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Biochimica et Biophysica Acta 1410 (1999) 215–228

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Review

# Nitric oxide, mitochondria and neurological disease

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Received 31 March 1998; received in revised form 15 May 1998; accepted 19 May 1998

**Abstract**

Damage to the mitochondrial electron transport chain has been suggested to be an important factor in the pathogenesis of a range of neurological disorders, such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, stroke and amyotrophic lateral sclerosis. There is also a growing body of evidence to implicate excessive or inappropriate generation of nitric oxide (NO) in these disorders. It is now well documented that NO and its toxic metabolite, peroxynitrite ($ONOO^-$), can inhibit components of the mitochondrial respiratory chain leading, if damage is severe enough, to a cellular energy deficiency state. Within the brain, the susceptibility of different brain cell types to NO and $ONOO^-$ exposure may be dependent on factors such as the intracellular reduced glutathione (GSH) concentration and an ability to increase glycolytic flux in the face of mitochondrial damage. Thus neurones, in contrast to astrocytes, appear particularly vulnerable to the action of these molecules. Following cytokine exposure, astrocytes can increase NO generation, due to de novo synthesis of the inducible form of nitric oxide synthase (NOS). Whilst the $NO/ONOO^-$ so formed may not affect astrocyte survival, these molecules may diffuse out to cause mitochondrial damage, and possibly cell death, to other cells, such as neurones, in close proximity. Evidence is now available to support this scenario for neurological disorders, such as multiple sclerosis. In other conditions, such as ischaemia, increased availability of glutamate may lead to an activation of a calcium-dependent nitric oxide synthase associated with neurones. Such increased/inappropriate NO formation may contribute to energy depletion and neuronal cell death. The evidence available for $NO/ONOO^-$-mediated mitochondrial damage in various neurological disorders is considered and potential therapeutic strategies are proposed. © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* Mitochondrion; Nitric oxide; Glutathione; Astrocyte; Neuron; Neurodegeneration

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PII: S0005-2728(98)00168-6

## 1. Introduction

Within the central nervous system (CNS), and under normal conditions, nitric oxide (NO) appears to have a number of important biochemical roles, e.g. cGMP formation, following activation of guanylate cyclase and the regulation of the glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase [1–3]. Furthermore, NO has been implicated to play an important role in a number of physiological processes in the CNS, i.e. pain perception, synaptic plasticity and learning [2].

NO itself is generated by nitric oxide synthase (NOS), of which there are at least three isoforms [2]. These enzymes catalyse, in the presence of molecular oxygen, tetrahydrobiopterin and other cofactors, the conversion of arginine to NO plus citrulline [2]. All CNS cells appear to have the ability to synthesise NO, in vitro [2]. In general terms, neurones synthesise NO *via* activation of the constitutive, calcium-dependent, neuronal NOS isoform (nNOS or NOS1), whilst astrocytes, and other glial cells, generate NO in a calcium-independent manner following induction (de novo enzyme synthesis) of NOS (iNOS or NOS2). A third isoform, eNOS (NOS3), is reported in the CNS and has been assigned predominantly to the brain vasculature [2].

NO is a free radical and hence in many biological systems it has a short half-life due to its reactivity with other intracellular constituents, such as superoxide ($O_2^{\bullet -}$) [4]. The reaction between NO and $O_2^{\bullet -}$ results in the formation of the peroxynitrite anion ($ONOO^-$), which is cytotoxic [5]. This reaction is extremely favourable, so much so that NO can effectively compete with the superoxide dismutases for $O_2^{\bullet -}$ [4]. Since the toxicity associated with NO generation can, in many cases, be prevented by the scavenging of superoxide, formation of $ONOO^-$ is therefore considered to be an important factor in causing cellular damage [5].

Despite the physiological roles attributed to NO, excessive formation has been implicated in the pathogenesis of certain neurological disorders [6,7]. Whilst a number of mechanisms have been invoked to explain NO/$ONOO^-$-mediated neurotoxicity, this review will concentrate on the potentially important interaction of these molecules with the mitochondrial respiratory chain and how such mitochondrial damage may be an important factor in the pathogenesis of neurological conditions, such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, ischaemia and amyotrophic lateral sclerosis.

