Abstract—NO or its derivatives (reactive nitrogen species: RNS) inhibit mitochondrial respiration in two different ways: (i) an acute, potent, and reversible inhibition of cytochrome oxidase by NO in competition with oxygen; and, (ii) irreversible inhibition of multiple sites by RNS. NO inhibition of respiration may impinge on cell death in several ways. Inhibition of respiration can cause necrosis and inhibit apoptosis due to ATP depletion, if glycolysis is also inhibited or is insufficient to compensate. Inhibition of neuronal respiration can result in excitotoxic death of neurons due to induced release of glutamate and activation of NMDA-type glutamate receptors. Inhibition of respiration may cause apoptosis in some cells, while inhibiting apoptosis in other cells, by mechanisms that are not clear. However, NO can induce (and inhibit) cell death by a variety of mechanisms unrelated to respiratory inhibition. © 2002 Elsevier Science Inc.

Keywords—Free radicals, Nitric oxide, Mitochondria, Cell death, Apoptosis, Necrosis, Permeability transition, Respiration

INTRODUCTION

Nitric oxide (NO) and its derivatives (reactive nitrogen species, RNS) inhibit mitochondrial respiration and either stimulate or inhibit cell death depending on conditions. This review outlines these effects and explores the two responses. Related reviews on NO inhibition of mitochondrial respiration [1–5], NO effects on cell death [6–8], or both [9–11] have been published elsewhere.

NO is synthesized from L-arginine by three isoforms of NO synthase, two of which (eNOS and nNOS) are constitutively expressed and are acutely regulated by calcium/calmodulin and phosphorylation, while the third (iNOS) is induced during inflammation and produces higher levels of NO for a longer period [6]. There may also be a mitochondrial isoform (mtNOS), but its origin and status is still unclear [12,13]. NO may also be produced nonenzymatically from nitrite at low pH (< pH 5), e.g., during ischemia [14]. NO diffuses very rapidly both through water and membranes, so NO can easily diffuse from one cell to the next.

NO itself at physiological concentrations (unclear, but probably 0.1–100 nM) is relatively unreactive, and most of its physiological actions are mediated by NO binding to Fe$^{2+}$ in the heme of soluble guanylate cyclase causing activation and cGMP production [6]. However, NO may be converted to a number of more reactive derivatives, known collectively as reactive nitrogen species (RNS). At high concentrations (or within membranes), NO reacts directly with oxygen to produce NO$_2^-$, which rapidly reacts with another NO to give N$_2$O$_3$. NO$_2^-$ may oxidize or nitrate (add an NO$_2^-$ group to) a variety of molecules (including tyrosine), while N$_2$O$_3$ can nitrosate/nitrosylate (add an NO$^+$ group to) amines or thiols. NO reacts at the diffusion-limited rate with O$_2^-$ to produce peroxynitrite (ONOO$^-$), which can oxidize/nitrate other molecules or decay and produce other damaging species (possibly the hydroxyl radical OH$^+$ and NO$_2^-$). NO may indirectly (possibly via N$_2$O$_3$ nitrosate thiols (e.g., in proteins or glutathione) to give S-nitrosothiols (RSNO, e.g., S-nitroso-glutathione and S-nitroso-albumin). The NO$^+$ group is directly transferable between different S-nitro-
the mitochondrial inner membrane. Nitrosothiols (RSNO) inhibit creatine kinase, and aconitase. ONOO− stimulates proton leak through respiratory complexes (I, II, III, IV), the ATP synthetase (ATPase), and oxygen binds between them (and is rapidly reduced by them) when both metals are reduced ($a_{3}^{2+}$ and Cu$^{+}$). NO can either (i) bind to reduced cytochrome $a_{3}$ to give cytochrome $a_{3}^{2+}$-NO, or (ii) bind and reduce oxidized Cu$^{2+}$ to give Cu$^{+}$-NO and the NO$^+$ can rapidly hydrate to give nitrite (NO$_2^-$) [23–29]. Both forms of inhibition are rapid and reversible due to (i) debinding of NO, and (ii) debinding of nitrite. The first form of inhibition is competitive with oxygen and reversible by light, whereas the second is not, and these characteristics may be used to distinguish between them [27]. It seems that, at least in vitro, both forms of inhibition may occur simultaneously, but the first form is favored at high levels of cytochrome reduction and low oxygen, whereas the second form is favored by the opposite conditions [27]. However, the inhibition observed in cells in response to NO or iNOS expression appears to be largely competitive with oxygen [18,26,30,31] and reversible by light [32], suggesting that inhibition due to reversible binding to heme $a_{3}^{2+}$ may be more important in cells.

