observed neither on metabolic deprivation nor on depolarization, but only after treatment with excitotoxins [7,9]. In related experiments (results not shown) using 1^C-m.r. to study flux through metabolic pathways, we observed quite different patterns between these various insults. NMDA caused glycaemic coma, is probably always caused by rapidly decreased N-acetylaspartate and increased lactate, with slow decreases in pool sizes of amino acids [10], in marked contrast to the changes caused by severe hypoxia [11] or depolarization [12].

Our preliminary unpublished results on the effects of MK801 (Figure 2, Table 1) tend to confirm suggestions [8] that Zn^{2+} may be associated particularly with the NMDA receptor, and provide further evidence for the importance of non-NMDA receptors in the damaging consequences of metabolic deprivation.

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**Energy metabolism, ion homeostasis, and cell damage in the brain**
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Acute brain damage, as it occurs after ischaemia, intracranial haemorrhage, gross trauma, and hypoglycaemic coma, is probably always caused by cellular energy failure, secondary to reduced delivery of oxygen or glucose [1-3]. Under such circumstances, oxidative phosphorylation and ATP production are insufficient to sustain normal metabolic functions. A decline in ATP production per se is potentially harmful because spontaneous degradation of macromolecules and macromolecular assemblies is no longer matched by a correspond-

ing resynthesis. However, the secondary effects of energy failure represent an additional threat to cell viability. These effects encompass membrane depolarization, with presynaptic release of transmitters, including excitatory amino acids (EAA)s such as glutamate, and loss of ion homeostasis, as well as lactic acidosis (see [4,5]). Over-bombardment of glutamate receptors and Ca^{2+} influx, in turn, activate enzymes which degrade cell structure (e.g. phospholipases and proteases), as well as those altering the activity of proteins which regulate receptor function, gene expression and protein synthesis (mainly kinases and phosphatases). In this way, primary energy failure triggers a cascade of metabolic events which have potentially adverse effects.

Under certain circumstances, cell death occurs in settings in which energy failure is less obvious. For example, head trauma without lacer-
tion and major changes in blood flow may nonetheless give rise to damage, particularly to axons (for an overview, see [31]). Furthermore, brief periods of ischaemia are often followed by resumption of oxidative phosphorylation, restoration of ion homoeostasis, and recovery of electrophysiological functions; yet, neurons in selectively vulnerable regions die after a delay of hours or days (for review, see [6,7]). Very probably, this delayed cell death reflects metabolic cascades set in motion by the primary insult; although their nature remains a matter of speculation, these mechanisms probably encompass a perturbed signal-transduction pathway [8,9]. However, it cannot at present be excluded that delayed mitochondrial dysfunction contributes to delayed cell death [10].

In this paper, we discuss the coupling among mitochondrial metabolism, phosphorylation potential, and ion transport, with particular emphasis on Ca\(^{2+}\) homoeostasis. Three major questions are posed. First, what factors determine the rate of loss of ATP when oxidative phosphorylation came to a halt, and how is energy failure coupled to loss of ion homoeostasis? Second, what are the determinants of the resumption of oxidative phosphorylation and extrusion of Ca\(^{2+}\) from cells, once oxygen and/or glucose supply are restored? Third, is mitochondrial capacity for generation of ATP affected by a period of ischaemia which will allow short-term metabolic and functional recovery but nonetheless cause (delayed) damage?

**Ischaemia, energy failure, and loss of ion homoeostasis**

Considerable information exists on the effect of sudden anoxia on the rate of hydrolysis of ATP, and on dissipative ion fluxes [3,11,12]. Figure 1 summarizes data obtained in rats, demonstrating how sudden anoxia (caused by ischaemia induced by cardiac standstill) alters cellular energy state, as assessed by measured phosphocreatine (PCr) and ATP concentrations, as well as on the calculated concentration of ‘free’ ADP (ADP\(_{t}\)), and how changes in energy state are correlated to efflux of K\(^+\), as assessed by measurements of the concentration of extracellular K\(^+\) (K\(^+_e\)) (calculated from [13]). We interpret the data as follows. When anoxia brings oxidative phosphorylation to a halt, ATP production ceases and, because many ATP-dependent reactions continue unabated, ATP content is reduced. However, as soon as ATP hydrolysis leads to a rise in ADP\(_{t}\) concentration, the creatine kinase (CK) reaction is shifted in the direction of PCr hydrolysis and ATP formation. This means that ATP concentrations are maintained at > 90% of the control value for at least 30 s, i.e. as long as some PCr remains to provide ATP via the CK reaction. Then, the ATP content falls precipitously. Interestingly, the ADP\(_{t}\) concentration increases 3-fold during the first 30 s, demonstrating that although the ATP content remains close to normal, the ATP/ADP\(_{t}\) ratio decreases appreciably.