## 2. The mitochondrial electron transport chain

The primary function of the mitochondrial electron transport chain (ETC) is ATP synthesis. The ETC, present in the inner mitochondrial membrane, is comprised of more than 70 polypeptide components which are grouped into four enzyme complexes (Fig. 1). The polypeptides that constitute complexes I (NADH ubiquinone reductase), III (ubiquinol cytochrome *c* reductase) and IV (cytochrome *c* oxidase) are coded for by both nuclear and mitochondrial DNA, whilst complex II (succinate ubiquinone reductase) is exclusively coded for by nuclear DNA [8]. In brief, transfer of reducing equivalents from NADH or $FADH_2$ to molecular oxygen is coupled to the pumping of protons across the inner mitochondrial membrane resulting in the formation of a proton gradient. Dissipation of this proton gradient through the membrane sector of the mitochondrial ATP synthase (complex V) induces a conformational change in the active site of this enzyme which favours ADP phosphorylation and hence ATP synthesis [9].

In view of the fundamental role the mitochondrial ETC plays in energy metabolism, damage to one or more of the respiratory chain complexes may lead to an impairment of cellular ATP synthesis. However, each of the complexes of the ETC exert varying degrees of control over respiration and substantial loss of activity of an individual respiratory chain complex may be required before ATP synthesis is compromised [10]. Furthermore, within the brain, mitochondria appear heterogeneous in nature and, depending on cell type, display different threshold effects, e.g. in non-synaptic mitochondria, complex I has to be inhibited by approximately 72% before inhibition of ATP synthesis occurs, whereas in synaptosomes the threshold is 25% [10,11].

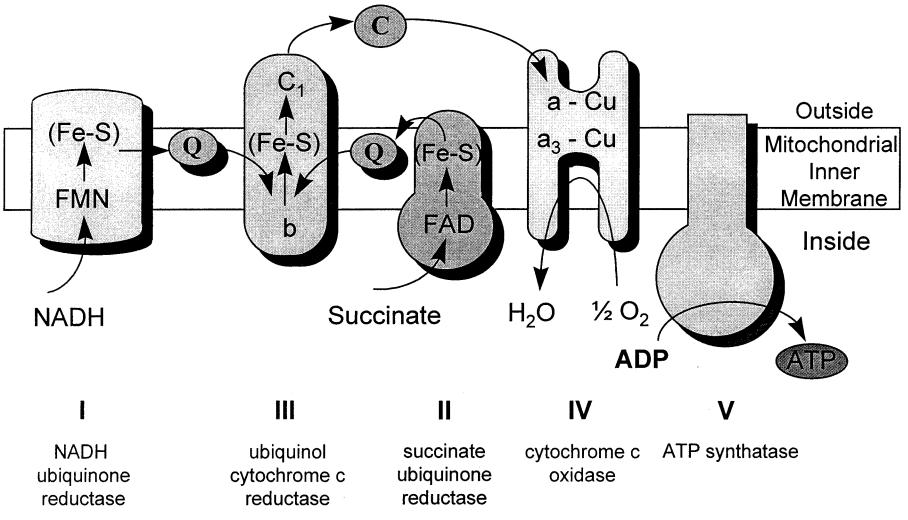


Fig. 1. Schematic of the mitochondrial electron transport chain. The four complexes of the mitochondrial electron transport chain, present in the inner mitochondrial, membrane are shown. In addition, ATP synthetase, which is sometimes known as complex V, is shown. Further details of this system are to be found in the text. Q and C represent the mobile electron carriers, ubiquinone and cytochrome *c*, respectively.

## 3. NO and the mitochondrial electron transport chain

### 3.1. Reversible complex IV inhibition

The interaction of NO with cytochrome oxidase (complex IV) of the ETC has been known for almost 60 years [12]. Since NO resembles dioxygen and has an unpaired electron, it has been used in electron paramagnetic studies to elucidate the possible mechanisms involved for oxygen binding to complex IV. Such investigations revealed that NO binds reversibly to the $Fe^{2+}$ centre of cytochrome $a_3$ and also to the $Cu^{2+}$ centre of complex IV [13,14]. Whilst such studies provided useful information with regards to complex IV structure and catalytic activity, the potential (patho)physiological relevance of the interaction of NO with complex IV was not considered until after 1987 when the importance of NO in biological systems became apparent [15].

Using biological systems, e.g. isolated heart mitochondria and synaptosomes, brief exposure to NO is reported to lead to the rapid inhibition of mitochondrial respiration at the level of cytochrome oxidase. [16,17]. Since, under the conditions employed, the inhibition of oxygen consumption appeared to be largely reversible, it has been postulated that NO may be a physiological regulator of mitochondrial respiration [17]. In support of this suggestion, Shen et al. [18] reported that administration of an NOS inhibitor to conscious dogs led to an increase in oxygen consumption that could not be attributed to haemodynamic changes. However, further work is needed to confirm or refute the suggested physiological role for NO in controlling mitochondrial respiration, particularly as complex IV may not display a high degree of metabolic control over oxidative phosphorylation [10].

