous TCA cycles in astrocytes and neurons

While it is clear that mitochondrial heterogeneity exists in the brain as a whole, it is less clear if mitochondria in individual cells may exhibit such heterogeneity. However, circumstantial evidence from detailed metabolic studies of cultured brain cells points toward such a possibility (Waagepetersen et al., 2001). The presence of multiple simultaneous TCA cycles in astrocytes, neurons and freshly isolated synaptic terminals provides additional evidence for mitochondrial heterogeneity (McKenna et al., 1990; McKenna et al., 1994; Westergaard et al., 1994; McKenna et al., 2000). Discrete subfractions of synaptic mitochondria have considerable heterogeneity of mitochondrial enzymes that are thought to reflect subcellular compartmentation and functional metabolic specialization (McKenna et al., 2000). Moreover, studies of the distribution of α-ketoglutarate dehydrogenase in mitochondria of individual astrocytes provide evidence of distinct subpopulations of mitochondria within individual cells (Waagepetersen et al., 2006). The question of subcellular compartmentation is of fundamental importance to the full understanding of the extent and intricacies of metabolic compartmentation in brain, particularly with respect to the distinct microdomains within astrocytes and neurons.

Partial TCA cycles can provide energy in brain

Under certain metabolic conditions with low levels of acetyl-CoA, such as in hypoglycemia, only a part of the TCA cycle may operate, particularly in neurons and synaptic terminals (Yudkoff et al., 1994; Erecińska et al., 1988; Sonnewald & McKenna, 2002). This truncated cycle, which enables utilization of glutamine and glutamate as energy substrates, consists of the steps from α-ketoglutarate to oxaloacetate and leads to aspartate production and sometimes accumulation (Yudkoff et al., 1994). The majority of the reducing equivalents produced in the complete TCA cycle are derived from these reactions (Figure 11-5). The energy production from this truncated TCA cycle corresponds to 75% of that produced from the entire cycle. It should be noted that de novo synthesis of glutamate in astrocytes is also a partial TCA cycle (that requires a redox shuttle to provide the 2 pyruvate to generate oxaloacetate and acetyl CoA), that produces ATP from the pyruvate and isocitrate dehydrogenase reactions, and consumes oxygen.

Other substrates (e.g., glutamate, glutamine, lactate, fatty acids, and ketone bodies) can provide energy for brain cells

All of the machinery for using monocarboxylic acids (lactate and ketones) for energy is present in adult brain, and they can be consumed when present in blood at high levels (Williamson et al., 1971) and in the presence of glucose (Itoh et al., 2003; Pan et al., 2002). D-β-hydroxybutyrate dehydrogenase, acetoacetate-succinyl-CoA transferase and acetoacetyl-CoA-thiolase are present in adult brain tissue in sufficient amounts to convert the ketone bodies into acetyl-CoA and to feed them into the TCA cycle. Cerebral utilization of ketone bodies appears to follow passively their concentrations in arterial blood, and in normal adults, ketone concentrations are very low in blood and cerebral utilization of ketones is negligible. However, in ketotic states resulting from starvation, fat-feeding or ketogenic diets, or diabetes, cerebral utilization of ketones is increased in proportion to the degree of ketosis (Williamson et al., 1971). During prolonged fasting for several weeks there is considerable uptake of ketone bodies by the brain (Cahill, 2006). If one assumes that the substances were completely oxidized, their rates of utilization would have accounted for more than 50% of the total cerebral oxygen consumption, more than that accounted for by the glucose uptake. Nevertheless, D-β-hydroxybutyrate

is incapable of maintaining or restoring normal cerebral function in the absence of glucose in the blood (Clarke & Sokoloff, 1999), suggesting that, although it can partially replace glucose, it cannot fully satisfy cerebral energy or carbon needs. One explanation may be that the first product of D-β-hydroxybutyrate oxidation, acetoacetate, is metabolized further by its displacement of the succinyl moiety of succinyl-CoA to form acetoacetyl-CoA, since the two processes compete for a common substrate (CoA).

Acetate, octanoate and other short-chain fatty acids are used by brain astrocytes *in vitro* for energy (Edmond et al., 1987), and these compounds are also used for *in vivo* brain imaging, biochemical, and NMR studies of astrocytic oxidative metabolism. Amino acids can also be oxidized *in vivo* by the brain for energy (Zielke et al., 2009). Glucose-derived carbohydrates and amino acids together form a large pool of carbon in brain, i.e., ≈15 μmol carbohydrate/g plus another 25 μmol/g in the aggregate amino acid pool, that are in a dynamic state of use and replenishment (Dienel & Cruz, 2003), and their degradation provides energy (McKenna 2007; Zielke et al., 2009; Hertz & Hertz, 2003). It is important that such backup systems are present to protect the brain when glucose is low (Siesjö, 1978). Under specific circumstances such as hypoglycemia, the net consumption of glutamate by its oxidation is an important mechanism for providing energy via a partial TCA cycle from α-ketoglutarate to oxaloacetate (see above). Malic enzyme or the combined action of phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate kinase is required for the complete oxidation of glutamate (see discussion of pyruvate recycling below). Leucine enters the brain at a high rate and can be converted into 3-hydroxybutyrate and subsequently oxidized for energy by brain cells. The use of amino acids for energy requires the disposal of the NH_3.

GLUTAMATE–GLUTAMINE METABOLISM IS LINKED TO ENERGY METABOLISM

Glutamate is synthesized in brain by addition of an amino group to the TCA cycle intermediate α-ketoglutarate, or by removal of an amino group from glutamine by the enzyme phosphate-activated glutaminase (PAG). Glutamate has many fates in brain, including conversion to glutamine by glutamine synthetase (GS) in astrocytes, conversion to GABA by the neuronal enzyme glutamic acid decarboxylase (GAD), oxidation for energy via the TCA cycle, protein synthesis, and glutathione synthesis (McKenna 2007; Sonnewald et al., 1993a)[and references therein].