During this period of relative energy failure with uphold ATP concentrations and rising ADP\(_{t}\) concentration, K\(^+_e\) increases gradually. Possibly, this reflects an early rise in the cytosolic Ca\(^{2+}\) concentration, [Ca\(^{2+}\)]\(_i\), with secondary activation of Ca\(^{2+}\)-activated K\(^+\) conductances. Two findings support this notion. First, our own data demonstrated phosphorylase \(b\) to \(a\) conversion at a time when cyclic-AMP concentration had not yet
increased [14]. Second, the direct microelectrode measurements of Silver and Erecinska [15] revealed an early rise in \([Ca^{2+}]\), which seemed unrelated to influx of \(Ca^{2+}\) from extracellular fluid. The mechanisms behind such a rise are not known, but it probably reflects the release of \(Ca^{2+}\) from intracellular stores, e.g. secondary to the rise in intracellular pH which is triggered by PCR hydrolysis [15]. At present, however, we cannot exclude the possibility that the rise in \(K^+\) is caused by activation of ATP-dependent \(K^+\) conductances. Thus, such channels may well be regulated by the ATP/ADP ratio (see [16]).

When ATP concentrations fall below 50% of the control value, a precipitous efflux of \(K^+\) occurs, together with cellular uptake of \(Ca^{2+}\), \(Na^+\) and \(Cl^-\). This sudden and seemingly unspecific activation of ion conductances may not reflect the fall in phosphorylation potential to a critical value, but rather the release of EAAs and the activation of post-synaptic \(N\)-methyl-\(d\)-aspartate (NMDA) and \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors. In support of this interpretation, chemical deafferentation of CA1 neurons dramatically prolonged the time between induction of ischaemia and influx of \(Ca^{2+}\) into cells [17]. The importance of energy failure is thus that it contributes to presynaptic depolarization and glutamate release, and that it prevents active transport of ions once their transmembrane gradients have been dissipated.

The main factors which influence the lag between induction of ischaemia and loss of ion homeostasis are metabolic rate and plasma glucose concentration. The inverse relation between metabolic rate and terminal depolarization time ([18], see also [19]) suggests that dissipative ion fluxes occur via conductances whose pre-ischaemic activity persists during the initial stage of ischaemia. Hyperglycaemia prolongs and hypoglycaemia shortens terminal depolarization time [11,20]. At least in part, these differences should reflect the ability of anaerobic metabolism of endogenous glucose stores to provide ATP for ion transport.

The rate of influx of \(Ca^{2+}\) into cells during terminal depolarization is influenced by the state of opening of \(Ca^{2+}\) channels (Figure 2), particularly that gated by NMDA receptors. Glutamate antagonists such as dizocilpine maleate (MK-801) slow down \(Ca^{2+}\) influx, as does acidosis [21]. The latter effect is probably due to \(H^+\) inhibition of both agonist-operated and voltage-sensitive \(Ca^{2+}\) channels [22].

**The importance of increased ion conductances**

At any given moment, the intra- to extracellular concentration ratios for ions such as \(Na^+\), \(K^+\) and \(Ca^{2+}\) depend on the balance between influx and efflux. Realizing that \([Ca^{2+}]\), also reflects binding and sequestration, we will discuss this 'pump-leak' relationship for \(Ca^{2+}\). Clearly, \([Ca^{2+}]\), can increase either because the leak pathways are activated, because efflux is inhibited, or because both factors contribute. We know that the first mechanism, i.e. an increased \(Ca^{2+}\) conductance, can give rise to massive translocation of \(Ca^{2+}\) from extracellular to intracellular fluid. For example, iontophoretic release of glutamate leads to marked reductions in extracellular \(Ca^{2+}\) concentration, \([Ca^{2+}]_c\), the maximal effects being observed in areas with a high density of NMDA and AMPA receptors [23]. Furthermore, the marked decrease in \([Ca^{2+}]_c\), which is
observed in spreading depression occurs in spite of the fact that energy metabolism is not compromised (see [24], see also [25]). Finally, translocation of Ca\(^{2+}\) from extra- to intracellular fluid in hypoglycaemic coma seems to be the cause rather than the effect of overt cellular energy failure [26]. This means that massive activation of Ca\(^{2+}\) conductances is an important determinant of the increase of [Ca\(^{2+}\)]\(\text{c}\), which occurs in disease. It further means that when an increase in Ca\(^{2+}\) conductances is combined with inhibition of Ca\(^{2+}\) extrusion, as occurs in ischaemia (or hypoglycaemia), [Ca\(^{2+}\)]\(\text{c}\) may rise to potentially detrimental levels (see [15,27]).

**Resumption of oxidative phosphorylation and ion transport**

Recirculation after a period of ischaemia is a potential threat to the viability of the tissue because generation of free radicals can cause damage to mitochondria or oxidized proteins involved in 'uphill' transport of ions (e.g. [28,29]). However, when recirculation is initiated after a 15 min period of dense ischaemia, cellular uptake of K\(^{+}\) commences within the first 2 min and is complete within the first 5 min [30]. Interestingly, active K\(^{+}\) transport is resumed in spite of minimal restoration of PCr concentrations, and at average ATP contents which are only 30-40% of control. The low PCr concentrations probably reflect the lingering (lactic) acidosis which, together with a persisting rise in ADP, shifts the CK reaction in the direction of ATP formation. In all probability, failure of normalization of PCr content after 5 min, when ADP\(_p\) is no longer increased, is related to persisting acidosis (see [30]). It should also be emphasized that the subnormal values of ATP after 5 min of recirculation does not reflect mitochondrial dysfunction but reduction of the size of the adenine nucleotide pool due to deamination and dephosphorylation of AMP during the insults [31,32].