### 3.2. Irreversible complex IV damage

Exposure of cultured astrocytes to lipopolysaccharide (LPS) and interferon-γ (IFN-γ) results in a 96-fold increase in iNOS activity [19]. Such treatment is accompanied by a marked decrease in cellular oxygen consumption which is only partially reversible by the administration of a nitric oxide synthase inhibitor or the scavenging of NO with oxyhaemoglobin [20]. Evaluation of the specific activities of the respiratory chain in homogenates prepared from these cells also revealed a significant irreversible loss of complex IV and complex II–III (see below) activity, which could be prevented by the addition of an NOS inhibitor or superoxide dismutase (+catalase to remove any hydrogen peroxide formed by superoxide dismutase) [19]. These findings therefore suggest that this irreversible loss of activity is mediated by $ONOO^-$.

Brief exposure of isolated brain mitochondria to $ONOO^-$ does not, however, result in a significant loss of complex IV activity [21]. In contrast, exposure of cultured neurones to $ONOO^-$ followed by 24 h incubation leads to a loss of complex IV activity [21]. The lack of an immediate effect of $ONOO^-$ upon complex IV suggests that intracellular, time-dependent, processes are involved for damage to this complex to occur.

Complex IV activity has been shown to correlate directly with the concentration of cardiolipin in the inner mitochondrial membrane [22]. Cardiolipin, a phospholipid rich in linoleic acid, is prone to peroxidation [22]. Since $ONOO^-$ may initiate lipid peroxidation [23], it is possible that this process causes loss of functional cardiolipin and a subsequent loss of complex IV activity. In support of this, Trolox, a vitamin E analogue and inhibitor of lipid peroxidation has been shown to protect complex IV in LPS/IFN-γ treated astrocytes [24]. However, as Trolox can react directly with $ONOO^-$ [25], it is likely that protection also arises as a result of the direct scavenging of $ONOO^-$.

### 3.3. Loss of complex II–III activity

The integrated activity of succinate cytochrome *c* reductase, i.e complex II–III of the ETC (Fig. 2), can be measured in a single assay [19]. Exposure of isolated brain mitochondria to exogenous $ONOO^-$ results in an irreversible loss of activity of complex II–III. [21]. Furthermore, in neurones exposed to $ONOO^-$ and analysed 24 h later, this component of the ETC is again significantly decreased in activity [21]. In addition, in cultured astrocytes exposed to LPS/IFN-γ, there is an irreversible loss of complex II–III activity [19]. Thus, in a range of systems, complex II–III appears to be particularly vulnerable to $ONOO^-$ exposure. Assay of complex II–III requires the endogenous quinone pool [26]. Recent observa-

