Review

ATP and Brain Function

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The importance of ATP as the main source of chemical energy in living matter and its involvement in cellular processes has long been recognized. The primary mechanism whereby higher organisms, including humans, generate ATP is through mitochondrial oxidative phosphorylation. For the majority of organs, the main metabolic fuel is glucose, which in the presence of oxygen undergoes complete combustion to CO₂ and H₂O:

\[ C₆H₁₂O₆ + 6O₂ \rightarrow 6O₂ + 6H₂O + \text{energy} \]

The free energy (ΔG) liberated in this exergonic reaction is partially trapped as ATP in two consecutive processes: glycolysis (cytosol) and oxidative phosphorylation (mitochondria). The first produces 2 mol of ATP per mol of glucose, and the second 36 mol of ATP per mol of glucose. In the latter case, 6 mol of ATP are contributed from the oxidation of 2 mol of NADH generated in the cytosol during glycolysis and transferred into the mitochondria indirectly through various "shuttle" systems. (In the α-glycerophosphate shuttle, the yield of ATP per NADH is reduced from 3 to 2 because the relevant mitochondrial dehydrogenase is a flavoprotein-linked enzyme). Thus, oxidative phosphorylation yields 17–18 times as much useful energy in the form of ATP as can be obtained from the same amount of glucose by glycolysis alone. It is therefore not surprising that limitation of O₂ supply produces very damaging effects on cellular function. The brain is one of the organs that is particularly sensitive to lack of oxygen and in humans at rest is responsible for 20% of total O₂ consumption although it accounts for only 2% of the body weight.

The role of energy in the maintenance of central nervous system (CNS) function has been discussed by Siesjö (1978) in his book Brain Energy Metabolism. Although the crucial observations and considerations he presented have largely withstood the test of time, a number of new advances, both technological and scientific, made since the book was published have prompted us to reconsider the topic. This review is not intended as a comprehensive guide through the literature; rather, it is aimed at raising issues that have either been neglected or not yet approached. Questions addressed include the following: First, what are the true, free, concentrations of the high energy phosphate compounds in brain? Second, how is ATP compartmentalized within the brain? Third, what are the roles of individual reactions that provide and maintain cerebral [ATP]? Fourth, what are the relative contributions to ATP synthesis from the various parts of the CNS? Fifth, how is the utilization of ATP distributed among the various endergonic functions of brain? Sixth, what are the relationships between energy level and ionic gradients in neurons and glia of mammalian CNS? Seventh, what other role(s) may ATP play in the function of the CNS? It will become evident from our discussion that only partial answers to these questions are currently available. However, the search for these solutions involves some of the most exciting areas of contemporary neurochemistry.

CONCENTRATIONS OF HIGH ENERGY PHOSPHATE COMPOUNDS IN MAMMALIAN BRAIN

Brain, like all other organs in the body, contains phosphorylated nucleotides that yield energy upon hydrolysis of their phosphate bond(s); the most important of these is the adenine nucleotide ATP. In addition, the CNS, in common with other excitable tissues, possesses another high energy reservoir, the creatine phosphate/creatinine (PCr/Cr) system,
which is linked to the adenine nucleotides through a rapid equilibration in the creatine phosphokinase reaction (see below). It is well established that under physiological conditions ATP is present in cells in much higher concentration than is either ADP or AMP, while the amounts of PCr and Cr are about equal and usually larger than those of ATP. The adenine nucleotides can influence cellular metabolism in one of two ways: as the chemical energy released in ATP hydrolysis (ΔG<sub>ATP</sub>), which is a function of [ATP]/[ADP][Pi], or as substrates or regulators of various processes. In either case, it is the concentrations of free nucleotides that is the relevant parameter. This is important to bear in mind because any binding of nucleotides to cell constituents and/or their sequestration will affect the [ADP]<sub>]<sub> and [AMP]<sub>], much more than [ATP]<sub>], because the total amounts of ADP and AMP are considerably smaller.

Systematic measurements of adenine nucleotides and creatine phosphate and creatine in brain began in the 1960s with the advent of rapid developments in analytical methods (Lowry et al., 1964) and in situ freezing techniques (Pontén et al., 1973). The amount of information generated by various laboratories throughout the world (see Siesjö, 1978 for a review of the earlier literature) has been enormous and no attempt has been made here to analyze it in any detail. However, a sample of typical results has been chosen from the literature and presented as ranges of values in Table 1. It provides a reasonably faithful illustration of the general picture and at the same time will serve as the basis for further consideration; the table also includes references to original publications from which the data were taken.

Inspection of the figures in Table 1 shows that total adenine nucleotides in brain are about 3 μmol/g wet wt whereas total creatine (i.e., PCr + Cr) is 10-11 μmol/g wet wt. The values are not much different from those found in liver (only adenine nucleotides can be compared here because liver does not contain PCr + Cr) but are somewhat lower (within a factor of 2–3) than those in heart and skeletal muscle. Moreover, there appear to be no major differences in the concentrations of these compounds in brain among various species of mammals: mouse (Goldberg et al., 1966; Folbergrová et al., 1970; Duffy et al., 1972; Gorell et al., 1976), rat (Pontén et al., 1973; Ljunggren et al., 1974; Lewis et al., 1974; Norberg and Siesjö, 1976; Veech et al., 1973; Kogure et al., 1980; Pulsinelli and Duffy 1983; Yoshida et al., 1985; Onodera et al., 1986), mongolian gerbil (Kobayashi et al., 1977; Nowak et al., 1985), or dog (Kintner et al., 1984). The [ATP]/[ADP] is, under physiological conditions, about 10 while the [ATP]/[AMP] is about 100. The level of ATP is lower than that of PCr, the [PCr]/[ATP] being 1.5 in gerbil, 1.7–2.0 in rat, and 1.8 in dog. The [PCr]/[Cr] reported in the literature varies from 0.6 in early measurements to 0.9–1.0 in later studies. In the earlier investigations (1964–1973), either the entire small animal, usually a mouse, or its head, was immersed in freon cooled in liquid N<sub>2</sub>. The freezing time is much longer in this technique than during direct, in situ application of liquid N<sub>2</sub> introduced by Pontén et al. (1973) and can result in some hydrolysis of PCr to Cr with consequent lowering of [PCr]/[Cr].

### Table 1. Levels of high energy phosphate compounds and inorganic phosphate in mammalian brain

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Chemical analysis (μmol/g wet wt)</th>
<th>ATP (mM)</th>
</tr>
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<tbody>
<tr>
<td>ATP</td>
<td>2.30–3.10&lt;sup&gt;±m&lt;/sup&gt;</td>
<td>2.24&lt;sup&gt;a&lt;/sup&gt;, 3.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ADP</td>
<td>0.21–0.56&lt;sup&gt;±m&lt;/sup&gt;</td>
<td>0.021&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>AMP</td>
<td>0.01–0.05&lt;sup&gt;±m&lt;/sup&gt;</td>
<td>0.00017&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>PCr</td>
<td>4.00–5.5&lt;sup&gt;±m&lt;/sup&gt;, 6.15&lt;sup&gt;g&lt;/sup&gt;</td>
<td>4.98&lt;sup&gt;e&lt;/sup&gt;, 5.1&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cr</td>
<td>4.80–5.6&lt;sup&gt;±m&lt;/sup&gt;, 7.63&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.02&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pi</td>
<td>2.20–2.8&lt;sup&gt;±m&lt;/sup&gt;</td>
<td>1.63&lt;sup&gt;g&lt;/sup&gt;, 1.73&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>ΣAN</td>
<td>2.80–3.20</td>
<td></td>
</tr>
<tr>
<td>ΣPCr + Cr</td>
<td>9.50–11.5</td>
<td></td>
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Metabolite ratio

<table>
<thead>
<tr>
<th>Metabolite ratio</th>
<th>ATP/PCr</th>
<th>ATP/Pi</th>
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<tbody>
<tr>
<td>PCr/ATP</td>
<td>1.50–2.0</td>
<td>1.50&lt;sup&gt;a&lt;/sup&gt;, 1.54&lt;sup&gt;a&lt;/sup&gt;, 2.12&lt;sup&gt;a&lt;/sup&gt;, 2.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ATP/Pi</td>
<td>1.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.29&lt;sup&gt;b&lt;/sup&gt;, 1.70&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PCr/Pi</td>
<td>1.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.88&lt;sup&gt;d&lt;/sup&gt;, 3.74&lt;sup&gt;d&lt;/sup&gt;</td>
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</table>