Transporters are required to carry glutamate and other amino acids across the mitochondrial membrane

Entry of glutamate into the mitochondria is essential for malate–aspartate shuttle activity and also for the oxidation of glutamate for energy. In neurons, glutamate enters the mitochondria via the aspartate–glutamate carrier aralar (Satrustegui et al., 2007), but in astrocytes, glutamate entry via the glutamate/hydroxyl carrier is thought to predominate (Berkich et al., 2007). The aspartate–glutamate carrier imports one molecule of glutamate plus a proton in exchange for the export of aspartate (Figure 11-4), whereas the glutamate carrier provides a net uptake of glutamate. Two isoforms of the glutamate carrier (GC1 and GC2), which are equally expressed in brain, have been identified (Fiermonte et al., 2002). Both mitochondrial glutamate carriers aralar and GC are essential for normal brain function. Mutations in the aspartate–glutamate carrier lead to impaired energy metabolism and hypomyelination (Satrustegui et al., 2007; Jalil et al., 2005), and mutations in the glutamate carrier are associated with neonatal myoclonic epilepsy (Molinari et al., 2009).

Metabolism of both glutamate and glutamine is linked to TCA cycle activity

The reactions catalyzed by glutamate dehydrogenase (GDH) and aspartate aminotransferase (AAT) or other aminotransferases convert the TCA cycle intermediate α-ketoglutarate to glutamate, and convert glutamate to α-ketoglutarate in the reverse reaction. Thus, the formation of glutamate is associated with TCA cycle activity. Brain AAT activity is high in comparison with flux through the TCA cycle. Therefore, the amino acids made by transamination of TCA cycle intermediates (i.e., aspartate and glutamate) in the mitochondrial matrix can quickly equilibrate with the TCA cycle intermediates as long as mitochondrial transport is not impaired. Because of this rapid exchange process label is transferred from TCA cycle intermediates to glutamate, and NMR studies have used labeling of glutamate from glucose metabolism as a surrogate marker of TCA cycle activity (Mason & Rothman, 2004; Henry et al., 2003; Gruetter et al., 2001).

In spite of the fact that the thermodynamic equilibrium of the GDH reaction favors glutamate production, the reductive amination of α-ketoglutarate is considered a minor pathway of glutamate production in brain (Yudkoff et al., 1993). This is most likely due to the high K_m of GDH for ammonia. However, the microenvironment in mitochondria of glutamatergic neurons with pronounced synthesis of glutamate from glutamine to produce ammonia may allow reductive amination to take place (Westergaard et al., 1996). On the other hand, oxidative deamination of glutamate in astrocytes is potentially important to sustain energy metabolism, as well as glutamate homeostasis (McKenna et al., 1996). Complete oxidative degradation of glutamate to CO_2 requires conversion of malate into pyruvate and reentry of acetyl CoA into the TCA cycle, i.e., pyruvate recycling (McKenna, 2007). Oxidation of glutamate via a partial TCA cycle can provide energy to neurons and astrocytes during normal metabolism (McKenna, 2007; McKenna et al., 1996), and particularly when glucose is low (Erecińska et al., 1988; Sonnewald & McKenna, 2002).

Glutamate participates in a number of metabolic pathways, and metabolism of glutamate and glutamine is compartmentalized

Glutamate participates in a number of metabolic reactions related to neurotransmission that are catalyzed by the enzymes glutamate dehydrogenase, a number of aminotransferases,

glutamine synthetase, phosphate-activated glutaminase and glutamate decarboxylase (Figure 11-6). Additionally, glutamate participates in metabolic pathways such as biosynthesis of ornithine, peptides (e.g., glutathione), and proteins. While all of these reactions occur in the brain as a whole, no single type of cell is able to perform all of these reactions (Yudkoff et al., 1993) due to selective cellular distribution of the enzymes (compartmentation) (Table 11-3). Hence, glutamate decarboxylase is mainly expressed in GABAergic neurons, whereas pyruvate carboxylase and glutamine synthetase are localized in astrocytes. Phosphate-activated glutaminase has a much higher activity in neurons than in astrocytes (Kvamme et al., 2001), but astrocytes are also capable of oxidizing glutamine for energy (Hertz et al., 2007; McKenna et al., 1993). A small glutamate pool that is the precursor for glutamine and a large glutamate pool with a slow turnover characterize the two glutamate compartments originally described in the brain (van den Berg & Garfinkel, 1971), which reflect the astrocytic and neuronal glutamate pools, respectively.