Mitochondrially associated NO synthases (i.e., eNOS, nNOS, or iNOS bound to mitochondria) or mtNOS (a possibly distinct NOS) may produce NO locally at mitochondria and, thus, has the potential to regulate respiration [12,13,33; Guilivi, this series]. For example, NO production from individual mitochondria was recently shown, though absent in NOS-knockout mice; thus, possibly this mtNOS is another splice variant of nNOS [33]. In cardiomyocytes from dystrophin-deficient mice, where this mtNOS was the only apparent form of NOS present, there was beat-to-beat formation of NO, which apparently inhibited cell shortening via the NO inhibition of cytochrome oxidase [33].

Endogenously produced NO may tonically inhibit respiration at cytochrome oxidase in some cells [25,26]. Inhibition is generally in competition with oxygen, so that NO can dramatically increase the apparent $K_m$ of respiration for oxygen [1,17–19]. In synaptosomes (isolated nerve terminals), half-inhibition of respiration occurred at 250 nM NO when the oxygen level was about 150 μM (roughly the arterial level), but the $K_i$ was about 50 nM NO at 30 μM O$_2$ (a median tissue level) [18]. According to this data, then, the presence of 50 nM NO would raise the apparent Km of respiration for oxygen from below 1 to 30 μM O$_2$—well into the physiological range. Thus, NO can make cells effectively hypoxic at
relatively high oxygen levels and potentially sensitize tissues to hypoxic damage. A variety of cells (including macrophages, microglia, astrocytes, endothelial cells, and aorta), when inflammatorily activated to express iNOS, have been shown to produce sufficient NO to not only inhibit their own respiration but also that of surrounding cells via reversible NO inhibition of cytochrome oxidase [30,31,34]. Recently, NO inhibition of cytochrome oxidase and its reversal by light was implicated in regulating the frequency at which fireflies emit bursts of light [35].

Whether NO can regulate mitochondrial respiration in vivo, either physiologically or pathologically, remains an important unresolved problem [2,3]. Hemoglobin and myoglobin both have very high capacities and rates of NO scavenging, which may protect tissues from significant NO inhibition of respiration in vivo [36,37]. The sensitivity of soluble guanylate cyclase to NO appears to be at least two orders of magnitude higher than that of cytochrome oxidase [38], which might indicate that the latter is not a significant physiological target for NO. However, inhibition of NOS in vivo has been shown to cause substantial increases in organ and whole-body oxygen consumption, apparently not due to changes in blood flow, consistent with a tonic inhibition of mitochondrial respiration in vivo [5,39]. On the other hand, it is still not known whether such effects of NO on oxygen consumption are mediated by cGMP, cytochrome oxidase, or other processes [2].

Irreversible inhibition of respiration by NO

Cells exposed to NO (or NO-producing cells) show immediate but reversible inhibition of respiration at cytochrome oxidase. However, after several hours of exposure to NO an irreversible inhibition develops, probably due to conversion of NO to RNS that inhibit respiration at multiple sites [15,20,40,41]. One of the more rapid effects is an inactivation of complex I, possibly due to S-nitrosoation of the complex [20,32], followed by inhibition of aconitase and complex II, possibly due to removal of iron from iron-sulphur centers [42–44] under conditions where peroxynitrite may be formed [44]. Peroxynitrite can inhibit complex I, complex II/III, cytochrome oxidase (complex IV), the ATP synthase, aconitase, Mn-SOD, creatine kinase, and probably many other proteins [1,15,45]. Peroxynitrite is a strong oxidant and can also cause DNA damage, induce lipid peroxidation, and increase mitochondrial proton (and other ion) permeability (probably by lipid peroxidation or thiol cross-linking) [46].

