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CNS energy metabolism as related to function

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Abstract

Large amounts of energy are required to maintain the signaling activities of CNS cells. Because of the fine-grained heterogeneity of brain and the rapid changes in energy demand, it has been difficult to monitor rates of energy generation and consumption at the cellular level and even more difficult at the subcellular level. Mechanisms to facilitate energy transfer within cells include the juxtaposition of sites of generation with sites of consumption and the transfer of ~P by the creatine kinase/creatine phosphate and the adenylate kinase systems. There is evidence that glycolysis is separated from oxidative metabolism at some sites with lactate becoming an important substrate. Carbonic anhydrase may play a role in buffering activity-induced increases in lactic acid. Relatively little energy is used for 'vegetative' processes. The great majority is used for signaling processes, particularly Na^+ transport. The brain has very small energy reserves, and the margin of safety between the energy that can be generated and the energy required for maximum activity is also small. It seems probable that the supply of energy may impose a limit on the activity of a neuron under normal conditions. A number of mechanisms have evolved to reduce activity when energy levels are diminished. © 2000 Elsevier Science B.V. All rights reserved.

Theme: Other systems of the CNS

Topic: Brain metabolism and blood flow

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1. Introduction

At times of peak activity, some regions in the CNS use as much energy as any other tissue in the body, including striated muscle (see below). Understanding the energy metabolism of neurons and associated glial cells is of importance for understanding the normal function of brain, and for understanding a variety of pathological states.

Energy metabolism was one of the earliest studied aspects of brain biochemistry, and a large body of information has been accumulated. Prominent publications include the classic studies from Lowry's laboratory [127], the extensive review by Siesjo [204], and more recent reviews by Erecinska and Silver [63,65].

Our understanding of brain energy metabolism continues to evolve; and some of the more recent studies, not all of them on brain itself, have provided information about the energy metabolism of cells that has required a considerable modification of our previous views. A number of these more recent revelations are the subjects of this review: (1) it is now clear that energy metabolism is highly organized within cells, and that special mechanisms have evolved to transfer energy efficiently from the site of generation to the site of consumption. As a consequence, there is heterogeneity between one part of a cell and another with respect to energy metabolism. (2) It has also become clear that glycolysis (glucose→lactate) plays an important role in some regions of the normally oxygenated brain and in some parts of cells, with the lactate generated at one site serving as substrate for oxidative metabolism at another. (3) The relative importance of glycolytic versus oxidative metabolism differs markedly between cell types, and symbiotic arrangements appear to have evolved between glia and neurons. (4) More is now known about why the brain requires so much energy, and it has become possible to make preliminary estimates of the various demands on the cells' energy supplies. (5) Comparisons between the capacity of cells to generate energy and their requirements for energy at times of maximal activity have raised the possibility of temporary energy imbalances under normal conditions. (6) Evidence has been obtained for mechanisms that reduce activity and preserve ATP at times of energy limitations.

This review addresses problems for which we do not yet have answers or have only speculative answers. No attempt has been made to review the chemical reactions that generate energy or to review research concerning the critical control points in the glycolytic and oxidative sequences. Much of the discussion assumes that energy generation is linked quite directly to energy consumption; i.e., that reductions in ATP and increases in ADP, AMP, and P_i are the principal controlling factors in both glycolytic and oxidative metabolism (see Ref. [62] and Refs. therein). The important problem of how regional blood flow (rCBF) is altered in response to changes in energy requirements is not discussed. See, for example, reviews by Brian et al. [31] and Harder et al. [84].

Since the mechanisms used to deliver energy to the processes that require it have been difficult to examine in the brain in situ (largely because of its inaccessibility and fine-grained heterogeneity, see below), this review cites studies on in vitro preparations of CNS including cultured cells, and also cites studies on tissues other than brain (e.g., muscle). I believe this to be warranted since energy transfer is such a fundamental requirement of all cell types that the mechanisms utilized can be expected to be quite

general. It should, however, be recognized that final conclusions must await the development of techniques applicable to the in vivo brain.

2. Experimental obstacles

Our continuing ignorance about many aspects of brain energy metabolism can be attributed to the difficulties inherent in devising revealing experiments. Physical access to the brain is restricted by the skull. Chemical access is restricted by the blood–brain barrier. Energy-related reactions proceed so rapidly that efficient quenching is required to harvest specimens that reflect the conditions that pertain in vivo. But the greatest difficulties facing the investigator are the problems of measuring *rates* versus *levels*, and of obtaining measurements with the appropriate spatial and temporal resolution.

2.1. Measuring rates

A great many studies have presented data about the *levels* of compounds involved in energy metabolism, e.g., the level of O_2, glucose, lactate, ATP, ADP, AMP, CrP. These data have provided information about the state of energy metabolism and about changes in the state of energy metabolism; but, generally, not about the rate at which energy is being generated and used. Rates are more difficult to measure.

An early approach to estimating rates used *stop-flow measurements* in which the supply of the substrates for energy metabolism was abruptly curtailed by circulatory arrest; the tissue was quenched after a few seconds; and measurements were made of the rate of change in the major sources of ~P [128]. As acknowledged at the time, interpretation of these data is complicated by the probability that energy usage did not remain at the physiological level during the time required to make the measurement. I believe, however, that this approach warrants further consideration because of its temporal resolution (see below) and because, with histochemical methods, it may be possible to localize the changes in energy metabolites with high spatial resolution, as pointed out by Lowry et al., in 1964.

Important information has been obtained by calculating rates using the *Fick principle*, as exemplified by the pioneering experiments of Kety [105]. This requires measurements of arterial concentration, blood flow, and venous concentration. Brain anatomy makes the latter two difficult to obtain with any degree of spatial resolution, though Tornquist and Alm [225] demonstrated that the Fick principle could be used to study the energy metabolism of retina.

Rates of *glucose consumption* have been estimated from the rate of accumulation of radioactive label on the glucose analogue, 2-deoxyglucose [104,211], or on glucose itself (e.g., Ref. [85]). The retention within the cell of deoxyglucose-6-phosphate has made deoxyglucose a remarkably useful indicator of glucose consumption, notwithstanding the fact that the retention appears not to be as complete as originally supposed [48,68,86,93]; but see also Refs. [41,154,252]. A more serious problem in using measurements of glucose uptake to estimate energy production arises from the fact that an appreciable portion of the glucose is converted to lactate (generating only two ~P) rather than being oxidized directly to CO_2 and H_2O (generating 36 ~P). Though most of the lactate is eventually oxidized, this may not occur in the cell that originally phosphorylated the glucose.

A relatively new and as yet largely unexploited technique for measuring rates of energy consumption has been developed by Goldberg and co-workers [43,255]. By exposing tissue to $H_2^{18}O$ and measuring the rate of appearance of labeled phosphates, they have been able to determine the rate of hydrolysis of ~P bonds. This has the advantage of measuring total energy consumption, regardless of whether the energy was generated glycolytically or oxidatively, and provides information about the turnover of specific ~P bonds. The application of this technique is restricted by the sophistication of the analytic techniques required, by the requirement for a high specific activity of $H_2^{18}O$ which limits its application to in vitro studies, and by the relatively large volume of tissue required for analysis.

Rates of glucose consumption can now be estimated, non-invasively, by using positron emission tomography (PET) to measure the rate of accumulation of ^{18}F fluorodeoxyglucose or of ^{11}C glucose; and rates of oxygen consumption can be estimated by using PET to measure the rate of accumulation of $^{15}O_2$. See review by Phelps et al. [171]. These approaches have the great advantage of being applicable to humans; but they have limitations with respect to spatial and temporal resolution, since they require voxels measured in hundreds of mm^3 and times measured in minutes.

A recently developed technique, using serial measurements with nuclear magnetic resonance spectroscopy (NMRS), has made it possible to follow the ^{13}C label on [1-^{13}C]glucose as it is transferred through substrates of energy metabolism in the in vivo brain [138,202]. Measurements of the flux of the ^{13}C into the glutamate pool (via α-ketoglutarate) have been of particular interest. α-Ketoglutarate and glutamate have been found to be in very rapid exchange, so they can be treated as a single combined kinetic pool; and the rate of entry of the ^{13}C into this pool has been used as a measure of the rate of pyruvate entry into the TCA cycle — and hence as a measure of the rate of oxidative metabolism. This approach has limitations with respect to spatial and temporal resolution, requiring voxels of several cm^3 and times measured in tens of minutes.

2.2. Spatial and temporal resolution

Spatial resolution is critical. There are often large differences in the energy metabolism of adjacent cells in the brain. Indeed, as will be discussed further below, there may be differences in the energy metabolism in different regions of a single cell. Most of the methods for measuring rates of energy consumption cited above require a sample size that includes 10^2 cells, or many more. Only microautoradiography of quick-frozen sections of tissue exposed to ^{3}H-labeled 2-deoxyglucose has provided resolution approaching the cellular level. However, the water-solubility of the labeled product (^{3}H-deoxyglucose-6-P) permits it to move to adjacent cells during the usual preparation of the autoradiograph. A number of ingenious, and more or less successful, attempts have been made to overcome this problem [201]. As an alternative to localizing a product of energy metabolism, Sharp et al. (ibid) localized the less diffusible products of immediate early genes (e.g., c-fos). This may identify the nuclei (and hence cell type) of cells having an increase in energy metabolism. Though the localization of the signal is improved, its relation to changes in energy flux is qualitative at best.

Information about the energy metabolism of a given cell type has sometimes been obtained by examining regions of the CNS where many identical cells are packed together, e.g., white matter tracts, Ammon's horn, retinal photoreceptors. But this approach has obvious limitations.

Isolated brain synaptosomes have proven to be a valuable experimental preparation for characterizing the energy requirements of the specialized portion of the neuron associated with transmitter release, even though the preparation is heterogeneous with respect to type of transmitter (see review by Erecinska et al. [62]).

It is probably fair to conclude that we have not yet been able to monitor the energy usage of any cell type as it operates normally in situ.

The *time required* to make a measurement is not critical in assessing the energy metabolism of resting neurons, but it may be very important in assessing an increase associated with activity since activity is usually phasic. It seems likely that, in order to monitor faithfully activity-related changes in neuronal energy metabolism, measurement times must be in the order of seconds.

Only a few of the techniques for measuring energy consumption or energy production have a temporal resolution in this range. Vanzetta and Grinvald [231] were able to measure changes in capillary pO_2 with a time resolution of 0.1 s by measuring the change in the phosphorescence emitted by an O_2-sensitive, exogenous phosphorescent probe bound to serum albumin. They observed a reduction in capillary pO_2 in the visual cortex of cats (indicative of a rise in O_2 consumption) beginning about 0.2 s after the onset of photic stimulation. Measurements of heat production (e.g., Ref. [229], and of spectroscopic shifts between oxyhemoglobin and deoxyhemoglobin [136] have provided evidence of changes in energy metabolism within a second. 'Stop-flow' measurements of changes in energy substrates have been obtained within a few seconds [128]. Microsensors have been developed that measure activity-induced changes in ECF oxygen and glucose in tens of seconds [92,126]. Rates of ~P hydrolysis have been measured with $H_2^{18}O$ labeling times as short as 20 s [76]. On the other hand, time in the order of minutes has been required for measurements by microdialysis, for measurements by the Fick principle, and for measurements of labeled substrate retention by PET or MRI. Measurement of 2-deoxyglucose accumulation by microautoradiography has required labeling times of tens of minutes.

The goal of defining the energy requirements of neuronal function will require new techniques that not only provide measurements on single cell types in situ, but that provide them in a time frame commensurate with the changes in electrophysiological activity. There will still remain the experimentally difficult problems of identifying which parts of the extended neuron are involved, which reactions are requiring the energy, which reactions (glycolytic or oxidative) are generating it, and how it is being transferred.

