Nitric Oxide and Its Congeners in Mitochondria: Implications for Apoptosis

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Apoptosis is an evolutionarily conserved form of physiologic cell death important for tissue development and homeostasis. The causes and execution mechanisms of apoptosis are not completely understood. Nitric oxide (NO) and its congeners, oxidative stress, Ca\(^{2+}\), proteases, nucleases, and mitochondria are considered mediators of apoptosis. Recent findings strongly suggest that mitochondria contain a factor or factors that upon release from the destabilized organelles, induce apoptosis. We have found that oxidative stress-induced release of Ca\(^{2+}\) from mitochondria followed by Ca\(^{2+}\) reuptake (Ca\(^{2+}\) cycling) causes destabilization of mitochondria and apoptosis. The protein product of the protooncogene bcl-2 protects mitochondria and thereby prevents apoptosis. We have also found that NO and its congeners can induce Ca\(^{2+}\) release from mitochondria. Thus, nitrogen monoxide (NO) binds to cytochrome oxidase, blocks respiration, and thereby causes mitochondrial deenergization and Ca\(^{2+}\) release. Peroxynitrite (ONOO\(^-\)), on the other hand, causes Ca\(^{2+}\) release from mitochondria by stimulating a specific Ca\(^{2+}\) release pathway. This pathway requires oxidized nicotinamide adenine dinucleotide (NAD\(^+\)) hydrolysis to adenosine diphosphate ribose and nicotinamide. NAD\(^+\) hydrolysis is only possible when some mitochondrial thiol groups are cross-linked. ONOO\(^-\) is able to oxidize them. Our findings suggest that NO and its congeners can induce apoptosis by destabilizing mitochondria via deenergization and/or by inducing a specific Ca\(^{2+}\) release followed by Ca\(^{2+}\) cycling. — Environ Health Perspect 106(Suppl 5): 1125–1130 (1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-5/1125-1130richter/abstract.html

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Mitochondria and Cell Death

Ca\(^{2+}\)-Induced Necrotic and Apoptotic Cell Death

Apoptosis is an evolutionarily conserved form of physiologic cell death important for tissue development and homeostasis. Its hallmarks are distinct morphologic alterations such as nuclear condensation, cell shrinkage, and bleb formation, and the absence of inflammatory responses of the affected tissue. Deranged apoptosis plays a major role in diseases such as cancer, acquired immune deficiency syndrome, autoimmune diseases, and neurodegenerative [reviewed by Thompson (1)].

Necrosis and apoptosis are distinct forms of cell death, the former being a passive process typified by gross damage and spillage of the intracellular content, the latter being a highly regulated and controlled process that avoids inflammation and damage to the surrounding tissue (2). Attention was drawn to Ca\(^{2+}\)-induced cell death many years ago [(3); for a recent review see Trump and Berezovsky (4)]. Excessive intracellular Ca\(^{2+}\) is thought to contribute to a final common pathway of cytotoxic events leading to formation of reactive oxygen species (ROS) and necrosis or apoptosis. These events include over-activation of protein kinase C, Ca\(^{2+}\)/calmodulin-dependent protein kinase II, phospholipases, proteases, protein phosphatases, xanthine oxidase, endonucleases, and nitric oxide synthase (NOS).

Although the exact role of Ca\(^{2+}\) in cell killing is unclear, a disturbance of mitochondrial Ca\(^{2+}\) handling can be fatal [for reviews see Nicotera et al. (5) and Richter (6)]. The normal Ca\(^{2+}\) uptake and release (cycling) across the inner mitochondrial membrane requires little energy (7). However, when the Ca\(^{2+}\) release pathway is stimulated by prooxidants, cycling may become excessive and lead to loss of the mitochondrial membrane potential (DY), general leakiness of the inner mitochondrial membrane, inhibition of adenosine triphosphate (ATP) synthesis, mitochondrial damage, and cell death (8).

Several conditions, molecules, or organelles such as oxidative stress, Ca\(^{2+}\), proteases, nucleases, or mitochondria are considered participants of apoptosis, but at present it is not always clear whether they are required for or are the consequence of apoptosis.

There is ample evidence that apoptosis is accompanied by oxidative stress [reviewed by Burkte and Sandstrom (9)]. A valuable tool used to elucidate the importance of oxidative stress is the protooncogene bcl-2, which stimulates an antioxidative response in cells and prevents apoptosis (10, 11).

The requirement of Ca\(^{2+}\) for apoptosis is also controversial (12). Early reports suggested that a rise of the intracellular Ca\(^{2+}\) leads to apoptosis via endonuclease activation, and more recent work indicated that apoptosis is accompanied by shifts of Ca\(^{2+}\) between various intracellular pools. It is worth noting that cellular Ca\(^{2+}\) handling and ROS production are related. Thus, increased mitochondrial Ca\(^{2+}\) release followed by reuptake driven by DY (Ca\(^{2+}\) cycling) stimulates ROS production (13).