The mechanism(s) by which NO and RNS inhibit complex I remain unclear. The S-nitrosothiols, S-nitrosoglutathione and S-nitrosoacetylpenicillamine, can rapidly inactivate complex I when added to isolated mitochondria, even in conditions when little or no NO is released, and such inhibition is reversed by light or reduced thiols (glutathione or DTT), suggesting that the inactivation is mediated by transnitrosation [32]. However, the inhibition of complex I induced by peroxynitrite (which is a poor nitrosating agent) is also partly reversed by light and reduced thiols [32] (as is the inhibition induced by NO treatment of cells [20]), suggesting that mechanisms other than nitrosation might be involved. It has been suggested recently that peroxynitrite inhibits complex I by tyrosine nitration [47] and NO-induced inhibition of complex I in isolated mitochondria was prevented by peroxynitrite scavengers [40]. An alternative (but not exclusive) target might be one or more iron-sulphur centers in complex I. High concentrations of NO can destroy iron-sulphur centers by binding and displacing the iron, and there is EPR evidence for such damage in complex I [48,49]. Damage must start with NO binding/reacting with the iron and/or cysteine residues that bind the iron, and this initial phase of inhibition might be reversible by light or reduced thiols. Whatever the mechanism, NO inhibition of complex I may be important in cell dysfunction or death. However, NO-induced thiol depletion seems to precede inhibition of complex I, and reduced thiols reverse the inhibition [20].

There is some controversy concerning inhibition of complex II and complex III by peroxynitrite: some authors show that peroxynitrite (and NO) inhibit complex II with little or no effect on complex III [15,49], whereas others find complex III inhibited and complex II unaffected by peroxynitrite [50].

Peroxynitrite has relatively little effect on the V max of cytochrome oxidase when added to mitochondria at levels that inhibit the other complexes [15]. However, it does have various damaging effects on isolated cytochrome oxidase, including particularly increasing the K m for oxygen [51]. High concentrations (>1 μM) of NO (possibly via NO 2 or N 2 O 3 ) can also induce an irreversible rise in K m for oxygen both in isolated cytochrome oxidase or in cells treated with NO [51,52].

Peroxynitrite and possibly NO itself inhibit cytosolic and mitochondrial aconitase, the latter being a component of the Kreb’s cycle and, thus, required for cellular respiration. Aconitase has a 4Fe4S iron-sulphur center, including one particular iron atom that can be removed by peroxynitrite and other oxidants [44], and in some conditions NO itself may S-nitrosate the iron-sulphur center [53]. Mitochondrial creatine kinase aids the export of ATP from mitochondria in muscle and nerves and is inhibited by S-nitrosothiols, probably by transnitrosation of a critical thiol [54–56].
NO-induced ROS, RNS, and MPT

Apart from inhibiting respiration, NO has two other effects on mitochondria relevant to the induction of cell death: (i) induction of ROS and RNS production from mitochondria, and (ii) induction of mitochondrial permeability transition (MPT) by RNS.

The mitochondrial respiratory chain can produce superoxide, which dismutates to hydrogen peroxide, and inhibition of the respiratory chain may enhance the production of these ROS. At moderate levels, NO can acutely increase $O_2^-$ and $H_2O_2$ production by inhibiting mitochondrial respiration, while at higher levels it inhibits $H_2O_2$ production by scavenging the precursor superoxide, resulting in peroxynitrite production [21,57]. NO may also apparently react with ubiquinol ($QH_2$) to produce $NO^-$ (which may react with $O_2$ to produce $ONOO^-$) and ubisemiquinone ($QH^+$) (part of which may react with $O_2$ to produce $O_2^{2-}$) [58]. Thus, NO may cause $O_2^-$, $H_2O_2$, and $ONOO^-$ production, but in other conditions it may produce $NO^-$, NO$_2$, or N$_2$O$_3$. Both NO and O$_2$ are more soluble in lipid bilayers than aqueous solution and, thus, reach higher concentrations within cell membranes that cell cytosol; as the reaction rate between NO and O$_2$ is proportional to the square of the NO concentration and proportional to the O$_2$ concentration, this reaction is much more rapid within cell membranes, including mitochondrial membranes, than in the aqueous phases [59]. Therefore, part of the ability of mitochondria to break down added NO is due to this simple reaction within the bilayer [59], which produces both NO$_2$ and N$_2$O$_3$. Mitochondria can also increase NO breakdown by reactions with superoxide, ubiquinol, and possibly cytochrome oxidase [29,57–60], and this might contribute to the regulation of NO levels in cells.