3. Organization of energy metabolism within cells

Providing ~P at appropriate rates to energy-consuming enzymes throughout the cell requires not only organized systems for generating the ~P but also organized means of delivery. The concept, once held, of a single intracellular pool of ATP — supplied by glycolytic and oxidative reactions, and drawn upon by various ATPases depending on their K_m values — is now recognized as no longer tenable. ['ATPase' describes any enzyme that requires the energy in the γ phosphate bond of ATP for its activity.] The movement of ATP within cells by diffusion is relatively slow — much slower than free diffusion in water. This is due in part to the tortuosity of diffusion paths through the tangle of microfilaments, tubules and organelles. But two factors of greater importance in limiting diffusion are the alterations in the physical properties of cytoplasmic water that result from its proximity to the surfaces of the structural elements, and the interactions of the diffusing solutes with these surfaces. In his review of this complicated subject Clegg [39] concludes that, as eucaryotic cells became too large for a 'solution-based metabolism', they ''devised a means of escaping the chaos of solution chemistry'' by developing ''intimate connections between cellular architecture and most, and possibly all, of the metabolic machinery''.

An assessment of diffusion within cytoplasm has been obtained by diffusion-weighted ^{1}H NMR spectroscopy (e.g., Ref. [170]), which has indicated that diffusion coefficients in intracellular fluid may be an order of magnitude lower than in extracellular fluid.

Three organizational features of the processes involved in energy metabolism serve to increase their efficiency: (1) the close spatial organization of the enzymes generating ATP; (2) the juxtaposition of sites of ATP generation with sites of consumption; and (3) when generation is separated from consumption, provisions to enhance the transfer of ~P to the ATPase and to provide a feedback to make generation responsive to consumption. These features are discussed briefly below with particular emphasis on the second and third.

3.1. Organization of enzymes that generate ATP

The organization within mitochondria of the enzymes of the citric acid cycle and the electron transport chain has been well characterized.

The glycolytic enzymes of the Embden Myerhof pathway are also spatially organized [39,78,109,119,193], so that the product of one reaction is made readily available as substrate for the next, and the overall sequence of reactions proceeds much more rapidly than would be predicted from comparable concentrations of the enzymes in free solution [113,139,242]. Green et al. [78] found that erythrocyte membranes catalysed the complete glycolytic series of reactions, with a 24-fold increase in activity per mg of protein compared to the whole hemolysate.

3.2. Colocalization of sites of ATP synthesis with sites of ATP consumption

3.2.1. Oxidative ATP synthesis: the Ca^{2+} link

An obvious, and long recognized, example of colocalization is the clumping of mitochondria where energy demands are high. An example of this is the palisading of mitochondria in the inner segments of retinal photoreceptor cells, where they are tightly packed against plasma membranes containing the unusually high concentration of Na^+,K^+-ATPase [141] required to pump out the Na^+ responsible for the dark current of phototransduction.

Using histochemical techniques to reveal the localization of a mitochondrial enzyme (cytochrome oxidase), Wong-Riley and co-workers found that mitochondria were concentrated at glutamatergic synapses, in the inner segments of retinal photoreceptors, and in unmyelinated axons [157,246,247]. Using immunohistochemistry, they demonstrated that cytochrome oxidase colocalized at subcellular levels with Na^+,K^+-ATPase and with NMDA-type glutamate receptors. They concluded that the localization of cytochrome oxidase ‘‘illustrates the ability of neurons to control their energy metabolism at an exquisitely local level’’. Interestingly, they also found that the level of cytochrome oxidase responded to changes in energy demands, as demonstrated by reversible reductions in the enzyme at sites containing voltage-sensitive Na^+ channels following sustained (1–4 weeks) administration of tetrodotoxin [247].

Ca^{2+} may link oxidative metabolism to energy consumption at the subcellular level (see Refs. [140,178] and Refs. therein). Increases in intracellular Ca^{2+}, $[Ca^{2+}]_i$, occur regularly in association with energy-requiring physiological functions, including: excitatory transmitter reception; exocytosis; Ca^{2+}-mediated second messenger systems; and sensory transduction in response to photic and auditory stimuli. Increases in $[Ca^{2+}]_i$, within the physiological range, increase the activity of three mitochondrial dehydrogenases and increase the rate of ~P generation [47]. The additional ~P thus provided, near the site of increased activity, may even anticipate the increase in energy consumption. This may account for the delay of only 200 ms, between photic stimulation and a rise in cortical O_2 consumption observed by Vanzetta and Grindvald (see above). Data are not yet available to assess the relative importance of a rise in $[Ca^{2+}]_i$, versus a rise in the ADP/ATP ratio, in eliciting an increase in oxidative metabolism in response to activity-induced increases in energy consumption.

3.2.2. Glycolytic ATP synthesis and its association with ion transport

Glycolytic enzymes may also be closely associated with major energy-consuming ATPases. Studies on erythrocytes and smooth muscle have provided compelling evidence for a link between glycolysis and ion transport, and there is evidence that this is also true of other cell types including cells of the CNS.

In erythrocytes, the binding of the glycolytic enzymes within the plasma membrane [78] makes glycolytically generated ATP immediately available to the energy-demanding transport systems in the membranes, as proposed by Schrier [193]. Proverbio and Hoffman [181], in experiments on erythrocyte ghosts, found that (unlabeled) ATP, that had been glycolytically generated and retained in a membrane pool, provided the energy for the Na^+,K^+-ATPase, rather than the [γ^{32}P]ATP that they administered exogenously. They concluded that there was a ‘microdomain’ within the membrane in which ‘‘ADP and ATP are compartmentalized in juxtaposition to the GAPD-PGK reaction sequence and the Na^+K^+ pump apparatus’’. Mercer and Dunham [144], using inside-out vesicles from erythrocyte membrane, found that glycolytic substrates supported Na^+ transport even in the presence of a hexokinase-glucose ATP sink, and that the glycolytically generated ATP was incorporated into a membrane-bound pool of ATP. This pool of ATP was decreased by added Na^+ and the decrease was inhibited by strophanthidin. They concluded that ‘‘membrane-bound glycolytic enzymes synthesize ATP and deposit it in a membrane-associated compartment from which it is used by the Na^+/K^+ pump’’.

Further evidence for an association between glycolysis and ion transport has been obtained in a series of experi-

ments on smooth muscle by Paul and co-workers. When they varied the energy required by Na^+K^+-ATPase (increasing it by an increase in extracellular K^+, or decreasing it by reducing intracellular Na^+ or adding ouabain), there were corresponding changes in glycolysis, but not in oxidative metabolism [165]. When they blocked glycolysis with iodoacetate, Ca^{2+} transport was interrupted [166]. However, when they blocked oxidative metabolism by removing O_2, muscle contraction was impaired [96], but Ca^{2+} transport continued [35]. Following the introduction of a hexokinase ATP sink, Ca^{2+} transport continued, indicating that the ATP generated by glycolysis was segregated and inaccessible to the exogenous hexokinase [166]. Further evidence for localization of energy metabolism within the cell was provided by the finding that the substrate for glycolysis was separated from the substrate for oxidative metabolism. Extracellular glucose, labeled with ^{14}C, was the sole precursor of the lactate generated by glycolysis, whereas glucosyl units from ^{14}C-labeled glycogen were a substrate for oxidative metabolism [130]. It should be noted however that, though glycolysis seems clearly to be the preferred source of ~P for the Na^+K^+-ATPase in smooth muscle, Na^+ pump activity could be maintained by oxidative metabolism alone [35].

Glycolytically generated ATP has been linked to ion transport in cardiac cells (e.g., Ref. [241]).

In CNS tissues, evidence for a link between glycolysis and ion transport has been obtained using several experimental approaches. Glycolytic enzymes and Na^+,K^+-ATPase have both been found to be bound at high specific activity within synaptosomal membranes [109,119]. Lipton and Robacker [124] concluded that the activity of Na^+K^+-ATPase in hippocampal slices (as reflected by increases in intracellular K^+ in response to increases in extracellular K^+) had a specific requirement for glycolysis. Raffin et al. [182], using K^+-sensitive electrodes, found that the maintenance of the normally low levels of extracellular K^+ in rat cortex required glycolytic as well as oxidative energy metabolism. Cultured neurons failed to maintain normal ion gradients when glycolysis was interrupted by removal of glucose while oxidative metabolism was being maintained by pyruvate [206]. The uptake of glutamate by cultured astrocytes (which depends on the Na^+ gradient maintained by Na^+,K^+-ATPase) caused an increase in glycolysis [168]. Glycolysis may play a particular role in Ca^{2+} transport [62] (and Refs. therein). A number of investigators have reported that interrupting glycolysis, while continuing oxidative metabolism, has an adverse effect on CNS function that appears to be out of proportion to any change in total energy status [50,123,151].

On the other hand there is the following compelling evidence that most of the Na^+ pumping by CNS cells is fueled by oxidative metabolism. Na^+ transport consumes about 50% of all of the energy generated in the CNS (see below). However, glycolysis accounts for only 5% of the energy generated by the CNS, with oxidative metabolism accounting for 95%. Thus, if all the glycolytic energy were used for Na^+ transport, it would provide only 10% of the energy consumed by the Na^+K^+-ATPase.

The relative contribution to Na^+ pumping of glycolytically versus oxidatively generated ~P can be assessed in in vitro CNS preparations by comparing the reduction in lactate production with the reduction in 0_2 consumption when the activity of the Na^+,K^+-ATPase is blocked with a cardiac glycoside. In rat brain synaptosomes, only one-tenth of the ~P used for Na^+ pumping was derived from glycolysis; and, when the pumping requirements of the synaptosomes were increased 7-fold by opening their voltage-sensitive Na^+ channels with veratridine, there was no increase in the relative contribution from glycolysis [58,59]. In isolated rabbit retina, only one-tenth of the ~P used by Na^+,K^+-ATPase was generated glycolytically (as assessed by the strophanthidin-induced reduction in lactate release and O_2 consumption), and virtually none of the ~P used to pump out the Na^+ that enters the photoreceptors via the 'dark current' (some 33% of the retina's total energy consumption) was generated glycolytically [4]. The maintenance of a near-normal $[K^+]_i$ in synaptosomes did not depend on there being glucose in the medium [42]. Cultured astrocytes maintained their Na^+ gradients (as measured directly [208] or assessed indirectly from glutamate uptake [218]) when glycolysis had been blocked with 2-deoxyglucose, as long as oxidative metabolism was still intact. (Conversely, ion gradients were preserved by an increase in glycolysis when oxidative metabolism was impaired, i.e., a Pasteur effect (e.g., Refs. [208,219]).)

In summary: a close association between glycolysis and ion transport has been well established in smooth muscle. It may also occur in brain, and may be of physiological significance. However, the Na^+,K^+-ATPase in brain is usually fueled by oxidative metabolism.

3.3. Transfer of ~P when source and sink are separated

Because diffusion through cytoplasm is slow over distances measured in μm (see above), a separation within the cell between the site of ~P generation and the site of ~P consumption imposes a potential limitation on the cell's ability to support energy-demanding reactions. This limitation may be viewed as having four interrelated components. (1) The continued generation of ~P may be inhibited by an accumulation of ATP and a depletion of ADP at the mitochondrion or glycolytic enzymes [17,71,98,114]. (2) The rate at which ~P can be transferred to an energy-requiring reaction may be limited by the rate of diffusion of ATP [145,224]. (3) The continued activity of the ATPase may be inhibited by a local depletion of ATP and an accumulation of ADP [188]. (4) The generation of ~P may be unresponsive to changes in the consumption of ~P in the absence of a rapid feedback between the ATPase and the mitochondrion or glycolytic enzymes.

On the basis of relatively recent studies, it now appears that two mechanisms have evolved that act in concert to reduce the potential limitations cited above. One depends on creatine kinase (CK) and creatine phosphate (CrP), and the other depends on adenylate kinase (AK).