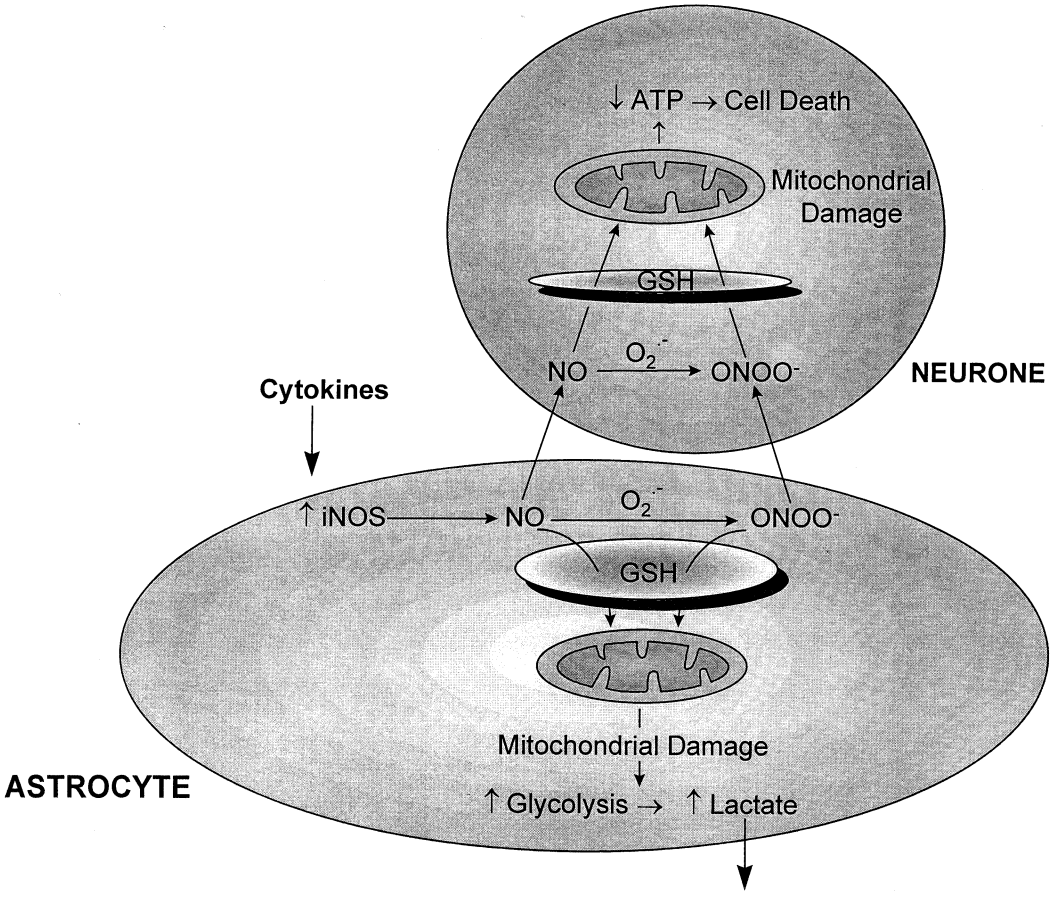


Fig. 2. Neuronal mitochondrial damage as a result of astrocyte generation of NO and $ONOO^-$. Exposure of astrocytes to cytokines leads to an induction of iNOS and marked generation of NO and $ONOO^-$ (formed from the reaction between NO and superoxide). Mitochondrial damage may occur in these cells, but ATP levels are maintained due to an increase in glycolytic flux. Further cellular damage is minimised due to the relatively high glutathione (GSH) concentration. Diffusion of NO and/or $ONOO^-$ into a neighbouring neurone may, if the cellular GSH concentration is low, lead to mitochondrial damage. Since these cells are unable to compensate by increasing glycolysis, a cellular energy deficiency state ensues which may lead ultimately to cell death.

tions suggest that NO and possibly $ONOO^-$ react directly with ubiquinol [27]. This finding may provide an explanation for the irreversible loss of complex II–III activity that occurs following $ONOO^-$ exposure and provokes the suggestion that oxidative loss of ubiquinone may, under certain conditions, occur in vivo. However, complex II itself also appears to be directly susceptible to NO/$ONOO^-$. Thus, direct exposure of isolated heart mitochondria to $ONOO^-$ and incubation of cultured oligodendrocytes with an NO donor, results in loss of succinate dehydrogenase (SDH – a component of complex II) activity [28,29]. In order to evaluate our findings further with isolated brain mitochondria, we have now assayed complex II and complex III separately and observed that brief exposure of brain mitochondria to $ONOO^-$ leads to loss of complex II activity only [30].

### 3.4. Loss of complex I activity

Using isolated heart mitochondria, Radi et al. demonstrated $ONOO^-$-mediated impairment of oxygen consumption with succinate as well as NAD-linked substrates [28]. Enzymatic analysis of the components of the respiratory chain indicated some loss of rotenone sensitive NADH dehydrogenase (used in this study to reflect complex I) activity [28]. However, NADH dehydrogenase was not as sensitive as SDH to the actions of $ONOO^-$ [28]. Later studies,

again with isolated heart mitochondria, revealed that NO caused reversible inhibition of complex I-, II- and IV-dependent respiration [31]. However, it is likely that this observation arises from NO-inhibiting complex IV which, as a consequence, leads to a secondary impairment of electron transport and hence glutamate/malate and succinate driven respiration.

Complex I in isolated brain mitochondria also appears to be relatively resistant to the direct actions of $ONOO^-$ [21]. However, we have noted that the sensitivity of this complex to $ONOO^-$ may be critically dependent on the concentration of mitochondria utilised in the incubations with $ONOO^-$ and whether respiratory substrates are present [30].

Induction of iNOS in astrocytes or brief exposure of cultured neurones to $ONOO^-$ does not result in any loss of complex I activity [19]. In contrast, when neurones are exposed to an NO donor for 24 h, marked loss of complex I activity occurs in conjunction with a loss of complexes II–III and IV [32]. Under such conditions, there is also a marked loss of the intracellular antioxidant, reduced glutathione (GSH) [32]. When compared to neurones, astrocytes appear resistant to exogenous $ONOO^-$ [21]. However, depletion of astrocytic GSH renders the cells more susceptible with marked complex I damage occurring following subsequent $ONOO^-$ exposure [33]. These findings imply that $NO/ONOO^-$-mediated complex I damage ensues only in the presence of a marked GSH deficiency.