The glutamate–glutamine cycle

Excitatory glutamatergic neurotransmission is characterized by synaptically released glutamate that is predominantly taken up into astrocytes and converted into glutamine, which subsequently is transferred back to the glutamatergic neuron; this process involves preferential localization of high-affinity glutamate transporters and glutamine synthetase in astrocytes and the enrichment of phosphate-activated glutaminase in neurons (Kvamme et al., 2001; Danbolt, 2001) (Figure 11-6). It is important to emphasize that the glutamate–glutamine cycle does not operate in a 1:1 stoichiometric fashion, because some glutamate in astrocytes is oxidatively metabolized in addition to being converted into glutamine (McKenna, 2007; McKenna et al., 1996; Yudkoff et al., 1994; Yudkoff et al., 1993; Hertz et al., 1999). Furthermore, some glutamine can also be oxidized in neurons after its conversion to glutamate and subsequently enter the TCA cycle as α-ketoglutarate. For complete oxidative degradation to CO_2, the carbon skeleton of glutamate has to exit the TCA cycle in the step of malate or oxaloacetate, be converted to pyruvate, and subsequently reenter as acetyl-CoA from pyruvate. This is part of the 'pyruvate recycling' pathway that has been reported in rat brain (Cerdan et al., 1990). Low levels of pyruvate recycling have been reported in cultured astrocytes (Waagepetersen et al., 2002) and neurons (Olstad et al., 2007). High levels of pyruvate recycling from alternative substrates have been reported in developing brain (Scafidi et al., 2010), and it is conceivable that this process becomes more pronounced *in vivo* during hypoglycemia. To the extent that the carbon skeletons of glutamate and glutamine are quantitatively oxidized to CO_2, there is obviously a need for replenishment of the glutamate pool, which occurs via the anaplerotic enzyme pyruvate carboxylase in astrocytes (see above). Any GABA that is oxidized must also be replaced by de novo synthesis (see below). Glutamatergic and GABAergic neurons are

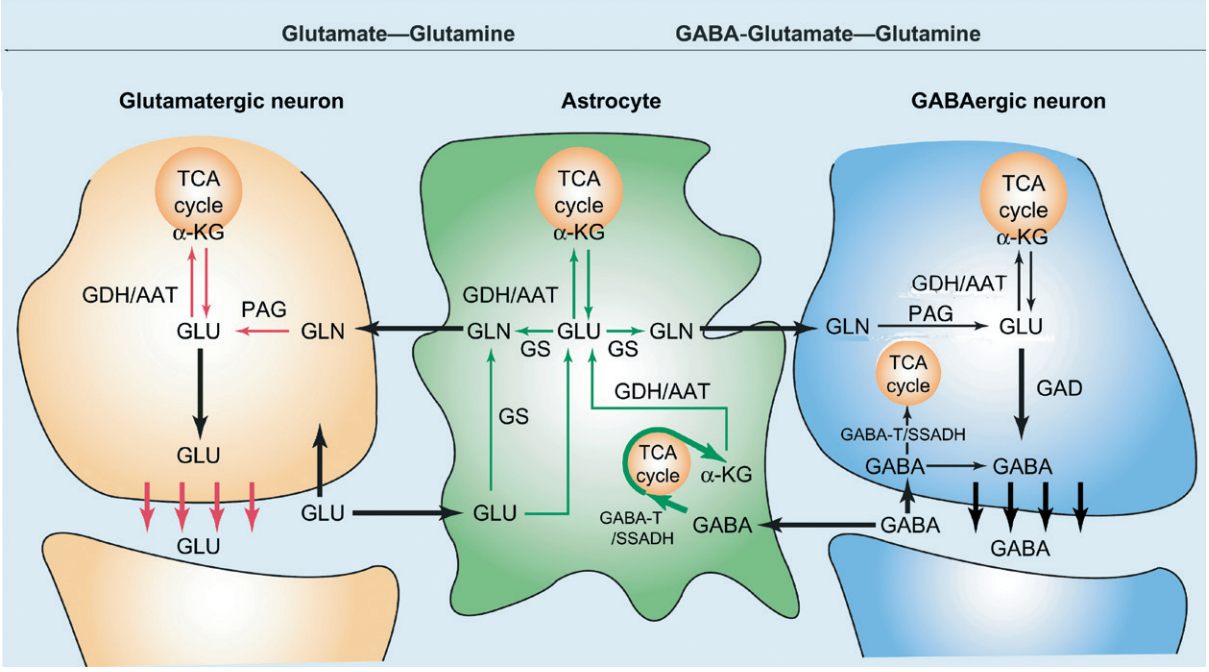

FIGURE 11-6 **Excitatory and inhibitory neurotransmission have essential interactions with astrocytic metabolism**. These processes are illustrated in the simplified schematic representation of key metabolic processes and release and uptake of neurotransmitters in glutamatergic and GABAergic synapses interacting with a surrounding astrocyte. The glutamate–glutamine cycle, including the glutamine synthetase (*GS*) reaction, is indicated in the glutamatergic neuron–astrocyte interaction. Analogously, the GABA–glutamate–glutamine cycle, including the GABA transaminase (*GABA-T*), succinate semialdehyde dehydrogenase (*SSADH*) and glutamate decarboxylase (*GAD*) reactions, is indicated in the GABAergic neuron–astrocyte interaction. The close association of the neurotransmitters, GABA and glutamate, to TCA cycle metabolism is indicated in all three cells. Glutamate is converted to α-ketoglutarate via either glutamate dehydrogenase (*GDH*) or aspartate aminotransferase (*AAT*). *GLN*, glutamine; *GLU*, glutamate; *α-KG*, α-ketoglutarate; *PAG*, phosphate-activated glutaminase; *TCA*, tricarboxylic acid.

incapable of replenishing their neurotransmitter pools unless precursors are received from surrounding astrocytes.

A specialized glutamate–glutamine cycle operates in GABAergic neurons and surrounding astrocytes

The neurotransmitter–glutamine cycle also operates between GABAergic neurons and astrocytes and is of quantitative importance for the maintenance of the neurotransmitter GABA pool (Sonnewald et al., 1993b). A prerequisite for the GABA–glutamate–glutamine cycle to operate is that GABA can be converted to glutamate in the astrocytic compartment. This is possible since the carbon skeleton of GABA has access to the TCA cycle by the concerted action of GABA transaminase and succinate semialdehyde dehydrogenase. After conversion to oxaloacetate and condensation with acetyl-CoA, α-ketoglutarate and thus glutamate can be formed (Figure 11-6). In GABAergic neurons the simultaneous presence of GABA transaminase, succinate semialdehyde dehydrogenase and glutamate decarboxylase provides a means by which the α-ketoglutarate dehydrogenase and succinyl CoA synthetase reactions can be circumvented. This is referred to as the *GABA shunt* of the TCA cycle, the activity of which constitutes approximately 10% of the normal flux in the TCA cycle (Figure 11-6).