3.3.1. The creatine kinase/creatine phosphate (CK/CrP) system

The importance of CrP in energy metabolism has long been recognized. It was initially thought to act merely as a reservoir of ~P to be drawn upon to meet transient high-energy demands. Though it is clear that this is the primary role of CrP in some cell types (e.g., [25]), it is now clear that the CK/CrP system plays additional roles in energy metabolism (see Refs. [17,98,189,235]). CrP acts as a carrier of ~P from its site of generation to its site of consumption; Cr acts as a ~P receptor in a feedback from the site of ~P consumption to its site of generation; and the high concentration and appropriate K_{max} of creatine kinase at sites of ~P production and consumption maintain the reactants at near-equilibrium levels and prevents the marked changes in the concentrations of ATP and ADP that would otherwise occur at these sites [145]. These several effects of the CK/CrP system on the energy metabolism of a cell are illustrated in Fig. 1. It is clear that, for the CK/CrP system to play any of the roles ascribed to it in a sustained fashion, CK must be present at both ends of the axis with a flux of CrP in one direction and an equal flux of Cr in the other.

Quantitative measurements of ~P flux through the CK/CrP system have been obtained by measuring the rate of transfer of the γ-phosphoryl of ATP to Cr [255]. In these experiments on in vitro muscle, replacement of about 40% of the water in the medium with $H_2^{18}O$ caused rapid labeling of the orthophosphate (P_i) being generated hydrolytically by ATPases. The labeled P_i became promptly reincorporated as the γ-phosphoryl of newly synthesized ATP, and the transfer of the γ-phosphoryl to Cr was measured by the rate at which the phosphoryl of CrP was labeled with ^{18}O. The rate of phosphoryl transfer from ATP to Cr corresponded closely to the rate at which ATP was being generated, and this was true both in resting muscle and in stimulated muscle in which ~P generation (and consumption) was several-fold higher. Earlier measurements of turnover of the phosphoryl of CrP had been made using the ^{31}P NMR saturation transfer technology [20], which measures unidirectional flux catalyzed by CK. The unidirectional flux rate was more than 10-fold greater than the *net* flux rate measured with ^{18}O labeling, indicating that the phosphoryls had undergone multiple exchanges between ATP and CrP.

The CK/CrP system appears to be closely associated with oxidative metabolism. Mitochondria contain high concentrations of CK [189,235], and there is evidence that CK is important for ~P transfer across both the inner and outer mitochondrial membranes [187]. In skeletal muscle, the rate of creatine phosphorylation corresponded with the rate of oxidatively generated ~P [255]. However, creatine phosphorylation does not depend exclusively on oxidative metabolism as shown by measurements of CrP in gly-

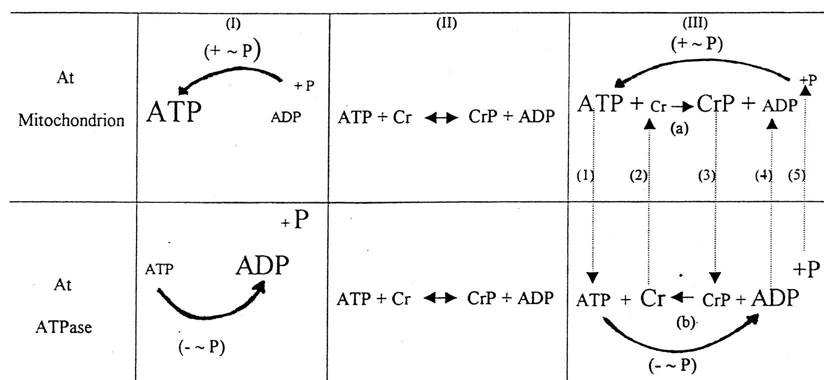

Fig. 1. Schematic to show qualitatively the effects of the CK/CrP system on the concentrations of reactants at sites of ~P generation and consumption, and on the diffusion of reactants between the two sites. Differences in print size indicate differences in concentrations. Panel (I) depicts the unidirectional reactions, with large energy changes, by which ~P is generated and consumed. Panel (II) depicts the reaction catalyzed by CK, which maintains the reactants at near-equilibrium. Panel (III) depicts the combination of the reactions in Panels (I) and (II). Solid arrows indicate net fluxes with respect to chemical transformations, and dotted arrows indicate net diffusion. The CK/CrP system reduces ATP and regenerates ADP at the site of energy generation, and reduces ADP and regenerates ATP at the ATPase. It provides an additional transporter of energy in the form of CrP and an additional feedback indicator of energy consumption, and promoter of energy generation, in the form of Cr. If the system depicted can be considered to be in isolation, the following relationships must be maintained at steady state:

$(+\sim P) = (-\sim P) = (5) = (1) + (3) = (2) + (4)$

$(1) = (4)$

$(2) = (3) = (a) = (b)$

colytic muscles and by evidence obtained with ^{31}P NMR of coupling between glycolysis and CrP utilization (see Refs. in Ref. [235]).

3.3.2. The adenylate kinase (AK) system

An important role for AK in buffering the ATP/ADP ratio and in ~P transfer has been recognized for some time [16,17,34]. The principal effects of AK are shown schematically in Fig. 2. They depend on the enzyme being present both at the site of ~P generation and at the site of ~P consumption, with diffusion of AMP from the site of consumption to the site of generation. The reaction catalyzed by AK then makes three important contributions to the cell's energy metabolism. (1) It prevents the marked increase in the ATP/ADP ratio that would otherwise occur at the site of ~P generation. (2) It prevents the marked decrease in the ratio at the site of the ATPase. (3) It makes it possible for the ATPase to utilize the energy inherent in the β-phosphoryl bond of ATP (as well as in the γ-phosphoryl bond), thus doubling the efficiency of the diffusion of ATP in the transfer of ~P. Though it is clear that, in the presence of AK, there is a net flux of ATP in one direction and of AMP in the other (see Fig. 2, Panel III), the direction of the net flux of ADP is not so clear. Since in steady state there can be no net flux of total nucleotide, the flux of ADP must equal the difference between the fluxes of ATP and AMP. If the AMP flux is greater than ATP flux, ADP net flux will be from the site of ~P generation to the site of ~P consumption, and ADP will act as a ~P carrier. If ATP flux is greater than AMP flux (which seems likely because of the low concentration of AMP; see below) net ADP flux will be in the opposite direction, and ADP will act as an additional ~P receptor. In Fig. 2, Panel III the net flux of ADP is left ambiguous as indicated by the double-ended arrow.

Zeleznikar et al. [256] measured the rate of AK-catalyzed phosphoryl transfer in muscle by using the $H_2^{18}O$ labeling technique (see above) to determine the rate at which ^{18}O-labeled γ-phosphoryls of ATP were being transferred to AMP to appear as ^{18}O-labeled β-phosphoryls in ADP, and (subsequently) in ATP. In resting muscle, much less (about one-fortieth) of the ~P was processed through the AK system than through the CK/CrP system, but AK-catalyzed phosphoryl transfer increased markedly (22-fold) with activity and even more (35-fold) with O_2 deprivation. The increase in AK activity observed when O_2 was removed (or KCN was introduced) closely paralleled a marked increase in lactate production, and was accompanied by a comparable decrease in the activity of the CK/CrP system [256]. Progressive inhibition of CK by 2,4-dinitrofluorobenzene (DNFB), in the presence of normal oxidative metabolism, caused progressive reciprocal changes in the CK/CrP and AK systems; so that, when CK was almost completely inhibited, the AK system accounted for almost all of the ~P transfer [56]. These observations suggest that (at least in muscle) the CK/CrP system and the AK system operate in parallel rather than in tandem.

In contrast to the CK/CrP system, the AK system appears to be more closely associated with glycolytically generated ~P. As indicated above, Zeleznikar et al. [254,256] found a close correlation between the rate of

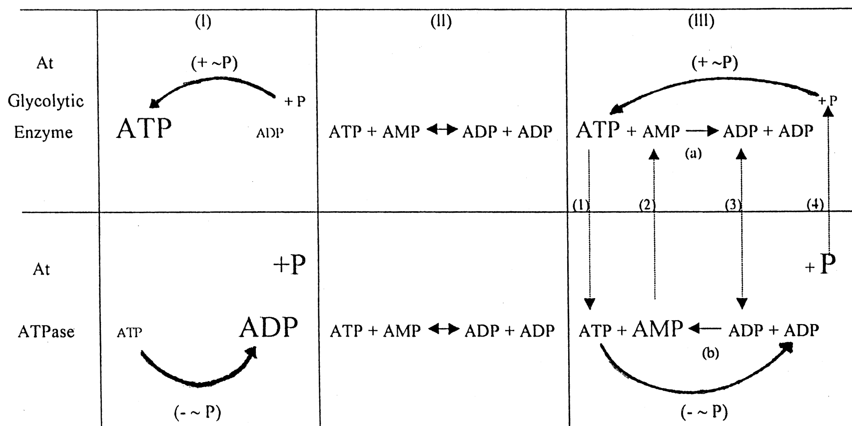

Fig. 2. Schematic showing effect of adenylate kinase (AK) on energy metabolites at sites of ~P generation and consumption. ATP source may also be a mitochondrion. As in Fig. 1, differences in print size indicate differences in concentrations. Panel (I) depicts the unidirectional reactions by which ~P is generated and consumed. Panel (II) depicts the reaction catalyzed by AK. Panel (III) depicts the combination. Solid arrows indicate net fluxes with respect to chemical transformations, and dotted arrows indicate net diffusion. Net diffusion of ADP is uncertain (see text). AK makes it possible for the ATPase to utilize two of the high-energy bonds of ATP, and provides a strong feedback signal in the form of AMP. In the system depicted, the following relationships must be maintained in steady state:

$(+\sim P) = (-\sim P) = (4)$

$(a) = (b) = 2 \times (2)$

glycolysis and the rate of AK-catalyzed phosphoryl transfer when the rate of glycolysis was markedly increased by O_2 deprivation or KCN. However, AK-catalyzed phosphoryl transfer does not depend exclusively on glycolytically generated ATP, since inhibition of CK with DNFB causes increases in AK-catalyzed phosphoryl transfer that greatly exceeded the rate of ~P generation by glycolysis [254].

3.3.3. The question of vectorial ligand conduction

It has been suggested [187,254,256] that creatine kinase and/or adenylate kinase may be positioned in linear arrays between a ~P generator and an ATPase; so that the substrates (the ~P carriers moving in one direction and the unoccupied carriers moving in the other) are passed directly from one enzyme molecule to the next in a series of linked reactions, analogous to what has been characterized as 'vectorial ligand conduction' in mitochondria [148] and as 'channeling' between glycolytic enzymes [213]. This proposal is supported by evidence (cited above) that the ~Ps undergo ten or more exchanges between ATP and CrP en route to the ATPase, and by evidence that the rate of ~P transfer (and the rate of feedback from the ATPase) is greater than expected from free diffusion of the substrates through the cytoplasm [16,235,254].

However the analogy with vectorial ligand conduction may not be very close. The distances involved in the transfer of ~P via the CK/CrP and AK systems would be measured in micrometers, so they would be several orders of magnitude greater than the distances involved in vectorial ligand conduction in mitochondria and in channeling between glycolytic enzymes (measured in nanometers). Though the CK and AK enzymes have been localized both at sites of ~P generation and at ATPases, there is as yet no histochemical evidence for linear arrays of the enzymes between these sites. An alternative explanation for the highly effective transfers observed is functional compartmentation (cf. Refs. [248,256]), with localized high concentrations of enzymes and substrates resulting in exceptionally rapid diffusion-based reactions.

3.3.4. How important are the CK/CrP and AK systems in providing ~P for ATPases?

As indicated above, the CK/CrP and AK systems facilitate processes that would otherwise depend entirely on the diffusion of ATP and ADP. These systems would therefore appear to be of little importance in situations in which close approximation provides for an immediate exchange of the ATP generated by a mitochondrion or glycolytic enzyme with the ADP generated by an ATPase; and their importance to the cell would be expected to increase as the distance between the site of ~P generation and the site of ~P consumption increases [91].