## 4. $ONOO^-$ and the mitochondrial permeability transition

Mitochondria may, upon exposure to oxidising species, undergo a permeability transition, i.e certain inner membrane proteins amalgamate to form a non-specific pore 2–3 nm in diameter that is specifically inhibited by cyclosporin A (CsA) [34]. Pore opening leads to loss of the mitochondrial membrane potential (and thus ability to synthesise ATP), and an ability to sequester $Ca^{2+}$, both of which may be important factors in necrotic cell death [35]. However, it also appears that this mitochondrial permeability transition is an important early event in programmed cell death (apoptosis) [35,36]. Pore opening leads to the release of mitochondrial cytochrome *c*, which acts as a pro-apoptotic signal [35,36]. Pore opening has been reported upon exposure of liver mitochondria to reactive oxygen species [37] and $ONOO^-$ [38]. The possible mechanisms involved in $ONOO^-$-mediated pore opening include: (1) $ONOO^-$-induced cross-linking of inner membrane protein thiol groups, leading to protein amalgamation and pore formation; (2) $ONOO^-$-induced lipid peroxidation, products of which are potent inducers of permeability transition; and (3) $ONOO^-$-induced mitochondrial respiratory chain dysfunction. It has been reported that inhibition of the kidney mitochondrial respiratory chain causes pore opening [39].

Whilst many of the phenomena associated with opening of the mitochondrial permeability pore appear to be prevented by CsA, it has been reported that peroxynitrite induces $Ca^{2+}$-independent swelling of rat liver mitochondria that, under the conditions employed, is insensitive to CsA [40]. Furthermore, we have observed that exposure of isolated rat brain mitochondria to $ONOO^-$ causes an increase in state 4 (ADP rate-limiting) respiration that is insensitive to CsA [30]. We attribute this to $ONOO^-$-induced lipid peroxidation that increases proton permeability of the inner mitochondrial membrane [30]. Thus, the precise experimental conditions and origin of mitochondria may dictate as to whether $ONOO^-$ induces the permeability transition.

## 5. Differential cell susceptibility

It should be apparent, from the above, that mitochondria from different tissue sources display differential susceptibility to oxidising species, such as $ONOO^-$. However, it is also now becoming apparent that, within the brain, there is a differential susceptibility of various brain cell types to $NO/ONOO^-$ [21]. Factors such as the cardiolipin concentration of the inner mitochondrial membrane, the cellular antioxidant status and an ability to maintain energy requirements, in the face of marked ETC damage, may be important in dictating sensitivity towards $NO/ONOO^-$.

Induction of iNOS in astrocytes leads, as discussed above, to marked damage to the ETC. However, despite such damage, cell death does not occur [19]. The apparent resistance of these cells, in this situa-

tion, appears to be mediated by a compensatory increase in glycolysis, i.e there was a marked increase in glucose consumption coupled with lactate formation [19].

In contrast to astrocytes, neurones appear to be particularly vulnerable to the actions of $ONOO^-$, i.e. brief exposure of neurones to an exogenous source of $ONOO^-$ results in irreversible mitochondrial damage and cell death within 24 h [21]. Such vulnerability may arise from an inability to sustain cellular energy demands by glycolysis and an inferior capacity to handle oxidising species such as $ONOO^-$. With regard to the latter suggestion, the intracellular GSH concentration may be particularly important, since $ONOO^-$ favourably reacts with thiol-containing compounds [21,41]. Various lines of evidence are now available to implicate a key role for GSH in dictating cellular susceptibility to $ONOO^-$; the astrocyte GSH concentration, in culture, appears to be approximately double that of neurones cultured under identical conditions [21], astrocytes display a higher specific activity of the rate-limiting enzyme of GSH synthesis, γ-glutamyl cysteine synthetase [42], GSH-depleted astrocytes display marked sensitivity towards $ONOO^-$ [33].

Another factor contributing to the relative resistance of astrocytes to $ONOO^-$ exposure may be their superior concentration of α-tocopherol (vitamin E) [42]. When compared to neurones, the concentration of α-tocopherol in cultured astrocytes is reported to be approximately 2.5 times greater [42].

## 6. Glutamate, NO and excitotoxicity

Stimulation of neuronal glutamate receptors can transiently elevate the intracellular calcium concentration [2]. Whilst some laboratories advocate an involvement of NO in the process of neurotoxicity, other groups have been unable to substantiate such claims [43–45]. Such discrepancies, may relate to the type of neurones used and the number of NOS-positive neurones that form the cell culture population. The number of NOS-positive neurones present in culture preparations may be dependent on a number of factors, including the number of days in culture [46,47]. Recently, using 'mature' neuronal cultures, i.e. containing cells capable of generating NO, we demonstrated that brief exposure to glutamate leads to an NO-dependent loss in oxygen consumption, ATP concentration, and mitochondrial complex II–III and complex IV activities [48].