Mitochondria in Apoptosis

Although the causes and execution mechanisms of apoptosis are not clearly understood, oxidative stress, NO (NO indicates nitric oxide independent of its redox state, whereas "NO, NO\(^-\), and NO\(^\cdot\)" refer to the nitrogen monoxide radical, nitrosonium ion, and nitrosyl anion, respectively) and its...
congeners, Ca\textsuperscript{2+}, proteases, nuclease, and mitochondria are important mediators of apoptosis. Recently, the role of mitochondria, particularly in apoptosis, was scrutinized (14,15). At present, their importance and exact role are elusive, but it is clear that mitochondria are both the target and the source of oxidative stress, NO, and Ca\textsuperscript{2+}. During apoptosis DY, which is the driving force for mitochondrial ATP synthesis, declines, and maintenance of DY prevents apoptosis. Because apoptosis is highly regulated and involves the activity of hydrolytic enzymes, chromatin condensation and vesicle formation apoptosis is likely to have a high energy demand. Indeed, it was recently proposed that apoptosis induced by intracellular Ca\textsuperscript{2+} overload in neurons requires active mitochondria (16). We have proposed (15) that the cellular ATP level is an important determinant for cell death.

Another line of evidence also puts mitochondria on the center stage of apoptosis; when they are destabilized, for example, by Ca\textsuperscript{2+} cycling, they release proteins, some of which induce apoptosis. One is cytochrome c, which acts with cytosolic factors to induce nuclear apoptosis (17). The other is a 50-kDa protein that by itself suffices to cause nuclear apoptosis (18). The present knowledge suggests that mitochondria function as a cellular sensors of stress into which very different apoptosis induction pathways converge and that mitochondria act as central apoptotic executioner (19).

Presently, a popular concept in apoptosis is the so-called mitochondrial permeability transition, a phenomenon related to the opening of a putative pore in the inner mitochondrial membrane. In the author’s opinion, the mitochondrial permeability transition represents the initial phase of unspecific damage to mitochondria that can, for example, be induced by mitochondrial Ca\textsuperscript{2+} cycling (20). Whether a pore opens or mitochondria are unspecifically damaged may not be relevant for apoptosis. The crucial event appears to be the release of proapoptotic factors such as cytochrome c or proteases from mitochondria.

**NO in Mitochondria**

**Biology of NO**

NO presently receives enormous attention. It mediates beneficial responses such as maintenance of blood pressure, inhibition of platelet aggregation, tumoricidal activities, or destruction of foreign invaders in the immune response, and is probably of major importance in long-term memory. However, when produced in excessive amounts, NO can become toxic, for example, during septic shock.

The dichotomy of NO is in part due to a broad array of redox species with distinctive properties and reactivities: NO\textsuperscript{•}, NO\textsuperscript{−}, and NO\textsuperscript{3−} [reviewed by Stamler et al. (21) and Stamler (22)], and the ability of NO\textsuperscript{•} to combine with superoxide anion radicals (O\textsubscript{2}−) to yield peroxynitrite (ONOO\textsuperscript{•}) (23).

ONOO\textsuperscript{•} is an efficient oxidant of thiols (24). Its in vivo formation was recently shown in a variety of cells (25). ONOO\textsuperscript{•} production is associated with the activation and expression of inducible NOS and implicated in the pathophysiology of diseases such as acute endotoxemia, inflammatory bowel disease, neurologic disorders, and atherosclerosis. Inhibition by superoxide dismutase, the O\textsubscript{2}− scavenger, of NO-mediated cytotoxicity suggests that ONOO\textsuperscript{•} contributes to the NO-mediated biologic effects (26).

NO\textsuperscript{•} is formed from NO by reduced superoxide dismutase (27). It is another NO congener that oxidizes thiols. NO\textsuperscript{•} is, like ONOO\textsuperscript{•}, neuroprotective at N-methyl-D-aspartate receptors because these compounds lead to disulfide formation at critical thiols of the redox modulator site of the receptor (28,29), which inhibits the Ca\textsuperscript{2+} entry into the cell.

**NO in Mitochondria**

**Nitrogen Monoxide and the Regulation of Cytochrome Oxidase**

The most-cited and best-understood physiological target of NO is guanylyl cyclase. NO binds to and stimulates it and thus controls cell functions via (cGMP), cGMP-gated channels, cGMP-dependent protein kinases, and phosphodiesterases. However, NO also binds to cytochrome oxidase and reversibly inhibits respiration as seen with the isolated enzyme, submitochondrial particles, mitochondrion, hepatocytes, brain nerve terminals, and astrocytes (30–36). Cytochrome oxidase inhibition is competitive with oxygen because of binding of NO to the oxygen binding site of the reduced enzyme (37,38). Why the inhibition is transient is not clear at the moment, but several findings point to consumption of NO as the underlying reason (39). Thus, cytochrome oxidase can reduce NO (40). NO can combine with O\textsubscript{2} to form NO\textsubscript{3−} and with O\textsubscript{2}− to form ONOO\textsuperscript{•}.

Concentrations of NO measured in a range of biologic systems are similar to those that inhibit cytochrome oxidase and mitochondrial respiration, and inhibition of NO synthesis results in a stimulation of respiration in many systems. It was, therefore, proposed that NO exerts a good part of its physiologic and pathologic effects on cells by inhibiting cytochrome oxidase (41).