We recall that a similar delay in resumption of oxidative phosphorylation and ion transport is observed when hypoglycaemic coma is terminated by glucose infusion [33,34]. Thus, the 1-2 min delay in ischaemic animals is not due to sluggish reperfusion.

These results, as well as previous ones (e.g. [35]), demonstrate that mitochondrial oxidative phosphorylation is quickly resumed after ischaemic insults which are followed by adequate reperfusion. In fact, such resumption has been described after ischaemic periods as long as 60 min [36]. Pre-ischaemic hyperglycaemia, which aggravates damage due to transient ischaemia (for literature, see [37]), represents a likely exception to this rule. Thus, although hyperglycaemic animals subjected to 15 min of transient ischaemia demonstrate prompt resumption of oxidative phosphorylation upon recirculation [35], those subjected to 30 min of ischaemia do not [38]. It remains to be shown, though, whether this is attributable to primary mitochondrial damage, or reflects failure of capillary reperfusion.

Although recirculation usually leads to prompt normalization of [K\(^{+}\)]\(\text{c}\), recovery of normal [Ca\(^{2+}\)]\(\text{c}\) values is slower, and typically occurs in two stages: a first recovery to ~70% of normal, and a slow, gradual return to control values (see [39,40]). Very probably, the fast recovery reflects extrusion of free and bound Ca\(^{2+}\), and the slow one gradual release of Ca\(^{2+}\) from sequestration sites such as mitochondria, and its extrusion by ATP-driven transport.

**Mitochondrial function and secondary delayed brain damage**

As stated above, transient ischaemia leads to neuronal cell death which is often conspicuously delayed. The mechanisms responsible have not been defined but may encompass a lingering depression of protein synthesis, or disturbance of intracellular signal transduction [6,8]. It is not unlikely that a major factor is a lingering perturbation of cell Ca\(^{2+}\) metabolism [3]. However, mitochondrial dysfunction cannot at present be excluded, and recent data suggest a genomic alteration of potentially detrimental consequences [10].

Also as stated above, mitochondrial function is quickly resumed when the tissue is recirculated after ischaemia. Furthermore, cellular energy state is rapidly normalized, also in the selectively vulnerable CA1 region of the hippocampal formation [41]. It follows from this that if mitochondrial dysfunction is responsible for the subsequent damage, it must develop after a delay, or encompass a reduction in mitochondrial capacity to meet increased functional demands. In this respect, it is of clear interest that transient ischaemia leads to a reduction of cerebral metabolic rate which may persist for at least 96 h [42], a reduction which is exaggerated by pre-ischaemic hyperglycaemia [43]. The reduction of metabolic rate is paralleled by a decrease in the activity of the pyruvate dehydrogenase complex in both normoglycaemic animals [44], and in those rendered hyperglycaemic [45].

A recent study from our laboratory has shed light on this problem [46]. Animals were subjected
Influence of seizure activity on CBF and CMRO₂ in control animals, as well as in those subjected to a 15 min period of forebrain ischaemia 6 h before seizure induction

A preceding period of ischaemia reduced CBF and CMRO₂ significantly; however, seizures increased CBF and CMRO₂ towards values observed in control animals, demonstrating retained metabolic capacity in post-ischaemic brain cortex. Adapted from [46].

These data demonstrate that although metabolic rate and blood flow are markedly reduced in the post-ischaemic period, the metabolic capacity is intact, and so is the ability of the cell to respond to metabolic challenge. It remains to be shown whether these conclusions apply to vulnerable regions such as the CA1 sector of the hippocampus, and to longer periods of recirculation.

In summary, we lack evidence that the sustained reduction of metabolic rate and blood flow in the post-ischaemic recirculation periods is due to mitochondrial failure, and that such failure is what causes delayed neuronal damage. However, definitive statements require additional data on post-ischaemic metabolic capacity in selectively vulnerable neuronal populations, and after longer post-ischaemic periods.

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Inborn and induced defects of the mitochondrial respiratory chain

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Introduction

The mitochondrial respiratory chain has become a focus of attention for both the study of inborn metabolic defects and the effect of endogenous and exogenous toxins on mitochondrial function. There is evidence that defects of the respiratory chain may be involved in a diverse range of human disorders ranging from myopathies through to Parkinson's disease (PD).

Inborn metabolic defects

Mitochondrial DNA mutations

Mitochondrial DNA (mtDNA) is a small 16.5-kilobase (kb) circular molecule encoding two RNAs, 22 tRNAs, seven subunits of complex I (ND1, ND2, ND3, ND4, ND4L, ND5, ND6), cytochrome b, three subunits of complex IV (COI, COII, COIII) and two subunits of complex V (A6 and A6L). Each mitochondrial contains 2–10 mtDNA molecules. Recent studies have suggested that there is relatively free flow of mtDNA between mitochondria within cells, leading to intracellular mitochondrial...