The sustained delivery of ~P to an ATPase will depend as much on the flux rate of the returning receptor as on the flux rate of the carrier. The contribution of the additional ~P carrier (CrP) and the additional receptors (Cr, AMP) will depend on their flux rates relative to the flux rates of ATP and ADP. Flux rates are determined by diffusion coefficients, and by concentration *differences*, according to Fick's Law. The diffusion coefficients of the solutes involved do not differ markedly [253]. However, there are some large differences between solutes with respect to their concentrations, and hence with respect to the potential for concentration differences between one site and another. The concentration in brain of CrP (~5 mM) is usually somewhat greater than that of ATP (~3 mM); the concentration of Cr (~6 mM) is much greater than that of ADP (~0.3 mM); and the concentration of AMP (~0.03 mM) is very low. [See references in Erecinska and Silver [63] for the values cited above.]

3.3.4.1. CK/CrP system. On the basis of the concentrations cited above, an exchange of Cr (~6 mM) for CrP (~5 mM) would be expected to be a more effective means of transferring ~P than an exchange of ADP (~0.3 mM) for ATP (~3 mM). Striking evidence for the functional importance of the CK/CrP system, when transfer distances are increased, was obtained in experiments on sea urchin sperm in which the introduction of the CK inhibitor, dinitrofluorobenzene (DNFB), caused loss of all movement in the distal two-thirds of the sperm tail [224]. Cain and Davies [34] have shown that inhibition of CK by DNFB seriously impaired striated muscle contraction. On the other hand, after assessing the diffusivity of the relevant compounds, Meyer et al. [145] concluded that CrP plays an important, but not a necessary, role in the transfer of ~P in skeletal muscle. It remains to be determined how essential the phosphocreatine shuttle may be for CNS cells, and at what sites it is most critical.

3.3.4.2. AK system. Because of the low concentration of AMP (~0.03 mM, see above), it seems unlikely that the AK system can play a major role in the transfer of ~P from one part of the cell to another. It might be argued that the AK system could nonetheless play an important role in *buffering* the changes in the ATP/ADP ratio at sites of ATP generation, and in buffering the reciprocal changes in the ratio at sites of ATP consumption. However, continued buffering of the ratio by AK depends on a continuing source of AMP at ATP generation sites and on a continuing sink for AMP at ATP consumption sites, so any sustained buffering will depend on AMP diffusion rates. The prediction of a small role for AK in ~P transfer was supported by measurements using the $H_2^{18}O$ labeling technique [256] showing that only a small fraction (about 3%) of the ~P consumed by normally oxygenated, resting muscle is transferred through the AK system. However, as noted above, progressive inhibition of CK by DNFB caused progressive increases in the activity of the AK system; and when CK was almost completely inhibited, ~P

transfer via the AK system accounted for almost all of the ~P generated [56]. This suggests that the AK system *does* play a role in energy transfer in muscle. Direct evidence, for or against, adenylate kinase playing an important role in energy transfer in the CNS is still lacking. The high k_m values of the enzyme in brain for its three substrates, reported in one study (see Ref. [65]), would, if correct, limit its role in the CNS. In the absence of an effective AK inhibitor, it has been difficult to assess its importance.

It is evident (see Figs. 1 and 2) that the continuing transfer of ~P to an ATPase requires the return of inorganic phosphate (P_i) to the site of ~P generation. The concentration of P_i (~2.5 mM; see Erecinska and Silver, [63]) is less than that of ATP and CrP, but its diffusion coefficient is somewhat higher [253]. It seems likely that the back-diffusion of P_i may sometimes be a limiting factor in ~P transfer [235].

3.4. Functional compartmentation of ATP within cells

It is clear that both the transfer systems just described and the juxtapositioning of sites of ~P generation with sites of ~P consumption preclude there being a single intracellular pool of ATP. Quantitative evidence for the expected heterogeneity of ATP turnover (functional compartmentation) in skeletal muscle has been obtained by Zeleznikar and Goldberg [255] using the $H_2^{18}O$ labeling technique. Their calculations depend on the fact that the four O_2 atoms in the γ-phosphoryl of the ATP are equivalent. Therefore, if there were only a single pool of ATP, continuing exposure to $H_2^{18}O$ would lead to a predictable pattern of progressive labeling of the γ-phosphoryl with up to four ^{18}O atoms. Instead, Zeleznikar and Goldberg's experiments demonstrated a more-than-expected multiple labeling of some of the ATP, indicating a subset (or subsets) of ATP that was turning over more rapidly than the rest. The fraction of more rapidly turning over ATP increased with muscle activity.

3.5. The role of GTP

When GTP is substituted for ATP as the energy carrier, the energy is made available only to GTPases. The relative magnitude of energy transfer by GTP has been little studied, but it appears to be remarkably large in some tissues. Experiments on rabbit retina using the $H_2^{18}O$ labeling technique [76] have shown that the γ-phosphoryl of GTP was turning over at a rate comparable to the γ-phosphoryl of ATP, and less than 20% of this could be accounted for by the turnover of cGMP (see Ref. [4]). The processes utilizing the great majority of the energy carried by GTP remain undefined. Cells contain a large number of GTPases (see review by Bourne et al. [29]), but their relative energy requirements remain unknown. Many of the GTPases (e.g., G-proteins) occupy proximal positions in signaling cascades, so their energy requirements are probably low. However, the energy used by GTPases that support ribosomal protein synthesis is substantial, and the energy used by GTPases that support vesicular transport in neurons may also be appreciable. The importance, if any, of separating the energy source for these processes from that of processes using ATP is not clear.

3.6. The physiological significance of the directed transfer of ~P

The localization of mitochondria or glycolytic enzymes near a particular ATPase gives that ATPase priority with respect to the energy being generated. When ~P is being transferred as CrP, a concentration of CK near an ATPase has a similar effect. GTP directs ~P to GTPases.

The directed transfer of ~P may be important for cell physiology. For example, it has been suggested [217] that the transfer of glycolytically generated ~P to ion pumps (as a consequence of colocalization) may serve to maintain ion gradients when energy is in short supply. When cerebral blood flow was moderately reduced, ion gradients were maintained [12,153,251] after protein synthesis [97,146,159,249] and electrophysiological function [12,153] were markedly diminished. Since the collapse of ion gradients (particularly Ca^{2+}) is an important step towards irreversible damage (e.g., Refs. [36,192,216]), the preferential maintenance of ion transport may have survival value for the cell. It is of interest to note in this connection that brain in vivo [131] and astrocytes in culture [217] were relatively little damaged by a marked reduction in oxidative metabolism if glycolysis remained unimpaired. It seems likely that there is much to be learned about the rationing of energy at times of increased demand or reduced supply.

Summary: Diffusion through cytoplasm is relatively slow. Diffusional exchange of ATP for ADP would be expected to limit the delivery of ~P to ATPases and to delay the response of energy metabolism to a change in energy consumption. Several measures have evolved to overcome these problems: the juxtaposition of the enzymes of energy metabolism next to major ATPases; the provision of an additional ~P carrier and receptor (Cr); the maintenance of the equilibrium between ATP and ADP and AMP at sites of ~P generation and consumption (with adenylate kinase). In addition, Ca^{2+} may provide a direct link between some types of activity and oxidative metabolism. These features create a marked heterogeneity of energy metabolism within a single cell. They also provide a means of directing ~P to particular energy-consuming enzymes (e.g., when energy supplies are limited). The transfer of ~P from ATP to GTP also serves to direct the delivery of energy. The significance of the directed transfer of ~P remains to be established.

4. The role of lactate in brain energy metabolism

Because the principal energy substrates entering the brain are glucose and O_2, and the principal products leaving are CO_2 and H_2O, it was conventionally assumed that brain cells generated virtually all of their ~P from the direct oxidation of glucose. It now appears that a (still undetermined but probably) substantial portion of the brain's energy comes from the conversion of glucose to lactate at one site (yielding two ~P per glucose molecule) and from the oxidation of the lactate at another site (yielding 36 ~P per glucose equivalent). The delay in recognizing the importance of lactate as an intermediate in glucose oxidation is attributable to the fact that the overall result is the same as when glucose is oxidized more directly; and distinguishing between the two alternatives (pathways I and II in Fig. 3) requires a degree of spatial and/or temporal resolution not usually available to the investigator. The following sections review evidence for the generation of lactate and for its oxidation, and also evidence for the movement of lactate from one site to another. [Some of the data to be cited were obtained on retina, which is peculiar in having a layer that is quite avascular; and some were obtained on cells in vitro, which may be more glycolytic than the corresponding cells in vivo. These data probably do not provide a quantitatively accurate reflection of processes in the brain proper in vivo.]

4.1. Evidence that lactate is generated and released by normally oxygenated brain cells

Isozymes of lactate dehydrogenase, containing the LDH5 subunit that favors the conversion of pyruvate to lactate, are widely distributed in brain, particularly in glia (e.g., [19,221,232]).

Measurements on rapidly quenched brains of rats and mice have shown lactate to be present at concentrations of about 1.5 mM, which is some 40–80% of the concentration of glucose [110,143,163,174]. On the basis of measurements by microdialysis, the concentration of lactate in rat brain ECF was estimated to be 1.1 mM [111]. Measurements by NMR spectroscopy in humans have indicated that lactate is present at concentrations of about 0.7 mM [180].

Brain lactate concentrations have been observed to increase markedly — up to 50% — in response to various types of physiological stimulation [45,66,180,191,227]. In addition, stimulation-induced increases in lactate synthesis have been calculated from the disparity between the increase in glucose uptake and the increase in O_2 consumption [69,133].

Lactate is transported across CNS plasma membranes, including the blood–brain barrier, by the monocarboxylate transporter [30,54,72,169,176].

Positive venous–arterial (V–A) differences in lactate concentration in cerebral vessels (higher in the venous blood) have been measured in normal human subjects [40,75,77,100]; and, on the basis of these measurements combined with measurements of the arterio–venous (A–V) difference in glucose concentrations, it has been calculated that some 4–16% of the glucose consumed by the brain appears as lactate in the venous blood. Blomqvist et al. [24] estimated that 12% of the glucose consumed by human brain is released as lactate, both on the basis of arterio–venous differences and on the basis of the difference between the uptake of D-[1-^{11}C]glucose and its oxidation as estimated by positron emission tomography (PET). Arterio–venous differences measured on the isolated, perfused dog brain showed that 15% of the glucose taken up by the brain was released as lactate [52]. Measurements on retina in vivo have indicated that from 40% (rabbit) to 80% (cat) of the glucose consumed is released as lactate [237,238]. Hyperoxia reduced lactate release by cat retina, but not by rabbit retina (ibid.).

It is important to note that the measurements of lactate release into venous blood cited above probably greatly *under*estimate the amounts of lactate being produced, since much of the lactate produced can be presumed to have been oxidized (see below) and to have left the tissue as CO_2 and H_2O. The fraction of lactate that is oxidized can be expected to be less in in vitro preparations in which the lactate being produced is continually eluted from the cells into the relatively large volume of incubating medium. Thirty percent of the glucose consumed by rat sympathetic ganglia appeared in the medium as lactate [90]. Eighty percent of the glucose consumed by isolated rabbit retina was released as lactate [4]. The great majority of the glucose consumed by cultured cortical astrocytes [168,236] and isolated retinal glia [173] was released into the medium as lactate.

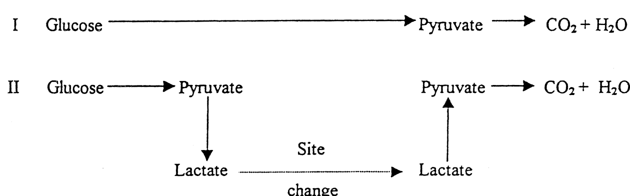

Fig. 3. Alternate routes of glucose oxidation.

4.2. Evidence that lactate oxidation can be an important source of ~P in brain

Isoenzymes containing the LDH-1 subunit of lactate dehydrogenase, which favors the transformation of lactate to pyruvate, have been found in both neurons and glia [19,221].