Several shuttles act to transfer nitrogen in brain

While the glutamate–glutamine cycle accounts for the transfer of the carbon skeleton of glutamate (between neurons and astrocytes) it does not account for the fate of the ammonia released in the glutaminase reaction and the source(s) of the ammonia utilized for amidation in the glutamine synthetase reaction. Ammonia diffusing into brain from blood can be toxic at high levels, and it is quickly incorporated into glutamine as the amide nitrogen (Cooper & Plum, 1987), probably mainly in the astrocytic endfeet surrounding the vasculature. In the metabolic communication between muscle and liver, the nitrogen transfer is well known to occur as an alanine–glucose exchange. An analogous mechanism involving alanine and lactate has been proposed for brain, according to which alanine is responsible for the transfer of the ammonia nitrogen between the neuronal (glutamatergic) and the astrocytic compartments (Waagepetersen et al., 2000). There is evidence that leucine and α-ketoisocaproic acid may also have a role in the transfer of amino groups between neurons and glia (Yudkoff et al., 1993; Yudkoff, 1997).

METABOLIC STUDIES IN BRAIN: IMAGING AND SPECTROSCOPY

Global assays of whole brain

Studies of brain metabolic rates started with assays of arteriovenous differences for various compounds across the brain and blood flow to calculate cerebral metabolic rates. Global studies identified glucose and oxygen as the primary fuels for brain, and these classical methods are discussed in reference (Clarke & Sokoloff, 1999). Global assays are still very useful for determination of overall changes in metabolic activity of brain, but, due to averaging of the rates from all structures, large changes in small structures cannot be detected.

Local rates of glucose and oxygen utilization, functional brain imaging, redox state, and metabolic pathway analysis

The cerebral metabolic rate of glucose (CMR_{glc}) was first studied using radiotracers, but analysis of incorporation of label into metabolites in various metabolic pathways is difficult and time consuming, and quantitative studies are limited by loss from tissue of diffusible products of glucose in time-varying quantities. To surmount this problem, Sokoloff and colleagues pioneered elegant radiolabeled tracer methods using glucose analogs to measure local rates of glucose utilization; these compounds compete with glucose for transport and phosphorylation, but have highly restricted metabolism beyond the hexokinase step so that labeled products are trapped in tissue (Clarke & Sokoloff, 1999). Radiolabeled 2-deoxy-D-glucose ([^{3}H- or ^{14}C]DG) was developed for use in autoradiographic studies in experimental animals (Sokoloff et al., 1977), and 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) was developed for human use in positron emission tomographic studies (FDG-PET) (Reivich et al., 1979). FDG-PET is now routinely used for various purposes, including cancer detection and developmental studies (Takahashi et al., 1999). These methods rely on quantitative intracellular trapping of the major phosphorylated metabolite, DG-6-phosphate(P) or FDG-6-P, which enables specific assay of the hexokinase reaction simultaneously in all regions of the brain of conscious subjects (Figure 11-7). Under steady-state conditions, the rate of each step in a multistep pathway is the same, and local rates of overall glucose utilization (CMR_{glc}) can be calculated from rates of DG and FDG phosphorylation (Sokoloff et al., 1977; Reivich et al., 1979).

Use of glucose labeled with radioactive (^{3}H, ^{14}C, ^{11}C) or stable (^{13}C) isotopes allows for assessment of the fate of specific carbon atoms of glucose as the glucose is metabolized via different major pathways (Figure 11-7). Labeling patterns and ^{13}C NMR spectra, also referred to as **m**agnetic **r**esonance **s**pectroscopic (**MRS**) spectra, are discussed below and illustrated in Figures 11-8 and 11-9. In brief, data obtained from MRS studies can be used to determine the metabolism of precursors via specific neuronal and glial pathways in brain; when combined with metabolic modeling, these data are used to calculate the rate of the tricarboxylic acid (TCA) cycle and cycling of glutamate and glutamine during excitatory neurotransmission (Mason & Rothman, 2004; Henry et al., 2003; Gruetter et al., 2001). Fluorescence microscopy is used to assess changes in the oxidation–reduction (redox) status of tissue under different conditions, and this approach takes advantage of the fluorescence of three endogenous cofactors, NADH, NADPH, and FAD, the concentrations of which are governed by cytoplasmic and mitochondrial redox states. Note that change in concentration does not report the rate of