Measurements of the high energy phosphate compounds by analytical methods give their total intracellular amounts. However, as mentioned earlier, the values relevant to this discussion are those for the free adenine nucleotides, and, more specifically, for those located in the cytosol where the overwhelming majority of energy-consuming reactions occur. It has been estimated that in liver 70-80% of ATP is cytosolic and that most of it (≥90%) is free (Murphy et al., 1988). Since there is no reason to suspect that the situation is different in brain, total ATP can be used as a reasonable approximation to its free cytosolic concentration. However, the same does not hold true for ADP and AMP, which appear not only to be compartmentalized but also mostly bound to various cellular constituents (Wilson et al., 1982). In principle, there are two approaches to the estimation of free cytosolic ADP concentration. The first makes use of the equilibrium in the cytosolic enzyme creatine phosphokinase (CPK) (Watts, 1973) and [ADP]_{i} is calculated from measured values of PCR, Cr, and ATP and a knowledge of the equilibrium constant, $K_{\text{CPK}}$, for the reaction:

$$[\text{ADP}]_{i} = K_{\text{CPK}} ([\text{ATP}]([\text{H}^{+}])([\text{Cr}]/[\text{PCR}]) \quad (2)$$

The results of such calculations have led to the somewhat unexpected finding (Veech et al., 1979) that [ADP]_{i} in brain is only 10-20 μM, i.e., about an order of magnitude lower than that measured directly on in situ frozen tissue (Table 1). Once the [ADP]_{i} is known, it can then be used to estimate the concentration of free cytosolic AMP, assuming a near equilibrium in the adenylate kinase reaction (AK) (Noda, 1973):

$$[\text{AMP}]_{i} = [\text{ADP}]_{i}^{2}/([\text{ATP}] K_{\text{AK}}) \quad (3)$$

This calculation yields a figure of less than 1 nM for the cytosolic [AMP]_{i}, a value 10- to 100-fold lower than that measured directly (Table 1).

The second approach involves the use of 31P NMR. During the past decade, it was hoped that this would be the technique of choice for determining free concentrations of phosphorylated high energy compounds in a variety of intact tissues including brain (see Prichard and Shulman, 1986 for review). The method has great advantages; it is noninvasive and allows constant monitoring of changes over long periods. However, at present, it suffers from three main drawbacks as an analytical tool. Firstly, it is rather insensitive, so that neither ADP nor AMP are "visible." Although this indicates that free concentrations of both adenine nucleotides are very small, their actual numerical values still have to be derived indirectly as described above for analytical techniques. Secondly, it cannot measure simultaneously all of the relevant components of the high energy stores because creatine does not contain phosphorus. Thirdly, there is no easy and immediate way to convert the areas under the signal peaks to concentrations of the corresponding compounds. Hence, the overwhelming majority of data are presented as ratios (e.g., PCR/ATP, PCR/Pi, and ATP/Pi) and the actual amounts, if given, are calculated by assuming that the total content of ATP and creatine measured by standard analytical methods in independent experiments in the same or other laboratories is directly applicable to the situation in areas observed by 31P NMR. In only one study, that of Nioka et al. (1987), were the ATP and total Cr measured on samples corresponding to the areas examined by NMR. However, despite these drawbacks, the results of such estimates (Table 1) show that there are no significant differences between the values of PCR and ATP observed by 31P NMR and recent measurements by analytical techniques. This means that if creatine phosphokinase and adenylate kinase reactions in brain are in equilibrium, which is a reasonably well supported assumption (see below), measurements of PCR, Cr, and ATP by either chemical analysis or 31P NMR allow reliable estimates of [ADP]_{i} and [AMP]_{i}.

The most neglected parameter that is indispensable for the evaluation of the amount of free energy available in ATP hydrolysis under cellular conditions is the concentration of inorganic phosphate. In only very few analytical investigations has Pi been measured (see, for example, Folbergrová et al., 1970 and Veech et al., 1973 for notable exceptions). On the other hand, 31P NMR studies have provided consistent and numerous data that show that under physiological conditions [Pi] is slightly less than 2 mM. This is somewhat lower than the 2.2-2.4 μmol/g wet wt obtained in the early 1970s from measurement of total cerebral Pi (Folbergrová et al., 1970; Veech et al., 1973).

In summary, this brief survey of the literature shows that in brain the cytosolic [ATP] is about 3 mM, [ADP]_{i} is about 20 μM, and [Pi] is 1.7 mM. The calculated [ATP]/[ADP]_{i} is then 150, [ATP]/[ADP]_{i}[Pi] is 88,235 M^{-1}, and $\Delta G_{\text{ATP}}$ equals 14.7 14.7 kcal/mol^{-1} at 37°C ($\Delta G_{\text{ATP}} = \Delta G_{\text{ATP}}^{0} + RT \ln ([\text{ATP}]/[\text{ADP}]_{i}[\Pi]$), where $\Delta G_{\text{ATP}}^{0} = 7.7$ kcal/mol at pH 7.2 and a free intracellular magnesium of 1 mM).

**IS ATP COMPARTMENTALIZED WITHIN THE BRAIN?**

In previous considerations of compartmentalization, it has been assumed that all components of the
high energy system are homogeneously distributed throughout the brain. This assumption may or may not be true. The CNS consists of at least two populations of cells (neurons and glia) and, moreover, within each population, environment typical of one part (e.g., cell body) may not necessarily be the same as that of the others (e.g., processes). In addition, various parts of the brain perform different functions and may differ in composition. Not surprisingly, a number of attempts have been made to compare the levels of ATP, ADP, AMP, PCr, and Cr in the gray and white matter as well as at different anatomical locations. However, no significant differences have been found (Gorell et al., 1976; Folbergrová et al., 1970; Pontén et al., 1973; Kogure et al., 1980; Pulsinelli and Duffy, 1983; Yoshida et al., 1985). In some instances, above average levels of PCr (or PCr and Cr) have been reported either in certain regions (e.g., cerebellum) (Ferrendelli and Chang, 1973; Agardh et al., 1981) or within particular areas of a given structure such as CA1 area (Pulsinelli and Duffy, 1983) or the molecular layer of the hippocampus (Lipton and Whittingham, 1982). There is no information in the literature about possible differences in energetic profiles of neurons and glia. However, the presence of CPK in both types of cells (Yoshimine et al., 1983) would suggest that neither is deficient in the additional high energy reservoir. The presence of PCr and Cr in the glia-derived C6 glioma cell line, and the high [ATP]/[ADP][Pi] in these cells (Erecińska and Silver, 1986), are consistent with the postulate that glia, like neurons, maintain a high energy level. Hence, it can be concluded that at the current level of resolution there seem to be no major differences in either ΔG or the concentrations of high energy phosphate compounds among the major components of the mammalian CNS. This, however, should not be taken to mean that there are no differences among the rates of ATP turnover, since there is no simple a priori relation between the level of ATP and the rate of its synthesis. For instance, in the electric organs of Torpedo and Electrophorus, resting [ATP] is only about 1 mM (Borrini, 1984; Chmoulovsky et al., 1974; Williamson et al., 1967) but metabolic activity can be increased severalfold in a very short period of time. On the other hand, PCr is >20 mM, which is consistent with the generally accepted idea that CPK and PCr are present in organs that have to respond rapidly to repetitive stimuli and mobilize their energy reserves quickly. This also means that more PCr and Cr and a larger [PCr]/[ATP] ratio may be typical of regions and/or cells endowed with high intrinsic activity. The greater concentrations of PCr reported in the molecular layer of hippocampus (Lipton and Whittingham, 1982) and cerebellum (Maker et al., 1973), regions that contain large numbers of synaptic contacts, are consistent with this view.