It has been known for some time that lactate may exceed glucose as the major energy substrate for the brain of young mammals [51,230]. Evidence that lactate can support brain energy metabolism (and function) in adults has

been provided by studies in which serum lactate levels have been increased considerably above normal. Nemoto et al. [155] demonstrated that, when blood lactate was increased to 8 mM in adult dogs, lactate provided about one fourth of the substrate for the brain's oxidative metabolism. Thurston et al. [222] found that increasing the plasma lactate to 20 mM in mice reduced the rate at which the brain utilized glucose by 40% while increasing the level of glucose and of citric acid cycle intermediates. Increasing plasma lactate during severe insulin-induced hypoglycemia led to recovery of neurological function [108,137,222].

Evidence for lactate oxidation has been regularly obtained in in vitro studies of nervous tissue. In early experiments by McIlwain [142] on human brain slices, lactate maintained O_2 consumption and maintained its increase in response to electrical stimulation as well as did glucose. Substantial rates of lactate oxidation have been measured in rat brain slices [95], chick sympathetic ganglia [116,117], and cultured astrocytes [223]. Winkler [245] found that oxidative metabolism and photoreceptor function in retina were maintained when glucose was replaced by lactate. Schurr et al. [197] found that lactate could support function in a hippocampal slice preparation in the absence of glucose, and in subsequent studies [196] they reported that lactate was superior to glucose in restoring function following a period of hypoxia. In another study on hippocampal slices [184], lactate was found to preserve ion homeostasis and synaptic transmission as well as did glucose; but, in this study, functions that were lost during anoxia were better restored by glucose than by lactate.

4.3. When does lactate play a role in the energy metabolism of the normally oxygenated brain?

Lactate is generated when the glycolytic enzymes of the Embden–Meyerhof pathway are separated from mitochondria. This may occur under three circumstances.

4.3.1. In intercapillary regions with low pO_2

The normal architecture of the CNS vasculature creates regions that are separated from capillaries by tens of μm [258]. The diffusion of O_2 and glucose into these intercapillary regions depends (according to Fick's law) on diffusion coefficients and concentration *differences*. Though the diffusion coefficient of O_2 in water is about 3.4 times that of glucose, its rate of diffusion is limited by a small concentration difference. Because of the low solubility of O_2 in water, the concentration of O_2 at the capillary is only about 0.09 mM, whereas the concentration of glucose is about 4 mM. The *difference* between the concentration at the capillary and the k_m of the relevant enzyme (cytochrome oxidase or hexokinase) at the intercapillary cell is about 40 times *less* for O_2 than for glucose. Furthermore, oxidative metabolism creates a sink for 6 times as many molecules of O_2 as of glucose. These factors account for the marked heterogeneity of pO_2 in brain tissue, with steep O_2 gradients that appear to correspond to the periodicity of the capillaries [67,199,205]. O_2 concentrations as low as 2 μM were measured in intercapillary sites. These concentrations, measured with micro O_2 electrodes 5–10 μm in diameter in the ECF, can be expected to be appreciably higher than the concentrations that existed within the cells. It therefore seems likely that the oxidative metabolism of cells furthest from the capillaries is normally limited by the availability of O_2. Whereas (as indicated above), glycolysis would not be limited by the availability of glucose. Most of the lactate generated in the normally hypoxic regions is probably oxidized in adjacent, better vascularized regions, rather than being eluted in the venous blood. In a study of the distribution of capillaries and enzymes in rat brain using histochemical staining techniques, Borowsky and Collins [28] found reciprocal patterns of staining for lactate dehydrogenase and cytochrome oxidase, with a negative correlation between capillary density and lactate dehydrogenase and a (weak) positive correlation between capillary density and cytochrome oxidase. They interpreted their results as consistent with the hypothesis that oxidative and glycolytic metabolism are partially segregated anatomically in brain. A striking example of neurons separated from capillaries is seen in the poorly vascularized inner retina where pO_2 is near zero [121]; and mitochondrial enzymes are much reduced, but glycolytic enzymes are in abundance [129].

4.3.2. Where there is segregation of glycolytic enzymes within a single cell

As discussed above, the plasma membranes of some cells have been shown to contain glycolytic enzymes that are functionally compartmentalized in association with Na^+,K^+-ATPase (e.g., Refs. [109,130,181]). The lactate generated at these sites presumably diffuses either out of the cell or to mitochondria in the cytoplasm for oxidation. It is not clear how often this situation pertains in the CNS or what its physiological significance may be.

4.3.3. Where glycolytic cells are juxtaposed to oxidative cells

There is evidence that some glia are primarily glycolytic and that the lactate they generate is transferred to adjacent neurons as substrate for their oxidative metabolism, in what has been characterized [19] as the 'astrocyte–neuron lactate shuttle.'

In a study of glia and neurons in primary cultures, Tholey et al. [221] found that the glia (astroblasts) contained predominantly an isoenzyme of lactic dehydrogenase (LDH) with the LDH-1 subunit that favors the formation of lactate from pyruvate; whereas the neurons contained predominantly an LDH isoenzyme with the LDH-5 subunit favoring the formation of pyruvate from

lactate. In an immunohistological study of slices of human brain obtained at autopsy, Bittar et al. [19] found that astrocytes were stained with antibodies to both the LDH-1 and LDH-5 subunits, whereas neurons were stained exclusively with antibodies to the LDH-5 subunit. Wong-Riley [246] reported that glia contributed minimally to the overall cytochrome oxidase activity in brain. The monocarboxylate transporter, found in the plasma membranes of both neurons and glia (see Refs. above) permits the movement of lactate down concentration gradients, from one cell to another, at rates sufficient to meet metabolic requirements [112].

In experiments performed on primary cultures, astrocytes consumed from 3 to 10 times more glucose (per mg of protein) than neurons [125,134]. In a histochemical study of freeze-dried sections of guinea pig retina using tritium-labeled deoxyglucose to assess glucose consumption, Poitry-Yamate and Tsacopoulos [172] found the glia (Mueller cells) to be heavily labeled in the inner retina whereas the neurons (ganglion cells) showed no labeling beyond background levels. Glutamate uptake by cultured astrocytes was associated with a marked increase in glucose consumption and lactate production [168]. Swanson [217] found that, with glucose present, cultured astrocytes continued to take up glutamate at about 60% of control values in spite of maximal inhibition of oxidative metabolism. Swanson et al. [219] found that dialysate recovered from rat brain after circulatory arrest contained less than one-fifth as much glutamate if the anoxic dialyzing fluid contained glucose than if it did not, evidence of glycolytically fueled glutamate uptake in in vivo brain. Poitry-Yamate et al. [173], in experiments on freshly isolated retinal glia (Mueller cells) and their attached neurons (photoreceptor cells), found evidence that lactate generated by the glia and released into the medium was taken up and oxidized by neurons in preference to glucose.

Further evidence for an astrocyte–neuron lactate shuttle has recently been obtained by Schurr et al. [195] in a study on rat hippocampal slices: (1) they observed an increase in lactate in the medium following the addition of glutamate (but not following the addition of NMDA, which simulates the excitatory effects of glutamate but is not actively taken up by cells), evidence that the energy for uptake was being provided by glycolysis. (2) When lactate was added to the medium, the neurons were protected from glutamate in the presence of 2DG, evidence that lactate was a suitable substrate for neuronal energy metabolism. (3) In the presence of α-cyano-4-hydroxycinnamate (4-CIN), a lactate transporter inhibitor, there was an increase in lactate in the medium, suggesting that lactate was being transported into a cell other than the cell that generated it. (4) In the presence of 4-CIN, neuronal function became more vulnerable to the damaging effects of high levels of glutamate or NMDA, suggesting that lactate uptake had been the source of substrate for their energy metabolism. Taken together, these findings provide support for the concept that one cell type (presumably glia) was generating lactate by glycolysis in the course of taking up glutamate and that the lactate thus generated was an important substrate for the oxidative metabolism of adjacent neurons. However, it should be noted that α-cyanocinnamates have been shown not only to inhibit plasma membrane lactate transport but also to inhibit mitochondrial pyruvate transport and to inhibit mitochondrial aldehyde dehydrogenases at the concentrations used by Schurr et al. [175]; so observations (3) and (4) above may be attributable to impaired oxidative metabolism rather than to inhibition of lactate transport.

Pellerin and Magistretti [168] have reviewed the evidence for a net flux of lactate from glia to neurons, with particular attention to its implications at glutamatergic synapses. They propose that neurotransmission increases, simultaneously, the glycolytic metabolism of synaptic glia (for extrusion of Na^+ that enters during re-uptake of glutamate) and the oxidative metabolism of the neurons (for extrusion of Na^+ that enters during depolarization). Each of these processes would facilitate the other, since the flux of lactate from glia to neuron would remove the end product of the glial glycolytic metabolism and provide the substrate for the neuronal oxidative metabolism. However, direct evidence for this concept is still lacking.

It is clear the glia are not exclusively glycolytic, and that neurons do not always use lactate rather than glucose as substrate for their oxidative metabolism. Evidence that glia may depend on oxidative as well as glycolytic energy metabolism has been obtained by Tildon et al. [223], who found that cultured astrocytes oxidized lactate at 3 times the rate of glucose. Swanson [217] found that glutamate uptake by cultured astrocytes, though very dependent on glycolysis, was also supported by oxidative metabolism as evidenced by inhibition by dinitrophenol and azide and facilitation by pyruvate. Cultured glia failed to maintain normal ion gradients when their oxidative metabolism was inhibited with rotenone, while glycolysis was being maintained with glucose [206]. Evidence that neurons metabolize glucose that has not been converted to lactate is provided by the presence in neurons of glucose transporters, particularly Glut-3 [135,152,239] and by the presence of hexokinase [101]. Neurons in primary cultures consume glucose [125,134].

It is of interest that histochemical studies have shown that both the glucose transporter [135,152,239] and hexokinase [101,102] are unevenly distributed amongst the different types of CNS neurons, which is consistent with some neurons being much more dependent on lactate as substrate than others, as suggested by Dringen et al. [53]. The question of which neurons normally use lactate, and what fraction they represent of the neurons in the brain as a whole, has not yet been answered.

4.4. Effect of an increase in physiological activity

As indicated above, physiological stimulation has been observed to increase brain lactate concentration

[45,66,70,180,191,227]. Physiological stimulation has also been observed to increase glycogenolysis [220]. From the magnitude and the rate of these increases, it has usually (and reasonably) been inferred that the stimulation had led to an increase in glycolysis that was out of proportion to any increase in oxidative metabolism. More direct evidence for this was obtained by Fox et al. [69] who found (using PET on humans) that physiological stimuli increased glucose uptake out of proportion to O_2 consumption. Madsen et al. [133] measured CBF and arteriovenous differences in rats and also found that activation increased glucose uptake disproportionately to O_2 uptake. Altering the rate of neurotransmission through rabbit retina with flashing light or with excitatory and inhibitory glutamate analogues had a marked effect on glycolysis but little effect on oxidative metabolism [1].

[It should be recognized that increases in oxidative metabolism are also regularly observed in response to physiological activity [69,90,94,115,136,183]. Oxidative metabolism is much more efficient than glycolysis; so, even if its proportional increase is less than the increase in glycolysis, most of the additional ~P required for activity is usually provided by oxidative metabolism (see brief review by Barinaga [13]).]

It is not clear why physiological activity should increase glycolysis out of proportion to oxidative metabolism. Two possibilities may be considered. (1) A *Pasteur effect* due to limitations imposed by the availability of O_2 or by the V_{max} of mitochondria. Consistent with this, Sappey-Marinier et al. [191] observed a decrease in the CrP/P_I ratio in human visual cortex during visual stimulation. (2) A *shift in the nature of the energy demands* that results in an increase in the rate of reactions that are normally served by glycolysis relative to reactions that are normally served by oxidative metabolism (see Paul [164] and Sappey-Marinier et al. [191]). For example, in response to an abrupt increase in synaptic transmission, a glycolytically fueled uptake of glutamate from the synaptic cleft may precede more sustained, oxidatively supported reactions associated with neurotransmission.