Reversible NO inhibition of respiration may result in local peroxynitrite production (due to local superoxide production), causing irreversible inhibition of respiration and further oxidant production—a vicious cycle that might contribute to cell death [40].

In addition to stimulating $H_2O_2$ production, NO or RNS can also inhibit catalase, deplete cellular glutathione, and inhibit glutathione peroxidase, thus increasing $H_2O_2$ levels in cells [6,21,61]. Indeed, NO and $H_2O_2$ have been found to be synergistic in killing cells, possibly in part by superoxide dismutase-catalyzed peroxynitrite production [62]. NO may also release iron from iron-sulphur centers and ferritin, potentially causing further oxidative stress [6].

RNS, S-nitrosothiols, or ROS cause MPT in isolated, calcium-preloaded mitochondria [63–65]. MPT is a dramatic increase in permeability of the inner mitochondrial membrane to small (up to 1.5 kDa) molecules (for reviews see [66–69]). Mitochondrial membrane potential and matrix calcium are known to determine the ability of other compounds to induce MPT; thus, inhibition of respiration by NO and subsequent decrease in membrane potential should favor MPT opening. On the other hand, NO itself can inhibit MPT due to the direct inhibition of respiration, preventing calcium accumulation in mitochondria [70]. cGMP (which is formed by guanylyl cyclase after stimulation by NO) may also inhibit MPT in cells via protein kinase G [71]. However, oxidants (such as tert-butyl hydroperoxide and phenylarsine oxide) at high concentrations can induce MPT even in the absence of calcium, and this effect is probably related to direct reaction of these compounds with functional thiols [72,73]. Therefore, NO at high concentrations can promote MPT probably due to either (i) the production of peroxynitrite, nitrosothiols, or NO$_2$/N$_2$O$_3$; or, (ii) depletion/oxidation of glutathione levels. NO/RNS may directly oxidize the protein thiols that regulate opening of the MPT pore [74,75].

MPT plays an important role in both necrotic and apoptotic cell death. MPT dissipates the proton motive force, causing uncoupling of oxidative phosphorylation, and reversal of the ATP synthase, potentially hydrolyzing cellular ATP, resulting in necrosis. MPT also causes rapid swelling of the mitochondria, such that the outer membrane can be ruptured releasing intermembrane proteins like cytochrome c. However, MPT-related cytochrome c release in cells can occur by other mechanisms that do not involve mitochondrial swelling and membrane rupture. Release of cytochrome c and matrix components, such as NADH, inhibits respiration, potentially causing necrosis. But release of cytochrome c and other apoptogenic intermembrane proteins, such as AIF and SMAC/Diablo, potentially triggers apoptosis [76,77]. Transient MPT opening may be a physiological process and usually does not cause cell damage, while longer, sustained MPT opening may cause either apoptosis or necrosis [78,79]. The mode of cell death after MPT opening is likely to depend on additional factors, such as activation of Bid/Bax/Bad pathway or availability of ATP (ATP depletion probably favoring necrosis) [67]. Calcium has been suggested to cause cytochrome c release from mitochondria and subsequent apoptosis by stimulating mtNOS to produce peroxynitrite, which then induces MPT or related processes [13].

NO-INDUCED CELL DEATH

NO-induced cell death is important in two contexts: (i) host cells kill pathogens using NO; and, (ii) in a variety of inflammatory, ischemic, and neurodegenerative diseases, NO kills host cells.

NO and RNS kill cells by a variety of different mechanisms (that are still not clearly defined), which differ
leading to ATP depletion-induced necrosis. 

Increased ATP content and turnover reduced GAPDH activity, increased glycolysis, and dephosphorylation. NO-producing macrophages or cells exposed to NO donors exhibit reduced GAPDH activity, increased glycolysis, and decreased ATP content and turnover [83–85], potentially leading to ATP depletion-induced necrosis.

**NO-induced necrosis**

Energy depletion-induced necrosis results from NO and/or its derivatives causing ATP depletion via four different mechanisms: (i) inhibition of mitochondrial respiration, (ii) induction of MPT, (iii) inhibition of glycolysis at glyceraldehyde-3-phosphate dehydrogenase, and (iv) activation of poly-ADP ribose polymerase (PARP; or synthetase, PARS).