**WHAT ARE THE ROLES OF INDIVIDUAL REACTIONS THAT PROVIDE AND MAINTAIN CEREBRAL ATP?**

Under steady-state conditions, the rate of energy consumption by endergonic reactions is exactly balanced by the rate of ATP synthesis; consequently, the concentration of ATP within the cell is maintained constant. The rate of glucose utilization by brains of various mammals is between 0.3 and 0.8 μmol/g wet wt/min (Table 2) and two rules apply: (a) higher rates are observed in smaller animals; (b)

**TABLE 2. Rates of energy production by mammalian brain under normal (rest), stimulated (seizures), and inhibited (deep anesthesia) conditions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
<th>Rest (μmol/min/g wet wt), 100%</th>
<th>Seizures, % of rest</th>
<th>Anesthesia, % of rest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose utilization</td>
<td></td>
<td>0.25–0.8&lt;sup&gt;a&lt;/sup&gt;–&lt;sup&gt;e&lt;/sup&gt;</td>
<td>200–400&lt;sup&gt;b&lt;/sup&gt;–&lt;sup&gt;i&lt;/sup&gt;</td>
<td>50–60&lt;sup&gt;n&lt;/sup&gt;–&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactate production</td>
<td></td>
<td>0.002–0.10&lt;sup&gt;d&lt;/sup&gt;–&lt;sup&gt;c&lt;/sup&gt;</td>
<td>400–1000&lt;sup&gt;f&lt;/sup&gt;</td>
<td>50&lt;sup&gt;n&lt;/sup&gt;–&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>O&lt;sub&gt;2&lt;/sub&gt; consumption</td>
<td></td>
<td>1.6–5.4&lt;sup&gt;e&lt;/sup&gt;–&lt;sup&gt;d&lt;/sup&gt;</td>
<td>200–300&lt;sup&gt;h&lt;/sup&gt;–&lt;sup&gt;j&lt;/sup&gt;</td>
<td>50–60&lt;sup&gt;n&lt;/sup&gt;</td>
</tr>
<tr>
<td>ATP production</td>
<td>Oxidative phosphorylation</td>
<td>9.6–32.4</td>
<td>200–400</td>
<td>50–60</td>
</tr>
<tr>
<td></td>
<td>Glycolysis</td>
<td>0.002–0.10</td>
<td>400–1000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ATP utilization</td>
<td>20–25&lt;sup&gt;k&lt;/sup&gt;</td>
<td></td>
<td>50&lt;sup&gt;q&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> ATP production = oxidative phosphorylation (O<sub>2</sub> uptake × 6) + glycolysis (lactate production × 1).
<sup>b</sup> ATP utilization = ΔCrP + 2ΔATP + Δlactate.
<sup>c</sup> Baláž (1970), <sup*d</sup>Borgstrom et al. (1976), <sup>e</sup>Hawkins et al. (1974), <sup*f</sup>Kintner et al. (1983), <sup*g</sup>Sokoloff et al. (1977), <sup*h</sup>Gjedde et al. (1975), <sup>i</sup>Lowry et al. (1964), <sup>j</sup>Brunner et al. (1971), <sup>k</sup>Blennow et al. (1979), <sup>l</sup>Duffy et al. (1975), <sup>m</sup>Chapman et al. (1977), <sup>n</sup>King et al. (1967), <sup>o</sup>Meldrum and Nilsson (1976), <sup*p</sup>Astrup et al. (1981), <sup>q</sup>Michenfelder (1974).
the white matter consumes less glucose than the gray matter. O$_2$ consumption varies from 1.6 to 5.0 μmol/g wet wt/min and faithfully reflects the rate of glucose utilization. Because combustion of 1 mol of glucose requires 6 mol of O$_2$, the close correspondence between these two sets of numbers (allowing for a factor of 6) indicates that more than 95% of the carbohydrate is utilized by, and more than 95% of ATP is derived from, mitochondrial oxidative phosphorylation. This conclusion is consistent with measurements that show that lactate production by the CNS under aerobic conditions accounts for 0–4% only (Kintner et al., 1984; Hawkins et al., 1974, Siesjö, 1978) of glucose metabolized. Since consumption of 1 mol of O$_2$ yields approximately 6 mol of ATP while generation of 1 mol of lactate produces 1 mol of ATP, it can be calculated (Table 2) that brain generates 25–32 μmol of ATP/g/min under nonstressed conditions, of which 1% or less is provided by glycolysis. Comparison of these figures with those from other organs is interesting. Isolated perfused rat heart, under a low work load, consumes 6–9 μmol O$_2$/g wet wt/min and derives about 3% of ATP from glycolysis (Nishiki et al., 1979). In the kidney, O$_2$ uptake is 3–6 μmol/g wet wt/min (Soltoff, 1986) while lactate production does not account for more than 5% of energy generated (Maxild, 1973). By contrast, vascular smooth muscle utilizes little O$_2$ (about 0.10 μmol/g wet wt/min) and derives about 30% of total ATP production from glycolysis (Paul, 1983).

In addition to glycolysis and oxidative phosphorylation, which are net energy producing processes, brain has two other mechanisms that help maintain a constant level of ATP. These are the creatine phosphokinase (Watts, 1973) and adenylate kinase (Noda, 1973) reactions. Creatine phosphokinase catalyzes a reversible transfer of phosphate between phosphocreatine and ATP:

$$\text{PCR} + \text{ADP} + H^+ \rightleftharpoons \text{ATP} + \text{Cr}$$

(4)

The apparent equilibrium constant for this reaction at 1 mM free Mg$^{2+}$ is $1.66 \times 10^9$ (Lawson and Veech, 1979). The large value of $K_{eq}$ for the reaction as written means that even at physiological pH 7.0–7.4, CPK favors formation of ATP and when the enzyme is sufficiently active and maintains near equilibrium, any ADP formed will be immediately rephosphorylated by PCR. Moreover, for near equilibrium to be sustained as total ATP and PCR fall, the absolute decrease in PCR must be larger than that in ATP because [PCR] and [Cr] are both significantly higher than [ATP] and [ADP]. Only after the PCR energy reserve has decreased to a low level do ADP and AMP concentrations rise appreciably.

This prediction is well supported by an abundance of observations in the literature that show that a fall in [PCR] precedes that in [ATP] under conditions of increased ATP utilization such as seizures (King et al., 1967; Duffy et al., 1975; Chapman et al., 1977; Astrup et al., 1979; Blennow et al., 1979) or during limitation in ATP synthesis in ischemia (Lowry et al., 1964; Ljuggren et al., 1974; Kobayashi et al., 1977; Kogure et al., 1980), anoxia (Duffy et al., 1972; Bachelard et al., 1974; Kass and Lipton, 1982; Kintner et al., 1983), and hypoglycemia (Norberg and Siesjö, 1976; Tews et al., 1965; Vannucci and Vannucci, 1978; Ratcheson et al., 1981). However, when the rate of energy depletion is very fast, the techniques that measure the levels of the high-energy phosphate compounds may not be sufficiently discriminatory to separate changes in [ATP] and [PCR]. In such situations, the falls in concentrations of these compounds may appear to occur almost simultaneously. It is also worth pointing out that another consequence of the relations between the adenine nucleotides and the PCR/Cr system is that transport of high energy phosphate occurs predominantly in the form of PCR (see Meyer et al., 1984).

Creatine phosphokinase exists in multiple forms: BB (or CK1, brain type), MM (or CK2, muscle type), MB (a hybrid), and the mitochondrial form (CK4) (Watts, 1973). During development, the brain-type isoenzyme appears first and, in the CNS, remains the predominant form throughout life. Early studies by Oguro et al. (1977) indicated that in adult rat brain the BB form accounts for 98% of total CPK with the 2% balance being made up by the MM form. Later studies by Norwood et al. (1983) suggest that the picture may be more complex. The BB form was found to contribute 64%, the MM form 21%, and the mitochondrial isoenzyme 15%. The total activity of the enzyme, in IU/g wet wt, has been reported as 207 (Booth and Clark, 1978, 25°C), 384 (Norwood et al., 1983, temperature unspecified), and 1000 (Oguro et al., 1977, 37°C), values that appear to be high enough to ensure near equilibrium in the CPK reaction. This suggestion is supported by recent studies with $^{31}$P NMR, which evaluated the flux between PCR and ATP directly and found it to be 1–2 μmol/g wet wt/sec in rat brain in vivo (Balaban et al., 1983; Shoubridge et al., 1982).

It has been reported that synaptosomal membranes contain an isoenzyme that is electrophoretically similar to muscle type CPK (Friedhof and Lerner, 1977; Lim et al., 1983). The same plasma membrane fraction was found to be enriched in enolase and pyruvate kinase (Lim et al., 1983). Thus, it
was postulated that ATP could be supplied locally to such processes as membrane-dependent ion transport and/or membrane protein phosphorylation. An earlier observation of Wood and Swanson (1964) that the microsomal fraction of brain can release Cr from PCr in the absence of ATP (but in the presence of small amounts of ADP) in an Na⁺-sensitive manner supports this suggestion.

The activity of CPK in neonatal brain is three- to sixfold less (Dawson and Fine, 1967; Norwood et al., 1983) than in the adult and may be too low to maintain near equilibrium. If this is so, then the PCr/Cr system would be unable to buffer the decline in ATP when its generation is impaired or utilization enhanced. In accord with this postulate, ³¹P NMR studies on neonatal brain showed that, in ischemia, ATP and PCr concentrations decreased at nearly the same rate (Norwood et al., 1983).