4.5. A possible role for carbonic anhydrase

The generation of lactic acid in one cell, and its oxidation in another, creates a source of H^+ in the first cell (e.g., glial) and a sink for H^+ in the second (e.g., neuron) as indicated in Fig. 4. This poses no threat to pH homeostasis under a steady state situation in which lactic acid is leaving the glycolytic cell (on the H^+ monocarboxylate transporter) at the rate at which it is being generated, and is entering the oxidative cell at the rate it is being oxidized. However, physiological activation can cause a rapid increase in the rate of glycosis. From measurements available for the resting concentration of lactate in brain (about 1.5 mM; see above) and for the fractional increase with stimulation (about 50% per min as measured by NMR or microdialysis; see [66,180,191], the rate of a

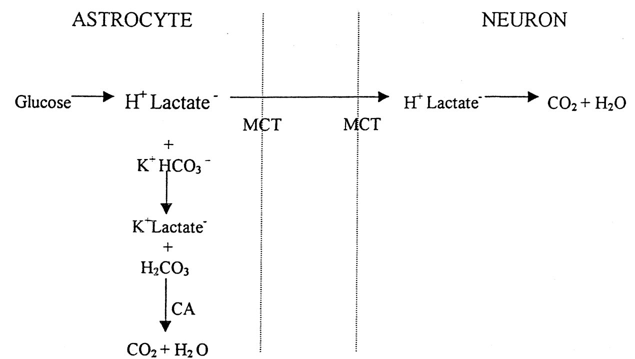

Fig. 4. Possible role of carbonic anhydrase in buffering acid load in glia caused by activity-induced increase in glycolysis. Dotted lines indicate plasma membranes of glial cell and neuron. Single arrows indicate net fluxes. MCT, H^+ monocarboxylate transporter; CA, carbonic anhydrase.

stimulation-induced increase in lactic acid is calculated to be about 0.8 mM min^{-1}. Since these measurements required a substantial volume of brain that included many different cell types, the increase must have been much greater at the cellular (and subcellular) sites most involved, perhaps in the order of 5 mM min^{-1}. The resulting fall in pH will be buffered in large part by the bicarbonate–CO_2 buffering system (see Fig. 4), which depends on the dehydration of H_2CO_3 (about 0.005 mM) to CO_2 (about 1.2 mM). The dehydration of H_2CO_3 is so slow in the absence of carbonic anhydrase ($t_{1/2}$ about 2 min; see Kiese and Hastings [107]), that carbonic anhydrase may be of critical importance for the maintenance of pH homeostasis in glia. (Buffering against a *rise* in pH, due to an activity-induced increase in the oxidation of lactic acid in neurons, would appear *not* to be a problem; because increases in oxidative metabolism are limited by the availability of O_2 and because the CO_2 produced by oxidative metabolism is potentially acidic.)

Carbonic anhydrase has been found in the brain in a wide range of animal species [8]. It has been localized in glia rather than in neurons [73,74,150,186]. It appears at about the time of onset of function [9,18,186]. Carbonic anhydrase inhibitors have been observed to cause a fall in pH in retina [226,250] and in cultured astrocytes [38]. However, the authors of these studies did not attribute it to the mechanism proposed above.

Summary: though the great majority of the glucose consumed by the brain is converted to CO_2 and H_2O, lactate generated at one site is sometimes oxidized at another site, even in another cell. Lactate should therefore be considered a normal product of brain energy metabolism, and an important substrate in addition to glucose. The implications of the separation of oxidative metabolism from glycolysis, when it occurs, are not well understood. The requirement for bicarbonate buffering caused by the generation of acid (lactic) at one site and its removal (by oxidation) at another may be one reason for the carbonic anhydrase in brain.

5. What is the energy used for?

Surprisingly little is presently known about the relative magnitude of the various energy demands on CNS cells. It seems reasonable to distinguish conceptually between the energy used for basic vegetative processes (e.g., protein synthesis), and the energy used for processes that underlie specialized physiological functions (e.g., neurotransmission and action potentials).

5.1. Energy requirements of vegetative processes

Protein synthesis is generally considered to be the most energy-demanding of the various housekeeping functions of cells. From measurements of the rate of incorporation of labeled amino acids, the protein in rat brain was found to be turning over at a rate of about 0.6%/h [55,198], and a similar value was obtained for protein turnover in rabbit retina [162]. Assuming a cost of four ~P per peptide bond, the energy required for protein synthesis in the retina was calculated to be 1.1 μmol ~P min^{-1} g^{-1} dry weight, which was only 1.3% of the ~P being generated by the retina [2]. The fractional energy requirements in retina of the other major anabolic processes, estimated on the basis of published values for rates of turnover and costs of synthesis, were 0.7% for nucleic acid synthesis and 0.6% for phospholipid synthesis [2]. The fractional energy requirement for maintaining physiological ion gradients across plasma membranes, *in the absence of signal-serving ion channels and carriers*, was estimated from the energy cost of ion transport in erythrocytes to be about 0.2%. In aggregate, the vegetative processes in retina, as identified above, were estimated to consume only 2.8% of the energy being produced. Even if important vegetative processes were omitted and if the estimates above were erroneously low, it seems clear that the vast majority of the energy being generated by retina (and by the brain proper) is being used for processes related to neurophysiological functions.

5.2. Energy requirements of function

Evidence for the high energy requirements of neurophysiological function is provided by the large changes that occur in total energy metabolism in response to changes in activity. The O_2 consumption of rabbit vagus nerve increased 3.4-fold when it was stimulated at 10 Hz [183]. Both the O_2 consumption and lactate production of rabbit sympathetic ganglia increased 40% with stimulation at 15 Hz [90,115]. Glucose utilization by various brain regions increased several fold in response to physiological stimulation or in response to pharmacological agents that affect physiological activity [87,210].

Measurements of *increases* in energy consumption with activity must often underestimate the *total* energy consumption ascribable to physiological functioning, since information processing usually involves modulation of ongoing reactions that continue (albeit at a slower pace) with the tissue 'at rest' (e.g., Ref. [3]). Furthermore, most of the measurements cited above were made on tissue samples containing a variety of cell types. The changes in energy usage in the cells particularly involved must have been a great deal larger.

5.2.1. Na^+ transport

Of the many function-related processes, the costliest with respect to the energy demands they create are the various reactions that cause Na^+ to enter cells. Though Na^+ enters cells by many routes, it is actively extruded by a single enzyme, Na^+,K^+-ATPase, for which specific inhibitors are available (e.g., ouabain, strophanthidin). By inhibiting the Na^+,K^+-ATPase and promptly measuring the reduction in energy usage, it has been possible to estimate the amount of energy used for the extrusion of Na^+. This represented about 50% of the total energy consumption of brain [11,244]. A similar value was reported for retina [4] and for cultured astrocytes [33] and synaptosomes [57], [though subsequent studies on cultured astrocytes [208] and synaptosomes [58,59] have indicated that Na^+ pumping accounted for only about 20% of the energy being produced]. The average half-time for the turnover of all of the intracellular Na^+ in retina was calculated to be only 0.5 min [4].

Little is yet known about which avenues of Na^+ influx are most responsible for the energy-demanding process of Na^+ extrusion. They include Na^+ entering by way of: voltage-sensitive Na^+ channels; cGMP-gated Na^+ channels; receptors for excitatory transmitters; Na^+/Ca^{2+} countertransport; and Na^+ cotransport with glutamate. Though some of these entry routes can be specifically blocked (e.g., voltage-sensitive channels by tetrodotoxin), the change in energy metabolism following such a blockade is usually uninterpretable because of its effects on all of the cells 'downstream'; i.e., postsynaptic.

[However, in isolated retina, it has been possible to measure the energy required to pump out the Na^+ entering through the cGMP-gated channels in the photoreceptor outer segments by first functionally isolating the photoreceptors from the cells downstream (with aspartate to saturate the postsynaptic glutamate receptors) and then measuring the drop in energy consumption when the cGMP-gated channels are closed with light. The energy used to maintain the 'dark current' was thus estimated to be 0.31 μmol ~P min^{-1} $retina^{-1}$, or 4.8 μmol ~P min^{-1} g^{-1} wet weight of total retina [4]. Since the Na^+,K^+-ATPase responsible is localized in the photoreceptors' inner segments and since the inner segments constitute only about 15% of the retina, the energy consumption of the inner segments in darkness was about 32 μmol ~P min^{-1} g^{-1} wet weight ($4.8 \div 0.15$), or about 450 nmol ~P min^{-1} $mg^{-1}$of inner segment protein. There is probably no other region in the body that consumes much more energy on a sustained basis. The energy consumption of active rat

cardiac muscle is about 33 μmol ~P min^{-1} g^{-1} wet weight [32]; and, in active skeletal muscle, it is about 214 nmol ~P min^{-1} mg $protein^{-1}$ [256]. Indirect evidence for the high energy metabolism of photoreceptor inner segments is provided by the tight packing of the mitochondria within them (e.g., Ref. [240]).

From the rate of photoreceptor inner segment ~P consumption (0.31 μmol min^{-1} $retina^{-1}$) and with data for the number of photoreceptors per retina (~8×10^7; see Ames et al. [6]) and for their length and diameter (15 and 2.0 μm; see Webster et al. [240]), it is possible to calculate the rate of Na^+,K^+-ATPase turnover per μm^2 of inner segment plasma membrane:

$$\frac{0.31\times10^{-6}\,\text{mol min}^{-1}\,\text{retina}^{-1}\times6.06\times10^{23}}{8\times10^{7}\times15\times2.0\times\pi}$$
$$=2.5\times10^{7}\,\text{min turnovers min}^{-1}\,\mu\text{m}^{-2}$$

With a maximum rate of turnover for a single molecule of Na^+,K^+-ATPase of about 6000 times per minute [99], some 4200 molecules of Na^+,K^+-ATPase would be required per μm^2 of plasma membrane. The density of Na^+,K^+-ATPase molecules in plasma membrane from the Henle loop of pig kidney was estimated to be between 3000 and 4500 per μm^2 by Vogel et al. [233], in experiments in which the Na^+,K^+-ATPase was visualized as particles on the surface of freeze-fractured membranes.]

In what follows, I assign the cost of Na^+/Ca^{2+} countertransport to Ca^{2+} influx and the cost of Na^+/glutamate cotransport to neurotransmission. The cost of pumping out Na^+ entering through gated channels is then calculated as the difference between the total cost of Na^+ pumping (as assessed by the response to ouabain or strophanthidin) and the costs (see below) of the countertransport of Ca^{2+} and the cotransport of glutamate. The gated influx of Na^+ is thus calculated to be responsible for 40–50% of the brain's total energy consumption.

5.2.2. Ca^{2+} transport

The energy required for reconstituting the gradients of ions other than Na^+ is not well known. The restoration of K^+ gradients may be viewed as a concomitant of the restoration of Na^+ gradients. Ca^{2+} is probably the most energy demanding of the other ions. Ca^{2+} flux from intracellular organelles into cytoplasm, or from extracellular fluid (ECF) into cytoplasm, is often an essential feature of the physiological functioning of neurons. Most of the transport back into organelles is by Ca^{2+} ATPases that transfer two Ca^{2+} per ~P [178], whereas the majority of the Ca^{2+} transport out across the plasma membrane is by countertransport with Na^+ with one Ca^{2+} ion moved per ~P consumed by the Na^+,K^+-ATPase [62].

Estimates of the energy required are still quite uncertain and are based in part on measurements made on PC12 cells and synaptosomes (see Refs. [62,178] and Refs. therein). The cost of transport within the cell appears to be similar to the cost of transport out of the cell, with the two together consuming perhaps 3–7% of the cell's energy supply.