It is important to note that inhibition of mitochondrial respiration in cultured cells or in vivo, induced for example by anoxia, cyanide, or carbon monoxide, is alone sufficient to cause rapid death of a wide range of cells, including particularly neurons and cardiomyocytes; thus, NO inhibition of respiration in these cells should also be sufficient to cause rapid death. However, respiratory inhibition does not kill other cell types, such as skeletal muscle or cell lines derived from tumor cells, due to their high glycolytic capacity. An important implication of NO raising the Km of cytochrome oxidase for oxygen is that NO should sensitize cells to hypoxia-induced cell death; however, this has not been experimentally tested.

NO does not inhibit glycolysis directly; however, peroxynitrite directly oxidizes the active-site cysteine residue of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), causing irreversible inhibition [80], and S-nitrosothiols can apparently S-nitrosate the active-site cysteine residue causing reversible inhibition, but this can further react with NADH or glutathione to cause irreversible inhibition [81]. Reduced thiols like glutathione can prevent such inhibition, and the dehydrogenase is not rate limiting for glycolysis so that effective inhibition of glycolysis may only occur when glutathione is substantially depleted in the cell. On the other hand, NO/RNS can cause slow depletion of cellular glutathione [20,82], potentiating the inhibition of glycolysis, respiration at complex I, and caspases and thereby converting NO-induced apoptosis into necrosis.

In addition, NO, or more likely RNS, can oxidize the active-site cysteine in GAPDH, inhibiting its dehydrogenase activity and inducing an acyl phosphatase activity in the enzyme [83]. This results in uncoupling of glycolytic flux from ATP synthesis by substrate level phosphorylation. NO-producing macrophages or cells exposed to NO donors exhibit reduced GAPDH activity, increased glycolysis, and decreased ATP content and turnover [83–85], potentially leading to ATP depletion-induced necrosis.

In cells exposed to NO, glycolytic generation of ATP is critical to cell survival because moderate levels of NO invariably inhibit mitochondrial respiration and, thus, mitochondrial ATP production. In the absence of glucose or sufficient glycolytic capacity, moderate levels of NO cause necrosis simply due to respiratory inhibition and consequent ATP depletion (and apoptosis is prevented by the lack of ATP [86,87]). Therefore, in many cells necrosis induced by NO (or NO-producing cells) is prevented by glucose [87,88]. If, however, MPT is chronically stimulated by NO derivatives, then respiration may be inhibited, oxidative phosphorylation uncoupled, and glycolytic ATP hydrolyzed by reversal of the ATPase; thus, necrosis might proceed even in the presence of rapid glycolysis. However, there is to date little direct evidence that MPT is involved in NO-induced necrosis. The rate at which ATP is being consumed by the cell also may be important in determining the sensitivity to energy depletion-induced necrosis, as suggested by the synergy between NO and impulse frequency in neuronal death [89].

Another potential cause of inhibition of glycolysis is depletion of NAD+ due to activation of PARP. PARP is a nuclear protein, which, when activated by DNA strand breaks (possibly caused by peroxynitrite or NO2/N2O3), catalyzes multiple ADP-ribosylation (using NAD+ as substrate) of proteins including particularly itself [90]. If DNA damage is extensive, then PARP activity is so high that cytosolic NAD+ is depleted (causing inhibition of glycolysis) and adenine nucleotides may also be depleted (either because they are substrates for NAD+ synthesis or because glycolysis is inhibited). Thus, PARP inhibitors or PARP knockouts can protect against NO-induced cell death in some cell types and conditions; however, these inhibitors are ineffective in other cells and conditions [90–92]. But activation of PARP may contribute to energy depletion of the cell, possibly in synergy with inhibition of respiration, inhibition of glycolysis, and/or activation of MPT (Fig. 2). Recently, it has been suggested that PARP activation can by some undefined means cause mitochondrial permeabilization and release of factors such as AIF to cause a form of apoptosis that is not caspase mediated [93].

**NO-induced apoptosis**

NO can induce apoptosis in cells or conditions where respiratory or glycolytic ATP production is sufficient. Although specific respiratory inhibitors can induce apoptosis in such conditions, induction is weaker and slower so respiratory inhibition cannot fully explain NO-induced apoptosis [65,87]. NO-induced apoptosis is mediated by downstream caspases (such as caspase-3) and is blocked by caspase inhibitors [65,87,94]. Cytochrome c
is generally released, suggesting that NO-induced apoptosis is normally mediated by mitochondria; but, in some cell types, early activation of caspase-8 or caspase-2 is observed, indicating that NO-induced apoptosis may be triggered nonmitochondrial pathways [94–96].