Creatine phosphokinase is distributed widely in the CNS between both neurons and glia (Yoshimine et al., 1983). Although earlier studies (Dawson and Fine, 1967) showed no large differences in the level of this enzyme among various regions of brain, more recent evidence (Maker et al., 1973) indicates that CPK activity may be increased in areas rich in synaptic contacts, such as the molecular layer of the cerebellum. Moreover, it has been observed that a rise in CPK activity parallels the neonatal increase in the Na⁺/K⁺-ATPase in a fraction of brain containing nerve endings (Gaballah and Popoff, 1971). This suggests that higher levels of PCr and CPK are characteristic of regions in which energy expenditure for processes such as ion pumping may be large.

An important and seldom recognized consequence of the operation of the CPK reaction is a rise in the concentration of intracellular inorganic phosphate that occurs before appreciable changes in the levels of ATP and ADP. This aspect of the reaction may play an important regulatory function (Davuluri et al., 1981) because Pi can enhance the rate of cellular glucose metabolism by activating both glycogenolysis, through stimulation of phosphorylase (Morgan and Parmeggiani, 1964) and glycolysis, by stimulation of phosphofructokinase (Passoneau and Lowry, 1962), and also by relieving inhibition of hexokinase by glucose-6-phosphate (Rose et al., 1964).

Equation (4) shows [H⁺] to be one of the components in the CPK reaction. When PCr is hydrolyzed, a proton is taken up in a stoichiometry of 1:1 in the physiological pH range (Lawson and Veech, 1979). This means that hydrolysis of 5–6 mmol of cytosolic PCr would consume an equivalent amount of H⁺ and, with the cerebral buffering power of 15–20 mmol/pH unit (Moody, 1984), this could cause an internal alkalinization of up to 0.3 pH unit. Danforth (1965) showed that alkalinization promotes conversion of phosphorylase b to a; in addition, it activates phosphofructokinase (Trivedi and Danforth, 1966). Thus, it is possible that stimulation of lactate production, which seems to be the earliest response of CNS to increased activity (electrical stimulation, seizures), occurs at least in part through pH modulation of carbohydrate breakdown. It should be added, however, that in most situations hydrolysis of PCr occurs when energy synthesis is limited. Thus, the initial alkalinization associated with H⁺ consumption in the CPK reaction is followed by rapid acidification caused by proton release during ATP breakdown.

Since H⁺ is a reactant in the CPK reaction, alterations in intracellular pH must affect the concentrations of other components: as H⁺ rises, the sum of ADP and PCr decreases while that of ATP and Cr increases. At alkaline pH values, opposite changes occur. Attempts have been made, therefore, to use the reaction to determine the cytosolic pH (Lewis et al., 1974; Yoshida et al., 1985; Kass and Lipton, 1982). Such calculations would be valid if the [ATP] and [ADP] measured corresponded to the free concentrations of these nucleotides. Since, as discussed above, ADP is mostly bound to intracellular constituents, cytosolic [H⁺] cannot be determined reliably from calculations based on total ADP. However, if [ADP]自由贸易 could be estimated independently, as for example from the combined equilibria in glyceraldehyde-3-phosphate dehydrogenase and glyceral kinase reactions (Veech et al., 1979; Connett, 1987), the method would provide a viable alternative to those currently in use. The finding that calculations of [ADP]自由贸易 by the above procedure give results identical to those derived from the near equilibrium in the CPK reaction (Veech et al., 1979) tends to validate the former approach and augurs well for its success.

An important side issue is the relation between H⁺ and energy metabolism. There are at least three aspects that have to be considered. Firstly, protons are turned over continuously during energy-yielding and -consuming reactions; hence, the balance between the two will determine H⁺ concentration and, consequently, pH自由贸易. Secondly, they are participants in some of the relevant reactions; thus, their concentration may affect the energetic state of the cell. Thirdly, some of the rate-controlling enzymes of ATP-producing pathways are dependent on pH; thus, the velocity at which they operate may be influenced by [H⁺].

The first issue has been cogently addressed in
reviews by Krebs et al. (1975) and Hochachka and Mommsen (1983), both of which provided detailed analyses. However, a few salient points should be made here because they constitute an integral component of the overall picture of regulation of cellular energy metabolism. Living cells continuously produce enormous quantities of H⁺ (150 g/day per human) (Krebs et al., 1975), the most frequent single contributor being the hydrolysis of ATP. The respiratory chain is also a major source of protons (during reduction of flavin and nicotinamide coenzymes, and cytochromes) as is CO₂ production and incomplete oxidation of fuels (Alberti and Cuthbert, 1982). The main sink for this metabolite is oxidative phosphorylation since H⁺ ions are removed when ATP is synthesized and reduced coenzymes and cytochromes are reoxidized. Because under normal aerobic conditions there is a balance between ATP synthesis and breakdown, intracellular [H⁺] remains virtually constant. On the other hand, when the rates of ATP synthesis and utilization become uncoupled, aerobic energy metabolism can produce either an increase or a decrease in pH; when the rate of breakdown exceeds that of synthesis and [ATP] falls, intracellular acidification occurs, while the opposite changes lead to alkalization.

It is a common belief that glycolysis is an important source of protons. Careful inspection of the reactions involved shows, however, that the end product of glycolysis is lactate (not lactic acid) and that the process is not generating H⁺ unless accompanied by hydrolysis of ATP (Krebs et al., 1975). However, even if glycolysis is stimulated in the presence of O₂, rapid hydrolysis and resynthesis of ATP and further metabolism of lactate maintain net production of H⁺ at a very low value, and consequently do not cause major changes in pH. This behavior is not true under anaerobic conditions when the rate of ATP breakdown (and H⁺ production) exceeds that of ATP synthesis and large quantities of protons accumulate. Detailed quantitative analysis of such a situation (Hochachka and Mommsen, 1983) shows that under intracellular conditions (i.e., free [Mg²⁺] 1 mM and pH 6.5–7.8) the total number of moles of H⁺ generated is always 2 per mole of glucose because of the opposite pH dependencies of H⁺ production by glycolysis and by ATP hydrolysis.

The amount of information available on the proton dependence of cellular energy metabolism is extremely small, although such knowledge is crucial if predictions are to be made as to whether deviations from the narrow range of pH at which most cells operate alter the free energy for ATP hydrolysis. In the only systematic study of which we are aware, Kashiwamura et al. (1984) found that intracellular [ATP]/[ADP] declined in isolated liver cells when pH was raised from 6.5 to 8.0, whereas there was little change in Pi. This behavior suggests that the amount of available energy declines at alkaline pH. It should be remembered, however, that there are two terms that contribute to the free energy of hydrolysis of ATP (∆G_ATP). These are ∆G⁰_ATP, the standard free energy change at 1M concentrations at a given pH, and the concentration-dependent term, RT ln[ATP]/[ADP][Pi]. The former is pH-dependent and its value becomes more negative (i.e., it increases) as pH rises from 6.5 to 8.0. The decrease in RT ln[ATP]/[ADP][Pi] found by Kashiwamura et al. (1984) was a mirror image of the increase in ∆G_ATP; consequently, ∆G_ATP remained essentially constant at physiological pH values.

The situation may be somewhat more complex in brain because, as [H⁺] rises, one would expect a decrease in [PCr]/[Cr] with a consequent rise in Pi. This has indeed been demonstrated recently by Njoka et al. (1987), who showed that during severe hypercapnia (pH of 6.85), intracellular inorganic phosphate concentration increased from 1.73 mM in control (pH 7.3) to 3.65 mM in dogs breathing high PCO₂. Since Pi is an independent variable in the RT ln[ATP]/[ADP][Pi] (see above), this rise in its concentrations could result in a decline in ∆G_ATP. However, more detailed work is required to evaluate these relationships accurately.

The final issue of interest concerns the pH dependence of regulatory enzymes in the energy-producing pathway. The most important of those in brain is phosphofructokinase, which controls carbohydrate breakdown during glycolysis. This enzyme is very sensitive to changes in [H⁺] and its activity decreases when pH falls (Trivedi and Danforth, 1966). Hence, if all other factors regulating phosphofructokinase remain constant, glycolysis should be seriously curtailed when [H⁺] rises. However, experimental evidence of large increases in lactate production during ischemia (see Siesjö, 1978 for review) does not support this suggestion and indicates that other stimulators of enzyme activity must overcome the inhibitory effect of increased [H⁺]. ADP, AMP, and Pi are the likely candidates (Ueda, 1979 for review). Furthermore, even if pH falls without any change in ATP, ADP, and AMP, a rise in inorganic phosphate generated through the alteration in CPK near equilibrium (see above) will stimulate phosphofructokinase, while a simultaneous fall in PCR would de inhibit the enzyme (Krzanowski and Matschinsky, 1969).