5.2.3. Processing of neurotransmitters

The energy required for processing neurotransmitters (as distinct from the energy consumed as a consequence of transmitter-activation of receptors in the postsynaptic neuron) includes the energy used for the uptake or synthesis of the transmitter; for the concentration of the transmitter within vesicles; for the translocation, docking, exocytosis and subsequent endocytosis of the vesicles; and for the reuptake (and sometimes chemical conversion) of the transmitter following its release into the synaptic cleft. Some of these processes take place in the presynaptic neuron, some in the postsynaptic neuron or perisynaptic astrocyte. Different transmitters are processed differently.

Studies on isolated synaptosomes (heterogeneous with respect to transmitters) have provided information about the energy requirements of transmitter processing within the presynaptic neuron. DeBelleroche and Bradford [44] and Blaustein [23] demonstrated that depolarization elicited exocytosis of neurotransmitters from isolated brain synaptosomes, and Sanchez-Prieto et al. [190] and Kauppinen et al., [103] found that a substantial amount of energy was required. Erecinska and co-workers [58,62] depolarized rat brain synaptosomes with KCl and demonstrated an increase in $[Ca^{2+}]_i$, a Ca^{2+}-dependent increase in transmitter release, and a Ca^{2+}-dependent increase in O_2 consumption that was not prevented by ouabain. It seems reasonable to ascribe the Ca^{2+}-dependent, ouabain-insensitive increase in O_2 consumption to the increase in energy requirements associated with processes related to neurotransmitter release. The depolarization-induced increase in O_2 consumption showed a nearly linear relationship to $\log[K]_e$; and, with 40 mM $[K]_e$, it equaled about 40% of the O_2 consumption of non-depolarized synaptosomes. In spite of this substantial increase in energy generation, there was a Ca^{2+}-dependent reduction in the energy state (ATP/ADP) of the synaptosomes [62], indicating that, in response to a very strong stimulus, the energy demands associated with transmitter release had exceeded the supply. [In assessing whether this occurs in vivo, it will be important to know how the synaptosomes' capacity to generate ~P compares with the capacity of nerve endings in situ.]

Not all of the energy requirements for the processing of neurotransmitters are presynaptic. In the case of glutamate, the major excitatory transmitter in the CNS, reuptake appears to be primarily into perisynaptic astrocytes. This is achieved by cotransport with three Na^+ ions [257] and requires one ~P per glutamate for extrusion of the Na^+. The glutamate is converted to glutamine by glutamine synthetase (one ~P per glutamate), and some of the glutamine returns by diffusion to the presynaptic neuron where it is reconverted to glutamate (no energy required),

as part of the neuronal–astrocyte, glutamate–glutamine cycle [64,200]. It has recently become possible to estimate the rate of the glutamine synthetase reaction in human brain (though with considerable variability) by using NMR spectroscopy to measure the flux of ^{13}C from glutamate to glutamine [138]. By comparing this rate with the rate of the TCA cycle (estimated in the same subjects from measurements of the flux of ^{13}C from [1-^{13}C]glucose to the C-4 of glutamate; see above), it appears that the glutamine synthetase reaction was consuming some 3–4% of all of the ~P being generated oxidatively. Since, as indicated above, an equal amount of energy was used for reuptake of the glutamate from the synaptic cleft, some 7% of the total energy production was being used for glutamate processing after its release into the synaptic cleft. [It is of interest that, in a study on rats in the same laboratory, Sibson et al. [203] demonstrated a continuing, close correlation between the rate of the glutamine synthetase reaction and the rate of the TCA cycle when both were progressively and markedly reduced by increasing depth of anesthesia. The parallelism under these circumstances between the energy used for glutamatergic transmission and the total energy being generated oxidatively (some 20-fold greater) was interpreted as evidence that most of the energy generated by the CNS is used for reactions related to function rather than for 'housekeeping' reactions.]

In summary: data on the energy costs of neurotransmission per se are scanty and uncertain. As an indication of the cost to the presynaptic neuron, measurements on isolated synaptosomes, showed a depolarization-induced increase in energy generation of up to 40%. In the case of glutamate, the cost of reuptake and resupply to the presynaptic neuron can be calculated from NMR spectroscopy to be about 7% of the energy metabolism of that region of brain. Though these measurements cannot be quantitatively combined, it seems reasonable to make a rough estimate that some 10–20% of CNS energy metabolism is used for the process of neurotransmission.

5.2.4. Intracellular signaling

Intracellular signaling systems usually consist of a succession of reactions, with amplification, so that the later events involve more molecules than the earlier ones (see recent review by Schulman and Hyman [194]). These sequential reactions include the activation and deactivation of proteins (e.g., plasma membrane receptors, G proteins, cyclases, phosphodiesterases, phospholipases, protein kinases) and the formation and removal of substrates that act as second messengers (e.g., cAMP, cGMP, inositol triphosphate, free Ca^{2+}). In a steady-state situation, the rate of protein activation must equal the rate of protein deactivation; and the rate of substrate formation must equal the rate of substrate removal. One arm of these turnovers usually involves the hydrolysis of a ~P bond. The total amount of energy required by these signaling systems remains undetermined. It seems likely, because of the amplification feature, that the later events in a series will require more energy than the earlier ones; and it also seems likely that second messenger turnover will require more energy than enzyme activation and deactivation.

Measurements of second messenger turnover have been difficult to obtain, because it has not been possible to introduce labeled substrate fast enough. The entry of most potentially useful substrates into cells that have not been permeabilized is much slower than the rates of the reactions in which they are subsequently engaged. H_2O is an exception. Goldberg et al. [76] measured the rates of turnover of cGMP and cAMP by measuring the rate at which $H_2^{18}O$ labeled the α-phosphoryls of the respective nucleotides. In rabbit retina, cGMP was hydrolyzed, and resynthesized, at a rate of 7.5 nmol min^{-1} g^{-1} dry weight during illumination, and this was estimated to account for 11% of the total energy consumption of the retina [4,6]. Retina must be considered atypical in this regard because of the particular role played by cGMP in phototransduction. The turnover of cAMP in retina was about one-third that of cGMP in darkness and only about one-fifteenth that of cGMP in bright light [76].

The level of free Ca^{2+} within cells can be monitored in real time using Ca^{2+}-sensitive fluorescent dyes; and studies of CNS cells using this technique have revealed rapid, several-fold changes in the free Ca^{2+} [179]. However, since the absolute concentrations of free Ca^{2+}, and of the IP3 that evokes its release, are only in the high nanomolar or low micromolar range, the energy required for this turnover is probably not large.

In summary: though little quantitative data is yet available, it seems likely that the many intracellular messenger systems, as a group, account for an appreciable fraction of neuronal energy requirements.

5.2.5. Other

Energy is required for axonal and dendritic transport in both directions (e.g., Ref. [88]), and for reshaping the cytoskeleton (e.g., Ref. [81]). The amount of ~P used can be assumed to be small. Some of it is derived from GTP [29].

5.2.6. Summary

The principal categories of energy-requiring processes as proposed above are listed below, together with very speculative estimates of the relative demands they may make on the energy produced by the brain as a whole. The categories are presented in order of increasing uncertainty with respect to energy demands, with the combined value for categories 5 and 6 selected to bring the total to 100%. There must, of course, be marked differences in energy requirements between different cell types, and between resting state and active state. The purpose of this listing is more to pose the problem than to present data:

1. Vegetative metabolism	5–15%
2. Gated Na^+ influx through plasma membranes	40–50%
3. Ca^{2+} influx from organelles and ECF	3–7%
4. Processing of neurotransmitters	10–20%
5. Intracellular signaling systems	20–30%
6. Axonal and dendritic transport; other	20–30%
Total	100%

[The dominant share of energy consumption is assigned to function-related processes in the above listing, which suggests that interruption of function would cause a substantial (presumably reversible) reduction in energy requirements. This approach has been proposed as a means of protection against ischemia (e.g., Refs. [5,10]).]

6. When energy demands exceed energy generation

If the capacity of CNS cells to generate energy is terminated abruptly (e.g., by circulatory arrest), cell function is lost following a brief latency, the duration of which reflects the level of *energy reserves*. If, on the other hand, the cells' capacity to generate energy is only marginally reduced (e.g., in climbers at high altitudes), function may or may not be impaired, depending on the *margin of safety* that exists between the amount of energy the cells can normally generate and the amount of energy they require. CNS cells clearly have small energy reserves, and they appear also to operate with a small margin of safety. In the section on *margin of safety* that follows, I also discuss the possibility that, even with a normal capacity to generate energy, CNS cells may not be able to meet the highest energy demands that are made on them if these are sustained.

6.1. Energy reserves

The paucity of the energy reserves has been demonstrated by experiments in humans in which the time to loss of consciousness after interrupting the circulation in the neck was found to be only 7 s [185], and the time to loss of vision after interrupting circulation to the retina was only 10 s [7].

These findings are consistent with measurements of energy metabolites in the brains of experimental animals. (Unless otherwise indicated, the measurements presented in the remainder of this paragraph are averages of the values cited in the reviews by Erecinska and Silver [63,65].) The total amount of preformed ~P in ATP, ADP, AMP and CrP is about 13.5 μmol g^{-1} wet weight. This would be sufficient to provide for the normal consumption of ~P (21 μmol g^{-1} min^{-1}) for only about 39 s. The amounts of endogenous substrates available for generating new ~P are also limited. The concentration of O_2 in brain is about 0.1 μmol g^{-1} [83] of which 90% is in oxy-hemoglobin in brain capillaries. This is enough to support the normal O_2 consumption (about 3.5 μmol g^{-1} min^{-1}) for 2 s. The brain contains about 2.8 μmol g^{-1} of glucose and about 4 μmol g^{-1} of glucosyl units in glycogen. It is evident from the relative amounts of the O_2 reserves and the glucose reserves that less than 0.5% of the glucose could be metabolized oxidatively following a circulatory arrest. If all of the glucose and glucosyl units were subjected to glycolysis, they would yield about 14 μmol g^{-1} of ~P. Thus the maximum amount of ~P available, or potentially available, following circulatory arrest would be about 28 μmol g^{-1}. If the consumption of ~P continued at the normal rate, the brain would be completely depleted of energy and of the capacity for generating energy in about 80 s.

Consumption, of course, does not continue at the normal rate following circulatory arrest; and ATP is still at 40–70% of its normal level 60 s after arrest, though CrP levels are more reduced [204]. ~P consumption would be expected to fall as the concentration of ATP falls relative to the K_{max} values of the various ATPases. The K_m for ATP of the regulatory site on Na^+,K^+-ATPase is near the normal concentration of ATP (e.g., Ref. [208]), so this major energy-consuming enzyme would be expected to be responsive to small reductions in ATP.

However, the situation appears to be more complicated, particularly when CNS cells are subjected to partial ischemia. A number of studies have indicated that energy deprivations causing relatively small reductions in [ATP] cause disproportionately large reductions in energy consumption. Evidence for this was obtained in studies on isolated rat brain synaptosomes [42]. When the synaptosomes' oxidative metabolism was impaired with 1 mM amytal, there was a 19% reduction in [ATP], but a 31% reduction in energy consumption (as assessed by ~P generation over 30 min). Much of the reduction in energy consumption was attributable to a reduction in Na^+ pumping. The 1 mM amytal reduced the ouabain-sensitive energy consumption by 60% and reduced the ouabain-sensitive ^{86}Rb uptake by 36%. However, in spite of this large reduction in Na^+ pumping, the transmembrane electrical potential was calculated to have fallen only from 57 to 54 mV, indicative of little loss of ion gradients. This suggests that the Na^+ influx had also been reduced, accounting (in part at least) for the reduction in energy requirements associated with the reduction in [ATP].

Other evidence has been obtained for a down regulation of energy-demanding functional processes early in the course of an ischemic challenge. In baboons subjected to CNS hypoperfusion, somatosensory evoked responses disappeared promptly when the blood flow was about 2 times higher than that associated with for loss of ion gradients [12]. In rats subjected to cardiac arrest, EEG activity stopped after 13 s, but it was more than 100 s before $[K^+]_e$ showed a marked increase [83]. Microelectrode recordings from rat brain subjected to hypoperfusion in vivo showed

that cortical electrical activity disappeared about 2 min before there was a substantial rise in $[K^+]_e$ [207]. A comparable response was observed during myocardial hypoperfusion, in which down regulation of contractile function led to a partial restoration of the energy balance [80].