Furthermore, there was a significant loss in the neuronal GSH concentration, which also could be prevented by the inclusion of a NOS inhibitor [48]. Whether GSH depletion, under these conditions, is due to an NO-dependent inhibition of cystine (a precursor of cysteine which is required for GSH synthesis) uptake [49] or arises from the direct reaction of $NO/ONOO^-$ with intracellular GSH is not known. However, these observations suggest that, in the presence of sufficient NOS-positive neurones, NO-mediated loss of mitochondrial function and cellular GSH is an important factor in glutamate neurotoxicity. Since NOS-positive neurones actually appear to be resistant to glutamate receptor activation, it has been proposed that these cells generate NO which is released to cause possible damage to NOS-negative neurones in close proximity [47].

## 7. Astrocyte-derived NO and neurotoxicity

In view of the ability of astrocytes to generate NO and $ONOO^-$ without cell death occurring, it is possible that in certain neurological disorders, astrocytic derived $NO/ONOO^-$ may cause mitochondrial damage to susceptible target cells, such as neurones (Fig. 2).

Whilst, induction of iNOS in mixed astrocyte/neuronal co-cultures does not appear to cause immediate neuronal cell death, it was observed that the 'NO-exposed' neurones displayed increased sensitivity towards glutamate [50]. Further studies, involving mixed astrocyte/neuronal cultures, demonstrated neuronal death 24–36 h after exposure to iNOS expressing astrocytes [51,52]. Investigations, utilising a co-culture system in which neurones and iNOS expressing astrocytes are cultured close to (1 mm apart), but not in contact with each other, demonstrated significant neuronal mitochondrial damage (complexes II–III and IV) and loss of ATP within 24 h [32,53]. Despite such mitochondrial damage, neuronal cell death did not occur [53]. In view of the marked sensitivity of neurones (when cultured alone) towards NO and $ONOO^-$, the lack of cell

death appears, at first, to be paradoxical. However, the co-culture of neurones with astrocytes leads to an increase (×2) in the neuronal GSH concentration [32]. This apparent upregulation of neuronal GSH status appears to arise from the trafficking of cysteine, a rate-limiting amino acid for GSH synthesis, from astrocytes to neurones [54]. These observations, therefore, lend further support to the critical role GSH plays in dictating cellular susceptibility to $NO/ONOO^-$ and suggest that results obtained from the 'mono' culture of neurones need to be interpreted with caution. Furthermore, in neurological conditions where induction of astrocyte iNOS may occur, severe damage to target cells, such as neurones and possibly oligodendrocytes, may not immediately occur until the GSH status of such cells is compromised. However, loss of GSH may eventually ensue as a result of the direct reaction between GSH and $ONOO^-$ and impairment of GSH synthesis (an energy-dependent pathway) due to mitochondrial damage.

## 8. Mitochondrial damage and cell death

Whether $NO/ONOO^-$-mediated mitochondrial damage is a direct cause of cell death is not established. However, it is of interest to note that toxic insults that cause severe mitochondrial damage, and hence a rapid and marked energy failure, are associated with necrotic cell death [55]. Impairment of the mitochondrial ETC may also play an important role in the initiation of apoptosis, e.g. as a result of the opening of the mitochondrial permeability transition pore (see above). Furthermore, glutamate neurotoxicity proceeds through the initiation of an apoptotic pathway only if functional mitochondria are present, suggesting an ATP requirement [55]. In support of this, exposure of PC12 cells to $ONOO^-$ and partial inhibition of the mitochondrial respiratory chain leads to cell death via apoptosis [56,57]. Whilst, such studies implicate perturbation of mitochondrial function in apoptosis, initiation of this process has been reported in a human fibroblast cell line lacking functional mitochondria [58]. The implication of these findings for neuronal cells is not clear. Furthermore, it is possible that these fibroblasts could be atypical in the sense that they could obtain substantial ATP, for an apoptotic programme to proceed, from glycolysis.

## 9. NO-mediated mitochondrial damage and neurological disease

Damage to the mitochondrial respiratory chain has been proposed to underlie the pathology of a number of neurodegenerative disorders [59]. However, as pointed out above, loss of specific activity of a particular component of the ETC does not necessarily equate to a cellular energy deficiency state, i.e. due consideration must be given to the degree of metabolic control a particular respiratory chain complex exerts over oxidative phosphorylation [10,11].

### *9.1. Parkinson's disease*

Decreased complex I activity is reported in the substantia nigra of post mortem samples obtained from patients with Parkinson's disease [60]. Furthermore, one study has also identified NADPH diaphorase (a putative marker for NOS activity) positive glial cells in the substantia nigra obtained from such individuals [61]. Since loss of nigral GSH is also considered to be an early and key event in the pathogenesis of Parkinson's disease [62], decreased scavenging of $NO/ONOO^-$ may occur leading to the possibility of mitochondrial damage. In view of the apparent specific loss of complex I activity, in Parkinson's disease, it is also of note that this component of the respiratory chain is particularly susceptible to the actions of $ONOO^-$ when cellular GSH levels are compromised [32,33].