How NO induces apoptosis is still poorly characterized [7,8], but the main routes may include: (i) MPT [7,65,97]; (ii) oxidation of mitochondrial phospholipids [98–100]; (iii) upregulation of proapoptotic proteins, such as p53 [100,101] and Bax, and downregulation of antiapoptotic Bcl-2 [102]; (iv) activation of MAP kinase pathways [103]; or, (v) activation of the endoplasmic reticulum stress-response pathway [104,105] (Fig. 3).

NO by itself usually does not induce MPT; however, peroxynitrite, S-nitrosothiols, NO2, and/or N2O3 may...
cause apoptosis by directly activating MPT, leading to cytochrome c release and caspase activation, and these events can be blocked with the MPT inhibitor cyclosporin A [7,65,97]. Apoptosis induced by “pure” NO donors, such as the NONOates, is less susceptible to inhibition by cyclosporin A but is inhibited by antioxidants (such as N-acetyl-L-cysteine, catalase, superoxide dismutase, ascorbate, and Trolox) and thus may require NO-induced ROS or RNS [65,94,106].

NO treatment of cells can cause oxidant-induced degradation of the mitochondrial phospholipid cardiolipin (probably due to peroxynitrite- or NO2-induced peroxidation), which is associated with irreversible inhibition of the respiratory chain and apoptosis [98]. Cytochrome c is normally bound to cardiolipin on the inner mitochondrial membrane, but peroxidation causes the release of cytochrome c [99]. Addition of peroxynitrite to isolated mitochondria causes lipid peroxidation and thiol cross-linking associated with increased proton and ion permeability, depolarization, and rapid swelling, which in the absence of calcium may be not mediated by MPT [46]. It has also been reported that addition of calcium to isolated mitochondria stimulates mtNOS, causing peroxynitrite production and (cyclosporin-insensitive) cytochrome c release associated with peroxidation of mitochondrial lipids [13]. Mitochondrial lipid peroxidation may be an important event in mitochondria-mediated apoptosis [107], but there is no direct evidence that lipid peroxidation mediates NO-induced apoptosis.

NO can upregulate Bax protein levels in cells and cause translocation of Bax from cytosol to mitochondrial outer membranes [102,103]. The mechanism by which NO initiates Bax translocation to mitochondria is unclear. NO-induced p38 MAP kinase activation has been shown to mediate Bax activation and translocation [103]. It was reported also that incubation of cells with mitochondrial uncouplers or with inhibitors of the respiratory chain can trigger Bax translocation to mitochondria, suggesting that a decrease of mitochondrial membrane potential is enough to induce Bax translocation [108,109], which leads to apoptosis if ATP levels are maintained. Thus, it is possible that NO triggers Bax association with mitochondria by inducing a collapse of membrane potential due to respiratory inhibition. Respiratory inhibitors (and hypoxia) can induce apoptosis in cell lines [65,87,108,110], possibly via a partial fall in ATP [111], an increase in ROS production, or the decrease of mitochondrial membrane potential, which may either activate MPT or induce Bax translocation to mitochondria, or otherwise activate Bax or Bak [103,108,110]. However, it is clear that NO can and usually does induce apoptosis by mechanisms unrelated to respiratory inhibition.

NO can also inhibit apoptosis induced by other agents. Several mechanisms have been suggested: (i) cGMP-mediated blockage upstream of cytochrome c release (cGMP has been shown to inhibit MPT in the presence of protein kinase G [71]), (ii) cGMP-mediated inhibition of ceramide synthesis, (iii) S-nitrosylation of caspases, (iv) energy depletion, (v) mitochondrial hyperpolarization, (vi) activation of MAP kinase pathways, (vii) activation of transcription factors NF-κB and/or AP-1, and (viii) increased expression of heat shock proteins and Bcl-2 [8]. Recently, there have been reports that NO can cause an increase in mitochondrial membrane potential in some cells (astrocytes, peritoneal macrophages) but not in others (rat cortical neurons, thymocytes) [112–114]; the increase has been suggested to inhibit apoptosis [112,113,115]. How NO increases mitochondrial membrane potential is unknown; it may be caused by a secondary stimulation of glycolytic ATP production, used by the ATPase to maintain mitochondrial membrane potential [112,113,115].