A number of mechanisms appear to act in concert to cause relatively large reductions in functional activity in response to an incipient energy imbalance:

1. Most commonly cited, is the hyperpolarizing effect of the opening of ATP-sensitive K^+ channels [15,83,158,214], which reduces exocytosis and prevents voltage-sensitive Na^+ channels from opening.
2. The increase in $[Ca^{2+}]_i$, which regularly accompanies an increase in activity or a decrease in energy supply, also hyperpolarizes neurons by opening K^+ channels [22,27,132]. In rat brain neurons made ischemic in vivo, a small increase in $[Ca^{2+}]_i$ was followed promptly by a loss of electrical activity, and these changes preceded the large losses of ion gradients by about 2 min [207].
3. Increases in $[Ca^{2+}]_i$ have also been shown to increase GABA formation by glutamic acid decarboxylase [60], and this could have an inhibitory effect on cells downstream. However, this was not the case in experiments on rat hippocampal slices in which anoxia *depressed* GABA receptor-mediated IPSCs (inhibitory postsynaptic currents) in one type of interneuron [106].
4. The release of excitatory neurotransmitters is energy-dependent at a number of sites (see above), and one or more of the processes involved appears to be sensitive to small reductions in [ATP]. The release of acetylcholine was reduced in response to a reduction in the level of ATP in the physiological or near-physiological range [247].
5. Adenosine is a potent inhibitor of glutamate-dependent neurotransmission, acting primarily through a presynaptic effect but probably also through a postsynaptic effect (cf. Refs. [89,156,160] and Refs. therein). A reduction in the phosphorylation state of a cell's adenine nucleotides causes an increase in the *formation* of adenosine in response to the increase in [AMP], and it may further increase the *concentration* of adenosine by inhibiting adenosine kinase [46,161]. Adenosine thus released into the ECF would affect both the cell of origin and adjacent cells. However, this would occur at the expense of a depletion of the nucleotide pool in the cell of origin [79].
6. An increase in extracellular lactate (which may occur early in the course of an energy imbalance) has been observed to reduce $[Ca^{2+}]_i$ and exocytosis, and it has been suggested that it was having a direct effect in blocking presynaptic Ca^{2+} uptake [26,243].

Down regulation by any of these mechanism would not only affect the cell directly involved but also the cells downstream, so that its effects would be expected to be cumulative. The protection that can be provided by reducing energy requirements is dramatically exemplified in aquatic turtles which are able to maintain normal ATP levels during anoxia by reductions of up to 10-fold in their energy requirements (see, for example, Refs. [37,89,167]).

In summary: it seems likely that a number of protective mechanisms have evolved against ischemia that serve to divert ~P from function-related processes that are not essential to cell survival to 'vegetative' processes that are. It should be noted that, if the energy imbalance becomes more severe and [ATP] falls to near the k_m of hexokinase for ATP, these protective measures are superseded by a vicious cycle in which further reductions in ATP lead to a reduction in its synthesis [61].

The effects of *severe* energy depletion have been the subject of extensive study because of their clinical importance. Loss of energy can be expected to affect virtually every aspect of the cells' metabolism. The failure of energy-dependent reactions initiates sequences of adverse secondary changes, which have been characterized as 'lethal cascades'. The event that determines the 'point of no return' with respect to viability has been of particular interest, but it probably differs depending on the type of cell and on the intensity of the ischemia. Apoptosis is clearly a lethal process. If, as seems likely, loss of viability may result from any of several lethal cascades that occur in quite rapid succession, corrective measures to interrupt any one of them would extend survival for only a limited period.

6.2. Margin of safety

Do all neurons have a margin of safety? That is, does their energy production always match their energy demands during physiological stimulation? That is clearly not the case for muscle (e.g., Ref. [229]). If the function of neurons is not energy-limited, what is their margin of safety; i.e., how much of a decrease in their capacity to generate energy or increase in energy demands is required before there is an impairment of function? Is an energy limitation sometimes a normal feature of CNS cell function?

These have been difficult questions to address experimentally. Evidence that energy imbalances (i.e., failure of energy generation to meet energy demands) may occur physiologically in brain has been obtained by Sappey-Marinier et al. [191]. In studies on human visual cortex, using ^{31}P NMR spectroscopy, they observed a reduction in the CrP/P_i ratio over 13 min in response to a strobe light flashed every 0.5 s. The electrical evoked potential in response to the strobe also declined over the same time period, suggesting that function was being limited by the energy available.

In spite of their unusual Na^+ pumping capacity (see above), retinal photoreceptors appear to be unable to maintain ion homeostasis in the face of the large cGMP-gated Na^+ influx that occurs in darkness. Measurements by electron probe microanalysis of the changes in intracellular ions during a shift from light to darkness showed an increase in $[Na^+]_i$ from about 19 mM to about 50 mM and a decrease in $[K^+]_i$ from about 138 mM to about 108 mM [212]. This could be due to a limitation imposed by the V_{max} of the Na^+,K^+-ATPase and/or to a limitation imposed by the amount of ~P available to the enzyme. The darkness-induced increase in Na^+ pumping by photoreceptor inner segments draws down the pO_2 in that region nearly to zero [121], so it is likely that the amount of ~P becomes a limiting factor in the maintenance of the ion gradients. Since Na^+,K^+-ATPase is a major energy consumer in most cells, their energy state would be expected to be sensitive to the level of activity of the enzyme if there were no margin of safety with respect to energy production. Evidence for this was obtained by Silver and Erecinska [208], who found that, when the Na^+ pumping of C6 glioma cells was blocked with ouabain, there was a 48% increase in the CrP/Cr ratio.

These findings suggest that features as fundamental to nerve cell function as energy balance and ion gradients can vary in response to physiological changes in Na^+ influx because of the normal limitations on energy generation. [It should be noted that, in interpreting the in vitro studies cited above (on photoreceptors and C6 glioma cells) with respect to what happens in vivo, one must consider whether the energy generating potential of the in vitro preparations is comparable to their energy generating potential in vivo. This may not always be the case (cf. Ref. [62]).]

The cells' capacity to generate ~P is determined by the V_{max} of the energy generating enzymes and by the amount of substrate available to them. A number of studies suggest that the supply of substrate, particularly of O_2, can be limiting even under physiological conditions. The pO_2 in the region of retinal photoreceptor inner segments falls to near zero in darkness [121], and there is evidence that increasing pO_2 above normal levels increases retinal O_2 consumption [4,177,237,238]. Relatively small reductions in arterial pO_2, comparable to those experienced at altitudes of 8000–10 000 feet, have been observed to impair CNS function as assessed in several ways: (1) a shift in the standing potential of the cat eye, attributable to a reduction in the pumping of Na^+ from the photoreceptors [122,215]; (2) a reduction in dark-adapted light sensitivity in humans and impairment of their peripheral vision (cited in Linsenmeier [122]); (3) impairment of short-term memory and complex task performance in humans [21]. Van den Berg [228] concluded that the waking brain operates near its maximal capacity for oxidative metabolism.

Glucose is less likely than O_2 to be limiting under physiological conditions. However, Swanson et al. [220] found increases in glycogenolysis in rat brain in response to sensory stimulation, which suggests that the demand for glucose transiently exceeded the supply.

Clinically significant energy imbalances (i.e., failure of energy generation to meet normal energy requirements) in the CNS are not always due to substrate depletion, but may also occur as a result of an impairment of the energy-generating enzyme systems in mitochondria. It now seems likely [14,234] that a number of the neurodegenerative diseases that appear with more advanced age and that often have a genetic basis may be the result of a chronic energy imbalance due to a mitochondrial enzyme deficiency in selected subgroups of CNS cells. Aging itself may be explained in part on this basis [120,147,149,234]. However, it may be difficult to determine whether the reductions observed in mitochondrial enzymes are primary, or whether they are secondary, and due to reductions in function for other reasons that led to a reduction in energy demands. Wong-Riley et al. [247] and Simonian and Hyman [209] have shown that the level of mitochondrial enzymes varies in response to the demand for energy.

The consequences for the cell of a marginal, but sustained, energy deficiency are not well understood. In relatively acute studies on animals, loss of electrophysiological function has been the first change observed, even without a measurable reduction in CrP [153]. Failure of protein synthesis is the most sensitive of the chemical changes noted [49,146,159] and may be an important factor in determining loss of viability [82]. The energy imbalance has to be considerably more severe before ion gradients are lost.

It has recently been proposed [118] that the difficult-to-meet requirement of maintaining the energy balance of excitable cells may have been an important factor in shaping the evolution of the brain. Because of limitations imposed by the proportion of cardiac output that could be allocated to the brain and by the volume within the brain that could be allocated to capillaries, the mechanisms for signal transfer and the nature of neuronal programming may have evolved with strict requirements for energy efficiency. The fact that the responses of most neurons are phasic, rather than tonic, is consistent with this thesis.

In summary: an imbalance between supply and demand is probably a normal occurrence in some cell types at times of high activity, and it may be a factor in normal information processing by CNS circuits. Energy imbalance may elicit a variety of reactions that reduce functional activity and conserve ATP.

7. Summary

1. The energy metabolism of the brain is proving to be considerably more complex than previously realized. Our understanding is still far from complete, and much

of it is inferential because techniques have yet to be developed for measuring the *rates* of reactions as they occur in situ and for measuring them with spatial resolution at the cellular level and with temporal resolution in seconds.

2. Energy metabolism is organized to link energy generation to energy consumption within the cell. ~P is often generated in close proximity to ATPases. When generation is separated from consumption, creatine kinase and adenylate kinase act to buffer the ATP/ADP ratio at both sites and to facilitate the transfer of the ~P, with creatine serving as a major carrier of ~P. There is evidence that activity-induced increases in Ca^{2+} elicit localized increases in oxidative metabolism. The features that facilitate energy transfer also serve to direct the ~P to particular reactions. This may be important at times of increased activity or reduced energy supply. GTP as an energy carrier also serves to direct ~P to a selected subset of reactions.
3. The ~P used by CNS cells is generated not only by the oxidation of glucose via pyruvate but also by glycolysis (glucose→lactate) and by the oxidation of lactate. These different energy-generating reactions are sometimes localized in different parts of a single cell. Different cell types differ markedly in this regard, and, in some regions, lactate generated in glia serves as substrate for the oxidative metabolism of adjacent neurons. The generation of lactic acid at one site and its removal by oxidation at another requires bicarbonate buffering and may be one reason for the carbonic anhydrase in brain.
4. The great majority of the energy used by CNS cells is for processes that subserve physiological functioning, and relatively little is used for the cells' 'vegetative' metabolism. Energy requirements may increase manyfold with increases in activity, reaching levels in localized regions that are comparable to the energy requirements of active striated muscle.
5. The greatest single demand on energy is for pumping out Na^+ that enters the cell through a variety of gated channels and also in the course of the cotransport of glutamate and the countertransport of Ca^{2+}. The energy cost of the Na^+ influx (as assessed by the activity of Na^+,K^+-ATPase) corresponds to about 50% of the total energy production of the brain.
6. Other important energy demands are related to the processing of neurotransmitters and to the continuing turnover of the components of the many intracellular messenger systems.
7. If the supply of substrate is completely interrupted, the total energy reserves of the brain (including glycogen) are sufficient to maintain normal energy demands for about 80 s.
8. The neurons are probably unable to generate enough energy to meet the requirements of maximal physiological activity. Energy limitation may therefore be a factor in shaping the normal operation of the brain's circuitry.
9. There appear to be substantial reductions in energy consumption when energy supplies are marginally inadequate, which prevent ATP from falling below critical levels. This occurs because some physiological functions are sensitive to small reductions in ATP or to changes in substrate levels that occur during an imbalance (e.g., increases in adenosine and lactate).

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