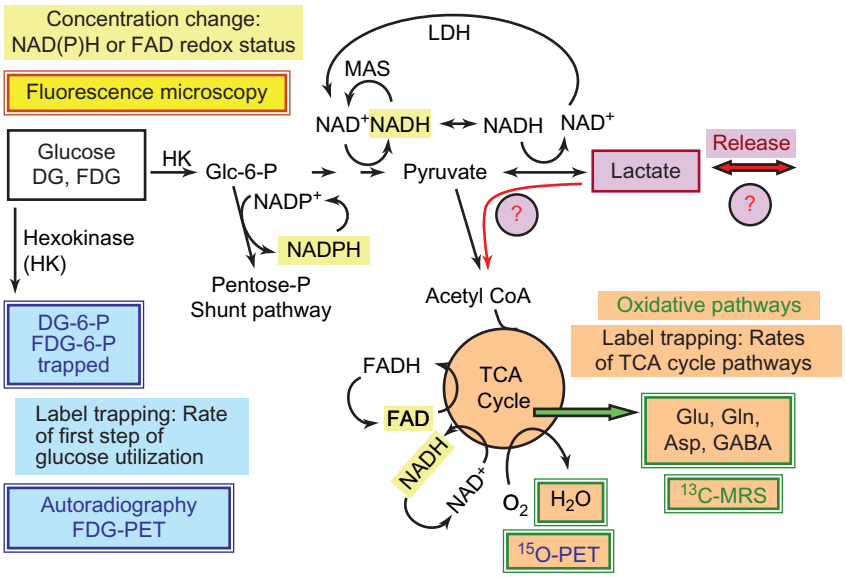

FIGURE 11-7 **Metabolic signals for brain imaging and spectroscopic studies: Interpretation requires understanding the basis of images.** Assays of the hexokinase reaction or of incorporation of labeled precursors into amino acids or water are used for pathway rate calculations. Note that these two approaches measure the initial and downstream oxidative steps of glucose utilization, respectively; together they can provide information related to release of lactate during brain activation. Fluorescence microscopy can measure changes in the concentration of NADH and NADPH (i.e., NAD(P)H) and of FAD, thereby evaluating redox status of cells and tissue. Abbreviations: DG, deoxyglucose; FDG, fluorodeoxyglucose; PET, positron emission tomography; TCA, tricarboxylic acid; LDH, lactate dehydrogenase; MAS, malate–aspartate shuttle; Glu, glutamate; Gln, glutamine; Asp, aspartate; GABA, γ-aminobutyric acid. Question marks associated with lactate denote uncertainties related to the quantity of lactate produced and released during cellular activity and contributions of lactate as a brain fuel.

flow of material through the pathway linked to the cofactor of interest. However, assays of product accumulation can be used to calculate rates. The combined use of all of the above technological approaches is particularly useful to evaluate changes in functional metabolism (Figure 11-7). DG and FDG report the first irreversible step of glucose utilization but do not provide information related to the downstream fate of the glucose carbon. Trapping of glucose-derived carbon by dilution in the large unlabeled amino acid pools derived from the TCA cycle enables assessment of the oxidative pathways but does not provide information about compounds present in low concentrations or the carbon (e.g., as CO_2 or lactate) that may be released from the cell in the upstream pathways. Thus, biochemical, imaging, and spectroscopic assays using multiple approaches as shown in Figure 11-7, various specifically labeled carbon atoms, and different precursors are required to understand the metabolic activities related to brain function.

Oxygen consumption can be measured directly by using **p**ositron **e**mission **t**omography (**PET**) (Figure 11-7) or polarography, or indirectly, by measuring the changing oxygenation of deoxyhemoglobin. The latter method has been exploited by the **b**lood-**o**xygen-**l**evel–**d**ependent (**BOLD**) **f**unctional **m**agnetic **r**esonance **i**maging (**fMRI**) or infrared spectroscopy methods. Inherent in all methods designed to measure cerebral oxygen metabolism directly is the rapid diffusion of oxygen across the blood–brain barrier. PET measurements of cerebral metabolic rate for oxygen (CMR_{O2}) are complicated to perform, but some intriguing results under certain conditions have been reported, such as the small or lack of increase in cerebral oxygen consumption during focal stimulation (Madsen et al., 1995; Fox et al., 1988). Assessment of cerebral oxygen metabolism by the BOLD mechanism, on the other hand (Ogawa et al., 1990), is heavily model dependent, as the apparent increase in venous deoxyhemoglobin during focal stimulation is a transient process critically dependent on the blood flow and volume changes. Nonetheless, imaging brain function using BOLD fMRI has led to renewed interest and insight into cognitive neuroscience, mainly due to its simplicity and ease of use. Other indirect methods to measure cerebral oxygen metabolism include measuring the turnover of carbon-13 label from glucose into glutamate C4, which through label exchange with the TCA cycle intermediate α-ketoglutarate can reflect the rate of the TCA cycle (Mason & Rothman, 2004).

A drawback to all methods discussed above, is that they cannot directly address the metabolic compartmentation inherent in the brain, i.e., the cellular and subcellular contributions to metabolism via specific pathways and local rates of metabolism of a substance. For example, studies in the 1960s using carbon-14 labeling reported that glutamate and glutamine have a different product–precursor relationship depending on whether labeled glucose or acetate was administered (Berl et al., 1970). These experiments deduced the existence of at least two TCA cycles associated with a large glutamate pool and a small glutamate pool, and acetate was subsequently demonstrated to selectively enter astrocytes, where it quickly labels glutamine via the small glutamate pool (Waniewski et al., 1998).