The second reaction that is believed to facilitate the storage and use of the high energy of the ade-
nine nucleotides is that catalyzed by adenylate kinase (Noda, 1973). In systems that utilize ATP with formation of ADP, this enzyme effectively makes available the energy residing in the β-phosphate group of the original ATP molecule as shown in the following equation:

\[ 2\text{ADP} \rightleftharpoons \text{ATP} + \text{AMP} \]  

(5)

The equilibrium constant for this reaction at 1 mM free Mg\(^{2+}\) is 1.05 (Lawson and Veech, 1979), which means that the process is readily reversible. Adult mammalian brain contains relatively large amounts of the enzyme, which is predominantly of type II (Pradhan and Criss, 1976). The operation of adenylate kinase is most clearly observed under conditions of rapid ATP breakdown, as for example in ischemia, where the increase in the level of AMP is much larger than that in ADP (see, for example, Ljunggren et al., 1974; Kobayashi et al., 1977; Kintner et al., 1984).

WHAT ARE THE RELATIVE CONTRIBUTIONS TO ATP SYNTHESIS FROM THE VARIOUS PARTS OF THE CNS?

It was stated earlier that in brain, as in any steady-state system, the metabolic rate (i.e., the rate of energy synthesis) is closely correlated with physiological function (i.e., the rate of energy utilization). Since ATP is required for a number of vital cellular processes, one would expect that the more “active” a cell, the more energy it will consume. Thus, the brain with its complex and diversified activities is likely to be composed of different groups of cells with varied functional demands that may change when the degree of physiological activity changes. From our foregoing analysis of brain energy metabolism, these considerations lead to two predictions. The first is that since glucose is the major source of energy for the CNS, the functional activities of various regions of brain should be reflected in the rates of regional glucose utilization. The second is that because oxidative phosphorylation provides over 95% of total cerebral ATP, density of mitochondria and/or the content and activities of their energy synthesizing enzymes should faithfully reflect the normal level of metabolic demand of a cell or its constituent parts. With respect to the first, autoradiographic techniques using radiolabeled glucose or 2-deoxyglucose have been developed (Sokoloff et al., 1977) to determine regional rates of carbohydrate metabolism. The validity of this approach as a quantitative measure of glucose utilization has been challenged on the grounds that the technique fails to recognize the substantial rate

of dephosphorylation of glucose-6-phosphate and consequently reflects only the velocity of the hexokinase reaction in situ (Huang and Veech, 1985). Nevertheless, analysis of regional distribution of radioactive 2-deoxyglucose has provided a wealth of information that, in general, is consistent with the results obtained by other techniques (see below).

It has been shown in the pioneering studies of Sokoloff and co-workers (1977) that in the conscious rat the uptake of 2-deoxyglucose in gray matter varies greatly from structure to structure (0.54–2 μmol/g/min), with the highest figure in areas related to auditory function. The values in white matter are much more uniform and 25–50% less than those in the gray matter. Thiopental anesthesia depresses the rate of glucose utilization throughout the brain but particularly in the gray matter. Similar results have been obtained by other authors and in various animals (e.g., Humphrey and Hendrickson, 1983; Kennedy et al., 1976; Livingstone and Hubel, 1982; Schwartz and Sharp, 1978). Furthermore, experimentally induced decreases or increases in the level of neuronal activity within specific sensory pathways were found to be accompanied by adjustments in the rate of 2-deoxyglucose uptake (Sokoloff, 1977; Shinohara et al., 1979). It has also been shown that regions rich in synaptic contacts such as the molecular layer of the hippocampus exhibit intense labeling (Meibach et al., 1981) and that during stimulation of nerves, axon terminals rather than the neuronal cell bodies are the sites of enhanced metabolic activity (Kadecaro et al., 1985). All of these results seem to indicate that regions of the CNS that are functionally more active consume more glucose and hence produce ATP more rapidly than the “dormant” areas with small metabolic demands.

The second prediction is based on the assumption that a correlation exists between functional demand and the capacity for oxidative metabolism. During short-term alterations in cellular work, when oxygen is abundant, the rate of ATP synthesis is controlled by two variables: the phosphorylation state ratio, [ATP]/[ADP][Pi] (Erecińska and Wilson, 1982; but see Brand and Murphy, 1987 for alternative views on regulation by the [ATP]/[ADP] or [ADP]) and the substrate input (redox state of the mitochondrial pyridine nucleotides) which is related to the activities of various rate-controlling dehydrogenases (for details of the mechanism(s) of regulation see Erecińska and Wilson, 1982, Brand and Murphy, 1987). However, during prolonged periods of increased energy usage, such as high levels of mechanical activity or enhanced rates of ion pumping, the cell responds by increasing either the number of mitochondria or the quantity of the respira-
tory chain enzymes or both (Erecińska and Wilson, 1982). It would not be unreasonable to suggest that in the CNS neurons perform more energy-consuming functions than do glia. This distal support is provided by the finding (Pysh and Khan, 1972), that although these two types of cells have about the same number of mitochondria, the crystal volume fraction of these organelles (i.e., crystal packing density) is considerably higher in neurons (17.3%) than in either astrocytes (11%) or oligodendrocytes (11.3%). Furthermore, whereas most of the glycolytic activity appears to be localized within neuronal perikarya and proximal dendrites (Friede, 1966; Kao-Jen and Wilson, 1980), the distal segments of dendrites often contain numerous mitochondria and the number of mitochondrial profiles per unit of cross-sectional area tends to be greatest in the smaller dendritic branches and axon terminals (Nafstad and Blackstad, 1966).

Yet another means of evaluating oxidative metabolism is to determine activities of enzymes, such as cytochrome c oxidase, that are crucial for this process in mitochondria. Systematic light and electron microscopic studies of Wong-Riley and co-workers (Wong-Riley, 1976, 1979; Kageyama and Wong-Riley, 1982, 1986) have shown that functionally active areas in the normal brain, which have elevated levels of glucose utilization (Sokoloff et al., 1977; Schwartz and Sharp, 1978), also exhibit enhanced activity of cytochrome c oxidase. (It should be noted that the method used to determine cytochrome c oxidase activity does not permit a distinction between an increase in the activity of the enzyme and an increase in the amount of enzyme with unaltered activity. Whatever the mechanism, the final outcome is, however, the same: a higher capacity for substrate oxidation and hence energy production.) Furthermore, experimentally induced decreases or increases in neuronal activity were found to be accompanied by long-term (over some days) adjustments in the levels of cytochrome oxidase activity. Such behavior supports the suggestion that changes in enzyme synthesis are involved in responses to chronically altered neuronal activity. The interesting fact that the pattern of cytochrome oxidase localization is consistent from species to species (Wong-Riley, 1976, 1979) indicates that the metabolic organization of structures with high functional activity is a very constant feature of mammalian brain.

The distribution of cytochrome c oxidase in several regions of the brain (e.g., cerebral cortex, hippocampus) has been analyzed in considerable detail. The results indicate that the oxidative metabolic activity may vary between (a) different laminae, (b) neurons and glia, (c) different neuronal types, (d) dendrites and soma of the same cell, (e) different types of dendrites, (f) different segments of the same dendrite, (g) different classes of axon terminals. However, a consistent pattern has emerged from these findings that can be summarized briefly in three points (a) in general, neurons have greater oxidative metabolic activity than glia (or endothelial cells), (b) many of the highly reactive neurons are ones that receive a strong excitatory input, and (c) high cytochrome c oxidase activity is seen in dendrites and synaptic terminals where the rates of ion transport would be expected to be high. The studies reviewed here of the distribution of oxidative activity in brain amply support the assumption that mammalian CNS is a heterogeneous structure in which the rates of energy metabolism are nonuniformly apportioned among various regions, cell types, and even different parts of the same cell.

HOW IS THE UTILIZATION OF ATP DISTRIBUTED AMONG THE VARIOUS ENERGONIC REACTIONS AND HOW ARE THEIR DEMANDS MET AND REGULATED?

In view of the correlation between the demand for energy and the rate of its production, it is important to identify the predominant reactions that consume ATP because it is their activities that will be largely responsible for the pattern of metabolic responses under physiological and pathological conditions.