### *9.2. Alzheimer's disease*

A decrease in complex IV activity has been reported in the cerebral cortex of individuals who died with Alzheimer's disease [63]. Whilst the exact mechanism for this loss of activity is not clear, it is known that this enzyme complex is particularly susceptible to oxidative damage [3,19,21,22,24]. In addition, there is now evidence to suggest that NO metabolism is affected in Alzheimer's disease. The glial derived factor, S100-β, which is over expressed in this condition, causes induction of astrocyte iNOS- and

NO-mediated neuronal cell death in a co-culture system [64]. Furthermore, β-amyloid is reported to activate NOS in a substantia nigra/neuroblastoma hybrid cell line [65]. Analysis of post mortem material has revealed, in Alzheimer brain tissue, the presence of nitrotyrosine residues [66]. Formation of nitrotyrosine is thought to occur as a result of the reaction between $ONOO^-$ and tyrosine residues in protein and is not detectable in age-matched control brains [66]. Studies using monospecific antibodies to iNOS have also revealed the presence of this isoform in neurofibrillary tangle-bearing neurones [67]. Despite evidence for activation of NO metabolism in Alzheimer's disease, analysis of the cerebrospinal fluid (CSF) nitrite+nitrate (stable degradation products of NO and $ONOO^-$) concentration revealed levels in Alzheimer's patients comparable to controls [68]. Whilst this observation does not dismiss a role for NO/$ONOO^-$ in the aetiology of Alzheimer's disease, it implies that if formation of these molecules occurs, it is of a localised nature and is of insufficient magnitude to cause a rise in CSF nitrite+nitrate concentration.

### 9.3. Multiple sclerosis

Considerable evidence is also available to support the suggestion that enhanced generation of NO and $ONOO^-$ occurs in multiple sclerosis (MS). Analysis of CSF from patients in the active phase of MS revealed an approximate 70% increase in the nitrite plus nitrate concentration [69]. Further support for the involvement of increased NO metabolism, in MS, comes from post mortem studies which have detected nitrotyrosine residues and elevated levels of mRNA coding for iNOS [70,71]. NADPH diaphorase activity, which can reflect NOS activity, has also been detected in astrocytes associated with actively demyelinating lesions [70].

Localised perturbation of the blood–brain barrier is proposed to be an important event in MS [72]. NO has been shown to cause a loss of barrier integrity in a cell culture model of the blood–brain barrier [73]. Whether NO-mediated mitochondrial damage is the cause of such damage remains to be established. Other experimental models also provide data to support a role for NO/$ONOO^-$ generation in MS; NO donors have been shown to impair oligodendrocyte (myelin generating cells) energy metabolism [29] and cause reversible axonal conduction block (a characteristic of MS), in vitro [74].

Whilst direct evidence for impairment of mitochondrial function in MS is not available, studies upon *N*-acetyl aspartate metabolism support the view that neuronal mitochondrial damage may be a factor in the pathophysiology of MS. *N*-Acetyl aspartate is believed to be synthesised exclusively in neurones [75]. Furthermore, inhibition of the mitochondrial respiratory chain is associated with an impairment of N-acetyl aspartate metabolism, both in vitro and in vivo [76,77]. Studies utilising NMR spectroscopy have revealed a reduction in the signal attributed to N-acetyl aspartate in MS plaques [78]. However, this decrease in N-acetyl aspartate concentration appears, in some cases, to be reversible [78]. Whether such changes actually reflect NO/$ONOO^-$-mediated damage to neuronal mitochondria is, as yet, unclear.

## 10. Ischaemia/reperfusion

Ischaemic brain damage is accompanied by an energy deficiency state and selective neuronal loss [79]. Under such conditions, there is an increase in the extracellular concentration of glutamate [2], which may be neurotoxic due to activation of nNOS (see above for further details). Excess NO generation, as well as causing impairment of energy metabolism and other metabolic processes, may also down regulate glutamate (NMDA) receptors [5], thereby minimising the effect of glutamate. In addition, NO can cause vasodilation and hence increase cerebral blood flow to the infarcted area [2]. These effects may provide an explanation for the contradictory results that have been obtained when non-specific NOS inhibitors have been evaluated in various models of ischaemia [2].