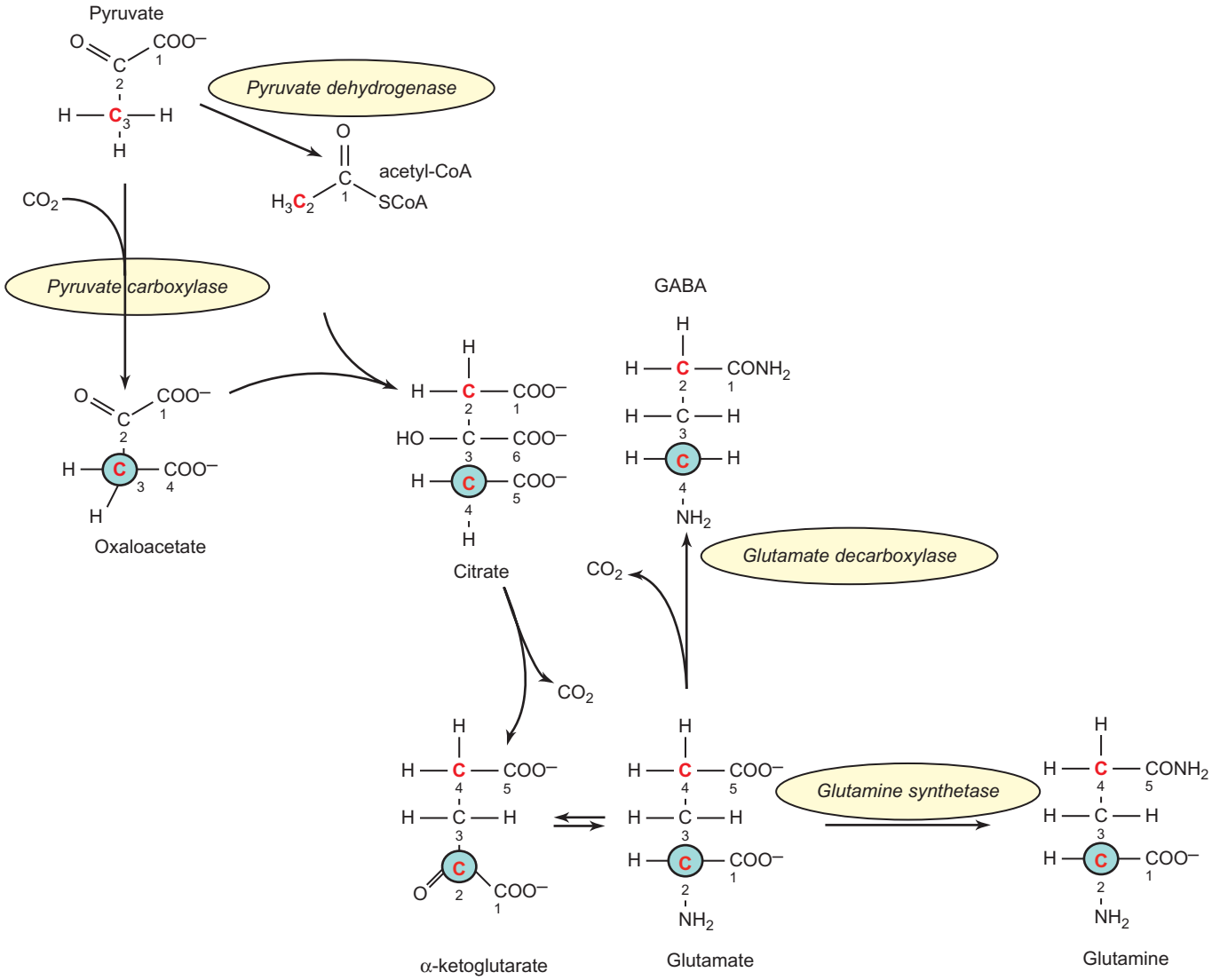

FIGURE 11-8 **Labeling pattern for metabolism of [1-^{13}C]-or [6-^{13}C]glucose by NMR spectroscopy.** Glucose labeled in the C1 or C6 position will give rise to pyruvate labeled in the C3 position (*red C*). Metabolism via the pyruvate dehydrogenase pathway gives rise to acetyl CoA labeled in the C2 position (red C), and subsequently to glutamate and glutamine labeled in the C4 position and GABA labeled in the C2 position (red C). Acetate or β-hydroxybutyrate labeled at the C2 position leads to a similar labeling pattern as metabolism of C1- or C6-labeled glucose via pyruvate dehydrogenase. Metabolism via pyruvate carboxylase (*blue circle around red C*), which occurs only in astrocytes, gives rise to glutamate and glutamine labeled in the C2 position and GABA labeled in the C4 position. Note that citrate, α−ketoglutarate, glutamate and glutamine can be labeled from metabolism via both pyruvate carboxylase and pyruvate dehydrogenase. See text for further details.

Carbon-13 nuclear magnetic resonance spectroscopy (NMR or MRS) for studying brain metabolism

^{13}C-NMR spectroscopy allows the simultaneous and separate detection of signals from different compounds as well as different atoms in a given molecule, so that metabolism via specific pathways can be determined. As an example, ^{13}C from glucose labeled at the C1 (or C6) position is incorporated via the PDH reaction into the C2 of acetyl-CoA, then into the C4 of α-ketoglutarate (Figure 11-8 and Figure 11-9), and finally into C4 of glutamate and glutamine and the C2 of GABA, which can be measured by NMR spectroscopy. In the TCA cycle, the label is scrambled at the level of succinate, leading to equal labeling of the C2 and C3 of aspartate (Figure 11-8) and, in subsequent turns, of glutamate and glutamine. In astrocytes, the metabolism of C3 labeled pyruvate via pyruvate carboxylase leads to labeling of the C3 position of oxaloacetate, which subsequently labels the C2 of glutamate and glutamine, and the C4 of GABA. Thus, labeling in glutamate and glutamine arises from multiple pathways, but the net de novo synthesis of glutamate and glutamine from glucose occurs only via pyruvate carboxylase in astrocytes (Figure 11-8). In addition to determining the amount of label in a given position in a molecule such as glutamate, the amount of molecules with label in different positions (isotope isomers or isotopomers) can be