The main function of the CNS (generation, processing, and transmission of impulses), depends on neuronal activity. Action potentials can be generated and synapses can transmit signals only if Na\(^+\), K\(^+\), and Ca\(^{2+}\) are maintained in electrochemical disequilibrium across the neuronal plasma membrane. This requires a constant input of ATP and one might expect that a substantial proportion of total energy produced in brain is utilized for this purpose. In addition, a variety of other cellular activities are supported by oxidative metabolism. Key processes include biosynthetic reactions such as synthesis of proteins and lipids; the phosphorylation reaction; fast axonal and dendritic transport; neurotransmitter synthesis, packaging, and transport; and active transport of substances across the plasma membrane and the maintenance of structural integrity of the cell.

The rate of energy (ATP) utilization can be estimated in vivo in three ways: from the rate of glucose consumption (\(\times 38\) ATP), from the rate of \(O_2\) usage (\(\times 6\) ATP; under aerobic conditions, glycolytic en-
ergy synthesis is negligible), and from the rate of depletion of the major energy reserves ($\Delta$PCr + 2ATP + $\Delta$lactate) during the first few seconds after depletion. All three methods give comparable results and yield a value of 22–32 \( \mu \text{mol/g} \) wet wt/min ATP utilized under normal conditions (Table 2). To determine the amount of energy normally expended in ion movements, ATP synthesis must be measured under conditions when electrical and synaptic activity in the brain are blocked. This has been done either by administration of thiopental to halothane-anesthetized dogs (Michenfelder, 1974) or by infusion of lidocaine and ouabain to pentobarbital-anesthetized animals (Astrup et al., 1981). Both of these measurements gave consistent results and showed that glucose and O\(_2\) consumption decreased by 50–60% from that in conscious animals; pentobarbital, halothane, or nitrous oxide anesthesia alone inhibit energy metabolism by 20–30%. This value agrees well with the reduction in oxygen consumption in slices of brain treated with ouabain or incubated in Na\(^+\)-free solutions (Whittam, 1962), and with declines in oxidative activity of nonmyelinated nerves treated with ouabain (Ritchie, 1967).

Such a large reduction in cerebral O\(_2\) uptake when ion movements are blocked indicates that these processes consume the major portion of cerebral ATP production. Only kidney expends a comparable amount of energy in preserving its ionic balance (Soltloff, 1986); other tissues, including heart (Gibbs, 1982), use considerably less (5–10%).

The major ions whose movements consume ATP are Na\(^+\), K\(^+\), and Ca\(^2+\). The main enzyme responsible for the maintenance of proper gradients of the first two ions is the Na\(^+\)/K\(^+\)-ATPase, which moves 3 Na\(^+\) from inside to outside against 2 K\(^+\) in the opposite direction, with a concomitant hydrolysis of 1 ATP (for review, see Schuurmans-Stekhoven and Bonting, 1981). Brain appears to contain two molecular forms of the enzyme, of which one may be present primarily in neurons (Sweedner, 1979). The \( K_m \) of the ATPase for Na\(^+\) is about 80 \( \mu \text{M} \) (Logan, 1980). For K\(^+\), it is about 2–3 \( \mu \text{M} \) (Logan, 1980) although it may be higher for the glial enzyme, which seems to saturate at 18–20 \( \mu \text{M} \) K\(^+\) (Grisar et al., 1979), and for ATP it is approximately 0.5 \( \mu \text{M} \) (Logan, 1980; Robinson, 1976). Thus, under physiological conditions, the activity of the neuronal enzyme is controlled predominantly by the concentration of intracellular Na\(^+\). A crude homogenate of the gray matter of beef brain was reported to have activity that corresponds to 50 \( \mu \text{mol ATP split/g wet wt/min} \), which is equivalent to 6250 pmol of the Na\(^+\)/K\(^+\) pump/g of tissue (Hansen and Clausen, 1988). By comparison, the density of the pump in heart muscle is 1500–2000 pmol/g and in skeletal muscle 300–800 pmol/g (Hansen and Clausen, 1988). Biochemical studies have suggested that the highest activity of the Na\(^+\)/K\(^+\) ATPase is present in glia-enriched and synaptosomal fractions (Grisar et al., 1979; Atterwill et al., 1984). On the other hand, histochemical and immunocytochemical methods seem to indicate that the Na\(^+\)/K\(^+\)-ATPase is confined predominantly to the plasma membrane of neuronal cell bodies and their processes, the reactivity of glial membranes being much smaller (Stahl and Broderson, 1976; Inomata et al., 1983; Pech and Stahl, 1984). The most intense staining was observed over the plasma membrane of the synaptic area, which agrees with the high rates of Na\(^+\)/K\(^+\)-stimulated ATP hydrolysis reported for isolated synaptosomes (Grisar et al., 1979; Atterwill et al., 1984; Abdel-Latif et al., 1970).

It is worth pointing out that these latter results correlate with studies on the distribution of cytochrome c oxidase activity (see above) and provide evidence for the postulate that high rates of ion transport and ATPase activity require a large supply of energy.

Another ion whose unequal distribution across the plasma membrane requires ATP is Ca\(^2+\). There are at least four mechanisms, all dependent directly or indirectly on energy, that maintain the low internal calcium concentration: the plasma membrane Ca\(^2+\) pump; the endoplasmic reticulum Ca\(^2+\) pump; Ca\(^2+\) uptake by mitochondria, which is driven by membrane potential and so linked to ATP indirectly; and the Na\(^+\)/Ca\(^2+\) exchange fueled by the Na\(^+\) gradient, which is maintained by ATP indirectly through the operation of the Na\(^+\)/K\(^+\)-ATPase (for review, see Carafoli, 1987). The system most relevant to our discussion, being linked directly to ATP splitting, is the plasma membrane Ca\(^2+\) pump responsible for net extrusion of calcium. The enzyme, which have been shown by numerous investigators (Gill et al., 1981, 1984; Sorenson and Mahler, 1981; Javors et al., 1981; Lin and Way, 1982; Michaelis et al., 1983) to be concentrated in the synaptic membranes, is activated by calmodulin (Kuo et al., 1979; Hakim et al., 1982). In the reconstituted liposomal system, the stoichiometry between transported calcium and hydrolyzed ATP approaches 1 and the enzyme functions as an obligatory Ca\(^2+\)/H\(^+\) exchanger whose probable stoichiometry is 1 to 2 (Carafoli, 1987). The \( K_m \) of this pump for Ca\(^2+\) is 0.2–0.5 \( \mu \text{M} \) and for ATP 25–40 \( \mu \text{M} \); the latter value increases to 300 \( \mu \text{M} \) in the presence of 100 \( \mu \text{M} \) Na\(^+\) (Gill et al., 1984).

Maximum velocities reported by various authors differ considerably (all at 37°C) from 2.2 (Gill et al., 1984) to 87 nmol/mg protein/min (Sorensen and Mahler, 1981). Although it is difficult to determine energy expenditure for the movement of Ca^{2+}, recent estimates from Blaustein's group (see, for example, Sanchez-Armass and Blaustein, 1987) are of interest. They calculated from the rates of Ca^{2+} efflux into low sodium, calcium-free media that the ATP-fuelled pump was extruding (net value) 10–20 pmol Ca^{2+}/mg protein/s (i.e., 0.6–1.2 nmol/mg protein/min). By comparison, net efflux of calcium mediated by Na^{+}/Ca^{2+} exchange at a low calcium load was 0.11 nmol/mg protein/s (i.e., 6.6 nmol/mg protein/min). If these calculations on synaptosomes in vitro reflect the situation in synapses in vivo, then direct utilization of ATP for calcium pumping would amount to 1.2 nmol/mg protein/min, or assuming that protein constitutes 10% of wet weight of brain (0.12 μmol/g wet wt/min). This latter figure represents 0.3–0.5% of total ATP expenditure of 20–32 μmol/g wet wt/min. However, the rate of the Na^{+}/Ca^{2+} exchange reaction that is already sixfold greater at low calcium loads than that of the calcium pump (see above) is also linked to energy utilization, albeit indirectly. The energetic cost of extruding one equivalent of Ca^{2+} via this pathway is the same as by the calcium pump because the number of Na^{+} that are moved in (3) for each Ca^{2+} transported out equals the number of Na^{+} that are extruded by the sodium pump per each ATP split (also 3). Using the same assumptions as in the paragraph above, the velocity of 6.6 nmol/mg protein/min corresponds to the rate of ATP hydrolysis of 0.66 μmol/g wet wt/min, i.e., 2–3% of total ATP consumption, and would probably rise much higher at larger calcium loads. It should be stressed that this calculation assumes that there is a perfect "coupling" between the Na^{+}/Ca^{2+} exchange and the Na^{+}/K^{+} ATPase with no energy losses during either of the reactions. Since any loss of energy would increase ATP consumption, the estimate above is a minimum for the energetic cost of calcium pumping.