Reperfusion, following ischaemia, may exacerbate the generation of oxidising species, in particular, superoxide [80]. Using a model of graded ischaemia, loss of brain mitochondrial function, at the levels of complexes I, II–III and ATP synthetase, has been reported [81]. Reperfusion was accompanied by a restoration of activity of these mitochondrial components, followed, after 2 h, by a dramatic loss of com-

plex IV activity [82]. The exact mechanism for this loss of complex IV activity is not known, but could involve the initiation of lipid peroxidation (see above).

Ischaemia is also accompanied by the formation of a gliotic scar which is comprised of reactive astrocytes [83]. Since a proportion of these astrocytes have been shown to express iNOS [84], excessive generation of $NO/ONOO^-$, by these cells, may be an important contributing factor to the mitochondrial damage associated with ischaemia.

Excessive generation of NO is implicated to play a role in the brain damage that occurs as a result of perinatal asphyxia. Thus, inhibition of NOS is reported to protect against hypoxic-ischaemia-mediated neurotoxicity [85,86]. Recently, loss of brain ATP concentration and mitochondrial complex II–III and IV activity has been demonstrated in a rat model of perinatal asphyxia [87]. Importantly, administration of an NOS inhibitor, to the mothers, prevented impairment of brain energy metabolism in the hypoxic pups [87].

### *10.1. Amyotrophic lateral sclerosis*

Oxidative stress is proposed to be an important factor in the pathogenesis of amyotrophic lateral sclerosis (ALS) (reviewed in [88]). With regards to the familial form of this disease, mutations in Cu,Zn superoxide dismutase (SOD-1) have been identified [89]. Furthermore, it has been proposed that such mutations increase $ONOO^-$ formation, due to decreased $O_2^{\bullet-}$ scavenging, and enhance the possibility of protein nitrosylation, i.e the active site of SOD is altered allowing greater access of $ONOO^-$ to the copper centre and so favouring the subsequent formation of a nitronium-like species which nitrosylates tyrosine residues [4]. Immunocytochemistry studies have also revealed, in the neurofilament aggregates associated with ALS a close association between SOD-1 and NOS activity [90]. Since, light neurofilament is rich in tyrosine, it is proposed that nitrotyrosine formation occurs which impairs neurofilament assembly and ultimately leads to motoneuron death [90]. Recently, increased nitrotyrosine immunoreactivity has been demonstrated in motor neurones of both sporadic and familial ALS, suggesting that $ONOO^-$-mediated oxidative damage may play a role in the pathogenesis of both forms of the disease [91]. Some evidence is now available to suggest that mitochondrial function is impaired in ALS. Thus, a significant reduction in complex IV activity is reported in the spinal cord (ventral, lateral and dorsal regions) of patients with sporadic ALS [92]. In addition, studies with a transgenic mouse model of ALS also suggest that axonal transport of organelles, in particular mitochondrial transport, is impaired and may be an important factor in ALS [93].

## 11. Therapeutic considerations

In view of the importance of antioxidants, in particular GSH, it is possible that agents that are capable of increasing the cellular concentration of this molecule may prove to be of therapeutic nature. Similarly, given the apparent key role of cardiolipin in maintaining optimum complex IV activity, prevention of lipid peroxidation or supplementation with linoleic acid may be of therapeutic use. It is also possible that some existing treatments, for which the mode of action is currently poorly understood, may involve alterations in NO metabolism. Such an example may be interferon-β which is used in the treatment of certain forms of MS [94]. Recently, we have found that preparations containing interferon-β impair the ability of astrocytes to induce NOS and hence generate NO when subsequently exposed to cytokines, such as interferon-γ [95].

## 12. Conclusions

There is now evidence, both direct and indirect, to support the concept that mitochondrial damage occurs in a range of neurological disorders, such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, ischaemia/reperfusion and amyotrophic lateral sclerosis. Furthermore, data is available to suggest increased generation of NO and $ONOO^-$ in these conditions. Whether $NO/ONOO^-$ generation is the direct cause of the mitochondrial damage remains to be demonstrated. However, data, from a wide range of relevant experimental models, clearly show that $NO/ONOO^-$ causes damage to the mito-

chondrial ETC. Further understanding of the interaction of these molecules with the mitochondrial ETC may lead to effective therapeutic strategies for the neurological disorders discussed. However, such studies must pay particular attention to the influence cellular environments have on dictating mitochondrial susceptibility.

## Acknowledgements

V.C.S. was funded by the Multiple Sclerosis Society of Great Britain and Northern Ireland and P.S.B. by the Brain Research Trust (UK). S.J.R.H. and J.P.B. are in receipt of a grant from the British Council – Acciones Integradas Programme. J.P.B. is also grateful to the Junta de Castilla y Leon and C.I.C.Y.T (Spain) for funding his research.

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