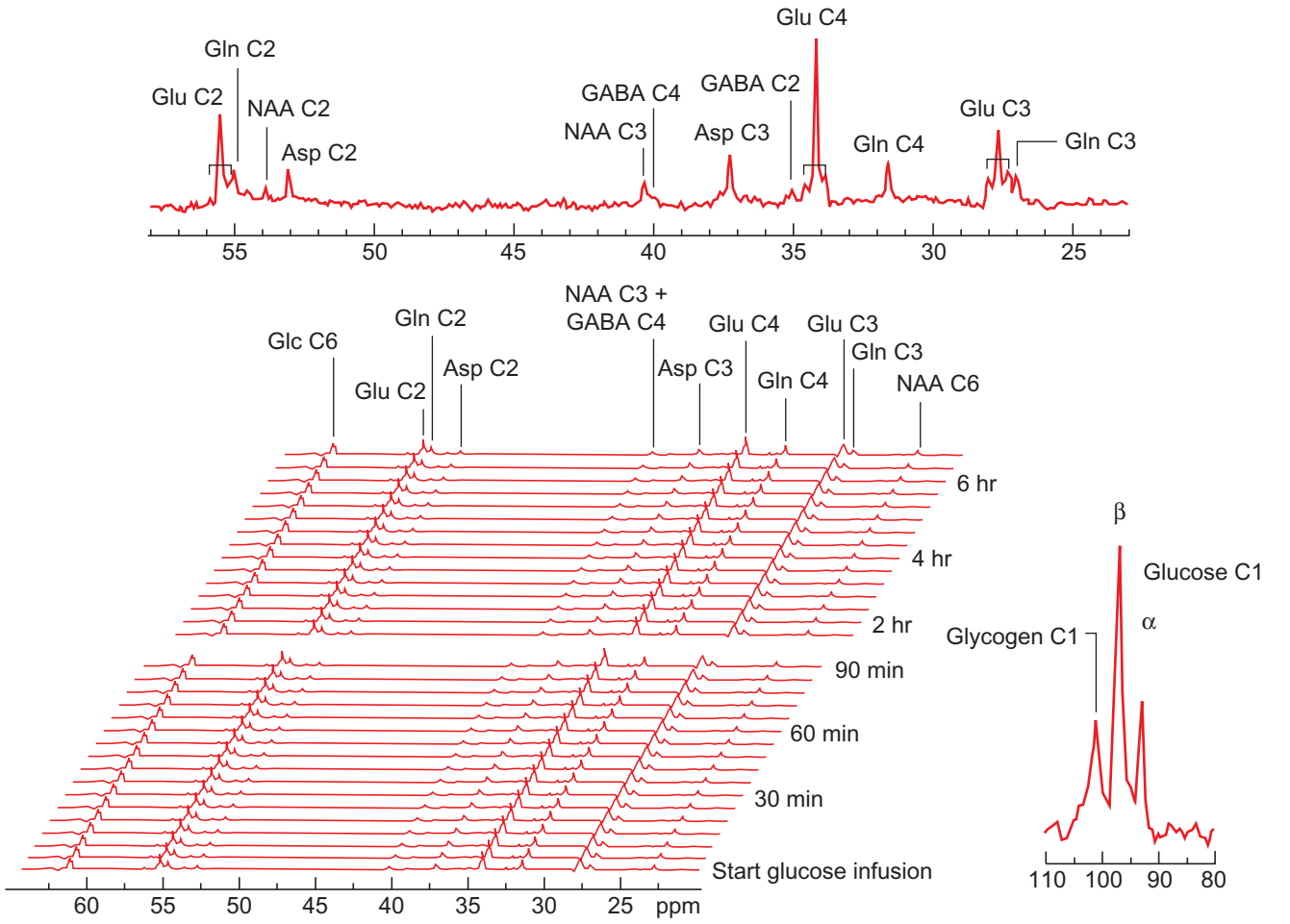

FIGURE 11-9 **Cerebral amino acids and carbohydrates incorporate ^{13}C label from infused glucose.** The **top panel** shows a ^{13}C NMR spectrum obtained from a gray matter–rich volume in the human head. (From Gruetter et al., 2001.) The **right panel** shows label incorporation into brain glycogen and glucose in humans. (From Öz et al., 2003.) The **stack plot** illustrates the rate of label incorporation into many compounds and carbons in the rat brain. (From Henry et al., 2003.) In all studies, glucose labeled at the 1 or 6 position was administered intravenously.

obtained. Furthermore, it can be determined if a ^{13}C atom has a labeled neighbor due to the magnetic interaction between adjacent ^{13}C nuclei (Cerdan et al., 1990). A labeling pattern similar to that obtained with glucose as a precursor is obtained with [2-^{13}C]acetate or [2,4-^{13}C]β-hydroxybutyrate (Pan et al., 2002). Because of selective uptake by astrocytes, acetate provides specific information about oxidative metabolism in these cells, and about the trafficking of compounds from astrocytes to neurons (Cerdan et al., 1990).^{13}C-β-hydroxybutyrate is taken up by both astrocytes and neurons, and can provide information about TCA cycle activity even when metabolism via pyruvate dehydrogenase is impaired (Pan et al., 2002; Kunnecke et al., 1993).

Cultured neurons and astrocytes are useful for studying subcellular compartmentation and identifying pathways of metabolism

Detailed biochemical information can be obtained from brain cell cultures, cell lines and tissue slices without interference from *in vivo* metabolism in peripheral organs such as liver or obstructions such as the blood–brain barrier. Cells taken directly from the organism and grown for at least 24 hours *in vitro* are considered to be primary cultures. Brain cells are obtained by initial mechanical and/or enzymatic dissociation of tissue from embryonic or newborn mice, rats or other animals; then different procedures are used, often in combination, to establish monotypic cultures from mixed cell suspensions. Because of the relative abundance of neurotransmitter glutamate, cerebellar granule cells are the preferred model system for excitatory neurotransmission, whereas GABAergic cerebral cortex neurons are the model for inhibitory neurotransmission; primary cultures of these neuronal types are important models since 90% of brain synapses utilize either glutamate or GABA as the neurotransmitter. Primary astrocytic cultures are also important models, since these cells are responsible for the removal and metabolism of neurotransmitter glutamate and for control of the pH and ion concentrations of the extracellular milieu in brain (Hertz et al., 2007). Astrocytes from different brain regions have different

properties, and astrocytic counterparts for studies using specific neurons are taken from the same brain region as the neurons (Qu et al., 2001). Co-cultures of astrocytes and neurons are a valuable tool for studying neuronal–glial interactions (Waagepetersen et al., 2002). It is important to recognize limitations of working with primary cultures, since a number of proteins including MCT1 are upregulated in cultured astrocytes and neurons and dilution of metabolites in large volume of extracellular space (culture medium) can influence transport and metabolism (McKennac et al., 2006c; Dienel & Hertz, 2001) [and references therein].