A general conclusion from this discussion is that, under normal conditions, the maintenance of ionic gradients requires 50–60% of the total O2 consumed, i.e., 12–16 μmol ATP/g wet wt/min, of which the major fraction is used by the Na^{+}/K^{+} pump.

Assignment of appropriate portions of ATP usage to the individual processes of so-called basal metabolism, which consume the remaining 40–50% of energy production, is difficult. The apportionment may be dependent on the characteristics of individual neurons and the level of their activity and may be affected by the relative sizes of the neuronal soma and its axonal and dendritic arborizations. One may expect that in brain, in addition to expenditure on protein and lipid synthesis, cell maintenance, and repair, a sizeable fraction of energy used is attributable to neurotransmitter metabolism, including transport, synthesis, and packaging and to phosphorylation reactions. A substantial body of experimental evidence accumulated during the past few years suggests that protein phosphorylation plays an important role in neuronal function (Nestler et al., 1984; Browning et al., 1985). Several classes of proteins undergo phosphorylation; these include enzymes (e.g., tyrosine hydroxylase, tryptophan hydroxylase, adenylate cyclase, and kinases), neurotransmitter receptors (e.g., β-adrenergic and nicotinic cholonic receptors), ion channels (Na^{+}, K^{+}, and Ca^{2+} channels), proteins involved in regulation of transcription and translation (RNA polymerase), and cytoskeletal proteins (actin and tubulin). Some of these may turn over rapidly and require a significant amount of ATP. However, identification of the proportion of total resting energy metabolism associated with each of these processes must await future studies.

Thus far, we have been concerned with the situation that occurs during sustained, nonstimulated cerebral function. However, it is well known that when the level of CNS activity rises, as for example during seizures, glucose and O2 consumption increase by up to threefold and lactate accumulation by seven- to 10-fold (Table 2), thus raising ATP production to about 100 μmol/g wet wt/min. Although it may be expected that increased ion pumping is responsible for this activation of oxidative metabolism, there are other energy-consuming processes such as neurosecretion or transmitter metabolism that may be stimulated at the same time. In an attempt to elucidate this problem, experiments were carried out on an in vitro preparation of rat posterior pituitary gland, which represents a relatively enriched population of axon terminals (Mata et al., 1980). It was found that electrical stimulation enhanced 2-deoxyglucose uptake by a process that was completely prevented by administration of ouabain. On the other hand, inhibition of neurosecretion was without effect on the increased metabolic activity. This suggests that the main energy-using process during electrical stimulation is ion pumping and that the key enzyme involved is the Na^{+}/K^{+}–ATPase.

At this point, it is appropriate to discuss very
briefly the mechanism(s) responsible for the enhancement of oxidative metabolism when cellular work is increased. Under such conditions, glycolysis and oxidative phosphorylation are stimulated independently. As mentioned earlier, the former is predominantly controlled by the operation of phosphofructokinase (Lowry et al., 1964). When energy expenditure is increased, the concentrations of activators of this enzyme (Pi, ADP, AMP) rise, whereas the concentrations of its inhibitors fall (PCr, ATP); consequently, the activity of the enzyme increases, thus stimulating flux through the glycolytic pathway.

When oxygen is abundant, the activity of oxidative phosphorylation is controlled by the interplay of two parameters: the phosphorylation state of the cytosolic adenine nucleotides ([ATP]/[ADP][Pi]) and the redox state of the intramitochondrial pyridine nucleotides ([NADH]m/[NAD+]m) (Erecińska and Wilson, 1982; Brand and Murphy, 1987). At a constant value for [ATP]/[ADP][Pi], respiration is inversely related to [NADH]m/[NAD+]m; when [NADH]m/[NAD+]m is constant, respiratory activity increases with a fall in [ATP]/[ADP][Pi]. However, owing to the near equilibrium relations that link the redox reactions between the NAD+ and the cytochrome c redox couples (Wilson et al., 1974), a decrease in the cytosolic [ATP]/[ADP][Pi] (i.e., the available ΔG) will cause a rise in the [NADH]/[NAD+]m. Such an oxidation of pyridine nucleotides has been observed in isolated mitochondria by Chance and Williams (1956) during state 4 to state 3 transitions and in intact brain during electrical stimulation (Lewis and Schuette, 1976; Lothman et al., 1975). This increase in intramitochondrial [NADH]/[NAD+] has two opposing effects on respiration. On the one hand, it lowers activity in the respiratory chain because NADH is the substrate for this multienzyme complex. On the other hand, it activates the key mitochondrial dehydrogenases (pyruvate, NAD-dependent isocitrate, and 2-oxoglutarate) that are inhibited by a high [NADH]/[NAD+]. Hence, an oxidation of the intramitochondrial pyridine nucleotides and consequent activation of the above enzyme will increase the flux of reducing equivalents to the respiratory chain. This may secondarily decrease the [NADH]/[NAD+]m to a level that will be determined by the balance between the change in the [ATP]/[ADP][Pi] and the extent of activation of the dehydrogenases. It is worth mentioning that if the intramitochondrial [ATP]/[ADP] falls in parallel with its cytosolic counterpart, this would also enhance activities of the dehydrogenases because all three are inhibited by a high [ATP]/[ADP].

Yet another mechanism, independent of those described above, may influence operation of the respiratory chain. It involves activation of the key rate-controlling dehydrogenases by increased levels of intracellular calcium. Such a stimulation raises the production of NADH irrespective of the alterations that occur in [ATP]/[ADP][Pi] and [NADH]/[NAD+]m and thus enhances ATP generation. This activation has been shown to occur for pyruvate dehydrogenase phosphatase (Denton et al., 1972), NAD-dependent isocitrate dehydrogenase (Denton et al., 1978) and 2-oxoglutarate dehydrogenase (McCormack and Denton, 1979). In the case of pyruvate dehydrogenase phosphatase, activation by Ca2+ leads to an increased amount of the active dephosphorylated form of the pyruvate dehydrogenase complex (see Wieland, 1983; Denton and McCormack, 1985). With the other two dehydrogenases, activation is of an allosteric nature and results in a decreased Km for the substrate. Whether this mechanism of dehydrogenase activation operates in brain in vivo remains to be established but two lines of experimental evidence suggest that it does. First, all of the conditions that increase cerebral activity lead to neuronal depolarization and transmitter release with consequent activation of the voltage-dependent and agonist-operated Ca2+ channels; hence, the concentration of intracellular calcium ion should rise. Second, high-frequency electrical stimulation has been reported to affect phosphorylation of the pyruvate dehydrogenase complex in hippocampal slices (Browning et al., 1979, 1981). Since glucose is the predominant fuel for brain, changes in the activities of pyruvate dehydrogenase would be expected to exert a major effect on cerebral oxidative metabolism.

Having discussed the mechanisms that control cellular energy production, it may be useful to delineate the most likely sequence of events that follow an increase in cellular work. The enhanced rate of ATP usage lowers the cytosolic [ATP]/[ADP][Pi], which directly stimulates respiration and, in addition, causes an oxidation of intramitochondrial pyridine nucleotides. The latter activates directly and/or indirectly the NAD-dependent dehydrogenases and leads to a more rapid generation of NADH, and hence ATP. When increased activity is accompanied by a depolarization of the plasma membrane, Ca2+-dependent voltage channels may open, allowing an increase in [Ca2+], and thus an activation of pyruvate dehydrogenase and other calcium-dependent dehydrogenases. The rapid acceleration of synthesis that ensues would tend to restore the level of ATP so that the high rates of respiration that occur with increased cellular activity
are accompanied by only small changes in the levels
of ATP, ADP, and Pi.

WHAT ARE THE RELATIONSHIPS BETWEEN
THE ENERGY LEVEL AND ION GRADIENTS
IN MAMMALIAN NEURONS AND GLIA?