**NO-induced excitotoxicity**

Neurons are exceptionally sensitive to NO or NO-producing cells [116]. One reason is that NO inhibition of neuronal respiration causes glutamate release and subsequent excitotoxic death of neurons [34] (Fig. 4). NO, hypoxia, and specific respiratory inhibitors all cause

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**Fig. 4.** Possible pathway of NO-induced neuronal excitotoxic cell death. ΔΨm = plasma membrane potential; NMDA-R = N-methyl-D-aspartate receptor.
rapid ATP depletion of neurons and glutamate release, apparently due to ATP limitation of the Na⁺ pump leading to decline of the plasma membrane potential and Na⁺ gradient, causing reversal of the Na⁺ -coupled glutamate uptake carrier [34,117]. High concentrations of extracellular glutamate (the main excitatory neurotransmitter in the brain) can kill neurons via activation of glutamate receptors, in particular the NMDA receptor, and such neuronal death is known as excitotoxic death. Respiratory inhibitors can strongly potentiate such death by depolarizing the plasma membrane, as depolarization is required together with glutamate for activation of NMDA receptors, which once activated act as ion channels allowing both Na⁺ and Ca²⁺ into the neuron [118].

Astrocytes and microglia (brain macrophages) become inflammatoryy activated in most CNS pathologies, including during infection, trauma, aging, after ischemia or stroke, and most inflammatory and neurodegenerative diseases [119]. These activated glia express iNOS and produce high levels of NO capable of inhibiting respiration both within themselves and surrounding neurons [34,120]. Thus, activated glia coincubated with neurons cause rapid inhibition of neuronal respiration at cytochrome oxidase, which then results in rapid glutamate release from the neurons and in turn causes excitotoxic death of the neurons [34,121]. This may be one important means by which brain inflammation, present in most CNS pathologies, causes neuronal death.

In some cases, inhibition of nNOS blocks glutamate-induced death of neurons, and cell death has been attributed to NO and calcium-induced mitochondrial depolarization, although mechanisms remain unclear [116,122–124]. NO and NO-producing astrocytes cause irreversible damage to respiratory complexes of neuronal mitochondria [9], but this now appears to be secondary to glutamate release and excitotoxicity [125]. Activation of PARP may also be involved in the neurotoxicity of NO [91].

CONCLUSIONS

To summarize, NO inhibits mitochondrial respiration in two ways: (i) reversible inhibition at cytochrome oxidase by NO, and (ii) irreversible inhibition or inactivation of complex I, complex II/III, cytochrome oxidase, the ATP synthase, creatine kinase, and aconitase by peroxynitrite or S-nitrosothiols. The reversible inhibition of cytochrome oxidase is caused either by NO binding to reduced heme a₃ in competition with oxygen (reversible by light or removing the bulk NO) or by NO binding to oxidized Cu₉₆ resulting in the formation of an inhibitory nitrite ion. Although NO from constitutive forms of NOS or mitochondrial NOS can regulate mitochondrial respiration in vitro and in cultured cells, it is still unclear whether this occurs significantly in vivo. iNOS produces higher levels of NO for a longer period, causing potent inhibition of respiration at cytochrome oxidase followed by irreversible inhibition of other mitochondrial components.

There are three main roles of mitochondria in NO-induced cell death: (i) NO inhibition of respiration can (if glycolysis is insufficient to compensate) induce necrosis (or excitotoxicity in neurons) and inhibit apoptosis via ATP depletion, (ii) RNS-induced MPT may induce apoptosis or necrosis, and (iii) RNS/ROS-induced signal transduction, ER stress, lipid peroxidation, or DNA damage may activate the mitochondrial pathway to apoptosis. [41]

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ABBRVATIONS

eNOS—endothelial nitric oxide synthase
FADD—Fas-associated death domain
GAPDH—glyceraldehyde-3-phosphate dehydrogenase
iNOS—inducible nitric oxide synthase
MAP kinase—mitogen-activated protein kinase
MPT—mitochondrial permeability transition
NMDA—N-methyl-D-aspartate
nNOS—neuronal nitric oxide synthase
PARP—poly-ADP ribose polymerase
RNS—reactive nitrogen species
ROS—reactive oxygen species