Metabolic assays in brain slices, axons, synaptosomes and isolated mitochondria

Brain slices are a step closer than cultured cells to the intact tissue *in vivo* without the confounding presence of the blood–brain barrier, and valuable information about metabolic pathways and pathological situations can be obtained from studies in slices (Bachelard & Badar-Goffer, 1993). Limitations of brain slices include lack of blood flow, so that substrates and oxygen must enter cells by diffusion from the medium and the cells on the cut ends of the slice are damaged. Subcellular preparations are also used to analyze particular aspects of metabolism, and synaptosomes can reveal information about the highly specialized metabolism in nerve terminals (McKenna et al., 1993; McKenna et al., 1994; Yudkoff et al., 1994), whereas axonal metabolism can be assessed in isolated optic nerve preparations (Brown, 2004). Isolated mitochondria are used to dissect the regulation of mitochondrial enzymes and TCA cycle metabolism and to compare synaptic and cell body mitochondria (Lai et al., 1977; McKenna et al., 2000), whereas the activity and regulation of cytosolic enzymes can be studied in the soluble fraction (Malik et al., 1993). Uptake studies, *in situ* hybridization, and Western blots have been used to assess age-dependent changes in transporter level and substrate transport rate (Vannucci & Simpson, 2003).

Concentrations of compounds in brain and regulation of metabolism in the intact brain

In vivo ^{1}H- and ^{31}P-NMR spectroscopy [usually referred to as magnetic resonance spectroscopy (MRS) for clinical studies] can be used to assess the concentration of clinically relevant compounds and high-energy phosphates (Jansen et al., 2006). Determinations of the *N*-acetyl aspartate (NAA), creatine, choline and lactate, and ratios of these compounds, are frequently used as indices of the integrity of metabolism and tissue in developing and diseased brain (Blumberg et al., 1997), and measurement of high-energy phosphates provides information about the energy status of the brain (Jansen et al., 2006). These methods, as well as ^{13}C-NMR spectroscopy, are used for research and clinically as NMR spectroscopy becomes more widely available. *In vivo* ^{13}C-NMR spectroscopy has the disadvantage that only compounds present in relatively high amounts can be detected *in vivo*, large doses of labeled precursor must be administered (sensitivity is lower than radioactive tracer methods), and the head must be immobilized, which necessitates the use of anesthetics in animal studies and complicating interpretation of results in terms of conscious resting or activated conditions.

Given the compartmentalization of compounds such as glutamate, glutamine, and glycogen and related enzymes (see above), important insights into the changes in pathway fluxes during activation and in brain diseases, as well as regulation of brain energy metabolism can be obtained by *in vivo* NMR spectroscopy studies (Figure 11-8). Since glutamate measured in tissue *in vivo* mainly reflects the neuronal glutamate pool, the flow of glucose-derived label from glutamate to glutamine has been interpreted to reflect glutamate neurotransmission. Conversely, flow of acetate-derived label from glutamine to glutamate and GABA measures the flux from astrocytes to neurons. Thus, ^{13}C-NMR spectroscopy enables one to calculate rates of the TCA cycle and glutamate neurotransmission *in vivo* (Mason & Rothman, 2004; Gruetter et al., 2001); it provides a translational bridge between the information obtained from cellular studies (see above) and studies in patients. In addition to observing the flux from glucose ^{13}C labeled at the C1 (or C6) position into the C4 of glutamate and glutamine, label can be observed directly in other positions of the molecule, such as the C2 position (Figure 11-9); this is important in assessing astrocytic pyruvate carboxylase activity, which is significant *in vivo* (Gruetter et al., 2001; Öz et al., 2003). Regulation of subcellular fluxes can be assessed by analyzing the flow of label into the other positions in glutamate and glutamine (Gruetter et al., 2001). Lastly, *in vivo* ^{13}C-NMR spectroscopy is currently the only method capable of assessing *in vivo* brain glycogen metabolism in the human brain (Öz et al., 2003; Öz et al., 2007) (Figure 11-9).

RELATION OF ENERGY METABOLISM TO PATHOLOGICAL CONDITIONS IN THE BRAIN

PET and NMR studies have shown that energy metabolism is perturbed in defined brain areas in patients with neurological or psychiatric diseases (Jansen et al., 2006; Blumberg et al., 1997). Detailed information about pathological conditions that adversely affect brain metabolism can be found in many other chapters of this book.

Acknowledgments

This work was based, for the most part, on the previous chapter by McKenna et al. in the 7th edition of *Basic Neurochemistry* (McKenna et al., 2006c), and also, in part, on the chapter by Donald D. Clarke and Louis Sokoloff in the 6th edition (Clarke et al., 1999). Some aspects of the material are covered in greater detail in those earlier editions of the chapter. We thank Ian Simpson for providing Figure 11-1 and Rolf Gruetter for providing Figure 11-9.

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