It was stated earlier that proper functioning of the
CNS requires a nonequilibrium distribution of Na\(^+\),
K\(^+\), and Ca\(^{2+}\), which is achieved at the expense of
energy consumption through the operation of the
ATP-linked ion pumps, the Na\(^+\)/K\(^+\) – and the
Ca\(^{2+}\)–ATPases. The activities of these enzymes are
dependent on ATP in two ways. Firstly, ATP is a
substrate for the respective reaction cycles and a
decrease in its concentration can limit the overall
process kinetically. This is, however, unlikely to
occur under normal in vivo conditions, because the
K\(_m\) values for ATP (Robinson, 1976; Gill et al.,
1981) are much lower than its cytosolic concentra-
tion. On the other hand, when the rate of energy
generation declines, as for example in pathological
conditions of ischemia and hypoglycemia, a lack of
ATP to maintain the pumps is one of the factors
responsible for massive movements of ions down
their concentration gradients (Hansen, 1985). Sec-
ondly, ion transport mediated by pump proteins is
obligatorily coupled to ATP splitting; hence, energy
has to be provided at a level sufficient to maintain
the required ionic disequilibria. Since the rate at
which ATP is used to support the steady state is
minimal when the pump reactions are very close to
equilibrium, it would be most advantageous ener-
getically if the free energy for ion transport were
close to the free energy of hydrolysis of ATP. It
should be remembered, however, that under intra-
cellular conditions the steady-state ion gradients re-
sult from the combined effects of ion pumps and
passive fluxes. Hence, for the steady-state gradi-
ents to be near the true equilibrium values, the ma-
ajor passive fluxes should be “turned off” in the
resting or “normal” state.

Calculation of the free energy for ion transport by
the pumps requires knowledge of the steady-state
extra- and intracellular concentrations of the rele-
vant ions and the stoichiometries of the process in-
vestigated. Furthermore, it involves the assumption
that ATP hydrolysis and ion movements are tightly
coupled through the pump proteins. Information on
the intracellular levels of Na\(^+\), K\(^+\), and Ca\(^{2+}\) in
brain has not been available until very recently
when their concentrations were measured with mi-
croelectrodes in neurons and glia, responsive cells,
and nonresponsive cells, respectively (Erecińska
and Silver, 1988). The relevant values are presented
in Table 3 along with the simultaneously determined
extracellular [Ca\(^{2+}\)], [Na\(^+\)], and [K\(^+\)] and the
membrane electrical potentials.

The first process to be considered is that medi-
ated by the Na\(^+\)/K\(^+\)–ATPase. As mentioned, this
pump extrudes three equivalents of Na\(^+\) and accu-
mulates two equivalents of K\(^+\) with concomitant
hydrolysis of one ATP. The overall process is elec-
trogenic with the \(\Delta G\) described by

$$\Delta G_{Na,K} = RT \ln \left( \frac{[Na^+]_e[Na^+]_i}{[K^+]_e[K^+]_i} \right) \frac{[K^+]_e^2}{[Na^+]_e^3}$$

(6)

where [Na\(^+\)] and [K\(^+\)] designate concentrations of
tassium and sodium, respectively, \(E\) is the cell
membrane potential, and \(R\), \(T\), and \(F\) have their
conventional meanings. Substitution of the appro-
 priate numbers from Table 3 into Eq. (6) yields a
figure of 8.34 kcal/mol for the free energy of trans-
porting Na\(^+\) and K\(^+\) through the neuronal pump
and 9.89 kcal/mol through the glial pump. These
values are 6.3 and 4.5 kcal/mol lower in neurons and
glia, respectively, than the cerebral free energy for
hydrolysis of ATP (14.7 kcal/mol).

A similar calculation can be done for the plasma
membrane calcium pump. This enzyme has been
reported to function as an obligatory Ca\(^{2+}\)/H\(^+\) ex-
changer with a probable stoichiometry of 1:2 (i.e.,
mediates an electroneutral process), which moves
one equivalent of Ca\(^{2+}\) from inside to outside for
each ATP hydrolyzed (Carafoli, 1987). The overall
\(\Delta G\) for the reaction is given by

$$\Delta G_{Ca} = RT \ln \left( \frac{[Ca^{2+}]_e}{[Ca^{2+}]_i} \right)$$

(7)

After substituting the appropriate values from Table
3, \(\Delta G_{Ca}\) is calculated to be 6.12 kcal/mol for the
neuronal pump and 6.16 kcal/mol for the glial en-
zyme. Both values are over 8 kcal/mol smaller than
the \(\Delta G_{ATP}\).

The overall conclusion from the calculations
above is that in cerebral neurons and glia neither the
Na\(^+\)/K\(^+\) nor the Ca\(^{2+}\)–ATPase is in thermody-
namic equilibrium. In other words, the steady-state ion gradients of Na\(^+\) and K\(^+\) and of Ca\(^{2+}\) fall short of those predicted by the equilibria of the respective ATP-driven pumps. The same discrepancy has been noted earlier by Tanford (1981) for the Na\(^+\)/K\(^+\)-ATPase of squid axon and red blood cell plasma membrane, and by Civan et al. (1983) for the same enzyme in basolateral epithelium of frog skin. On the other hand, in resting skeletal muscle, the Ca\(^{2+}\)-ATPase of the sarcoplasmic reticulum was estimated to operate close to equilibrium. It is interesting that the behavior of the plasma membrane calcium pump is so different. It should be noted, however, that estimates of the free energies depend very strongly on the stoichiometries of ion movements through the pump proteins: the plasma membrane enzyme appears to transport one equivalent of Ca\(^{2+}\) for each ATP split, whereas the sarcoplasmic reticulum ATPase moves two cations for the same amount of energy. Hence, if the stoichiometry of the former were underestimated, it could lead to an apparently lower calculated \(\Delta G_{\text{Ca}}\).

However, in the absence of information to the contrary and assuming that the calculations above reflect the true in vivo situation, the question that has to be answered is why the two most important cation pumps of the plasma membrane appear to operate so far from equilibrium. Tanford (1981) has considered two possibilities. The first is that it may be incorrect to assume that passive ion fluxes produced by other ion-translocating systems in the plasma membrane are very small. This would imply that although the pump itself is 100% efficient, a continuous high rate of ATP hydrolysis is required to maintain the steady-state ion gradients. The second possibility is that there are inherent limitations in the pumps themselves that could lead to the deviations from the stoichiometries accepted in our calculations. If this were true, the pumps under steady-state conditions would be less than 100% efficient but could operate close to equilibrium so that the rate of ATP utilization would be maintained at a low value. Whichever mechanism holds true under in vivo conditions, the considerations above amply demonstrate how closely ion movements are linked to cellular energy utilization.

**WHAT OTHER ROLE(S) MAY ATP PLAY IN THE FUNCTION OF THE CNS?**

In the closing section, we shall consider the fact that ATP may participate in yet another type of regulation of cellular metabolism, namely modulation of ionic permeabilities. This function, in contrast to phosphorylation, appears to involve binding of ATP to certain classes of channel proteins and does not require its hydrolysis. Best known among these proteins is a class of K\(^+\) channels that are present in heart, skeletal muscle, and pancreatic \(\beta\)-cells (see Stanfield, 1987, for review). In addition, there are some Ca\(^{2+}\)-dependent channels that are closed by ATP. It is, however, not clear whether all regulation is exerted by ATP itself or through the [ATP]/[ADP] because in some situations the two nucleotides have opposite effects on channel function and affect their reactivities reciprocally. Although ATP-controlled channels have yet to be described in neurons or glia, their existence in these cells seems very likely. Moreover, in view of the great diversity of ion channels in nervous tissue and the high rates of ion movements, this type of regulation may ultimately prove of the utmost importance in controlling CNS function.

**SUMMARY AND CONCLUDING REMARKS**

ATP plays a fundamental role in mammalian brain function. Under normal conditions, it is synthesized almost exclusively by mitochondrial oxidative phosphorylation with only a small contribution from glycolysis. The creatine phosphokinase and adenylate kinase reactions operate efficiently in both neurons and glia to help maintain a constant ATP level. The “resting” concentrations of the high energy phosphate compounds do not appear to differ significantly between regions. By contrast, the rates of ATP production vary widely throughout the brain, with more rapid synthesis in areas of higher consumption. Cellular energy production is utilized predominantly for the maintenance of ion gradients but neither the Na\(^+\)/K\(^+\) ATPase nor the plasma membrane calcium pump are in thermodynamic equilibrium. The absolute concentrations of ATP may be important in the regulation of processes such as phosphorylation reactions and ion channel activities; however, further studies are required to establish the exact nature and extent of ATP involvement.

It has been the intention of this review to discuss several aspects of some hitherto unrecognized relationships between ATP and cerebral function. We realize that this may have raised more questions than it has answered. Nevertheless, it is our hope that the considerations presented will prove thought-provoking and offer a stimulus for design of novel and daring experiments.
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