**Presence of Nitric Oxide Synthase in Mitochondria.** NO is present within mitochondria (42). This offers exciting new insights into the biology of NO. For example, because the enzyme is stimulated by Ca\textsuperscript{2+} and located in the matrix or the inner side of the inner mitochondrial membrane, this may provide a self-regulating system for mitochondrial Ca\textsuperscript{2+} homeostasis in which Ca\textsuperscript{2+} uptake by mitochondria would lead to NO formation. NO could promote Ca\textsuperscript{2+} release by collapsing DY via inhibition of cytochrome oxidase.

**Other Putative Targets of NO in Mitochondria**

NO not formed inside mitochondria may also have a profound impact on the organelles, as it is uncharged and can easily traverse membranes. For example, extramitochondrially formed NO could combine in mitochondria with O\textsubscript{2}− and form ONOO\textsuperscript{•}, which could then stimulate Ca\textsuperscript{2+} release from mitochondria with maintenance of DY. NO has been compared with ONOO\textsuperscript{•} as to its inhibitory capacity in mitochondria. Aconitase is the principal site of inhibition by ONOO\textsuperscript{•} (and O\textsubscript{2}−), whereas this enzyme is resistant to NO (43,44).

Another possibility would be a NO-catalyzed auto-ADP-ribosylation of mitochondrial NAD-binding proteins, as reported for several cytosolic enzymes (45,46). Also, mitochondria are rich in glutathione and contain key sulfhydryl enzymes such as the adenine nucleotide translocator or creatine kinase that are putative targets of nitric oxide congeners.

**Reactive Oxygen and Nitrogen Species as Regulators of Mitochondrial Ca\textsuperscript{2+} Homeostasis**

**Mitochondria and Cellular Ca\textsuperscript{2+} Homeostasis**

Intracellular Ca\textsuperscript{2+} regulates many processes. Its concentration is adjusted by binding to nonmembranous proteins, by mitochondria, and by membrane-bound Ca\textsuperscript{2+}-ATPases located primarily in the plasma, nuclear, and endoplasmic reticular membrane [reviewed by Carafoli (7)]. Mitochondria contain Ca\textsuperscript{2+}-sensitive targets regulated by moderate Ca\textsuperscript{2+} transients. These organelles are also able to take up...
large amounts of Ca<sup>2+</sup> and buffer the cytosolic Ca<sup>2+</sup>. They thereby act as safety devices against potentially toxic increases of cytosolic Ca<sup>2+</sup> [reviewed by Richter and Kass (8)]. Mitochondria take up and release Ca<sup>2+</sup> by separate routes. As a consequence, Ca<sup>2+</sup> is cycled across their inner membranes (7).

Mitochondria are of central importance for physiologic Ca<sup>2+</sup> handling. They act as a reservoir for Ca<sup>2+</sup>, provide much of the ATP used by Ca<sup>2+</sup>-ATPases, and Ca<sup>2+</sup> regulates the activity of intramitochondrial dehydrogenases as well as nucleic acid and protein synthesis (47).

The importance of mitochondria as short-term modulators of cytosolic Ca<sup>2+</sup> under physiologic conditions was until recently considered minor. However, there is now compelling evidence [reviewed by Rizzuto et al. (48) and Hajnoczky et al. (49)] that during physiologic cell stimulation, mitochondrial Ca<sup>2+</sup> transport directly participates in the modulation and maintenance of cellular Ca<sup>2+</sup> homeostasis. Several reports have additionally documented that physiologic cytosolic Ca<sup>2+</sup> pulses are relayed into mitochondria of brain, liver, and Xenopus laevis oocytes (50-52).

**Mitochondrial Ca<sup>2+</sup> Release**

In principle, Ca<sup>2+</sup> can leave mitochondria in three ways: by nonspecific leakage through the inner membrane, by reversal of the influx carrier, and by an Na<sup>+</sup>-dependent or -independent release pathway (7,53). Only the latter two are physiologically relevant because they operate when DY is high. The Na<sup>+</sup>-dependent pathway predominates in mitochondria of heart, brain, skeletal muscle, adrenal cortex, brown fat, and most tumor tissue. The Na<sup>+</sup>-independent pathway is important in liver, kidney, lung, and smooth muscle mitochondria, probably exchanges Ca<sup>2+</sup> with H<sup>+</sup>, and is linked to the redox state of mitochondrial pyridine nucleotides. Compounds causing their oxidation (and hydrolysis) promote Ca<sup>2+</sup> release from intact mitochondria. This release has recently been reviewed (8,47,54).

**Prooxidant-Induced, NAD<sup>+</sup>-Linked Ca<sup>2+</sup> Release**

**NAD<sup>+</sup> Hydrolysis Is Required for Ca<sup>2+</sup> Release from Intact Mitochondria.**

Hydrogen peroxide can stimulate a specific Ca<sup>2+</sup> release pathway from intact mitochondria by oxidizing mitochondrial pyridine nucleotides through the activities of glutathione peroxidase, glutathione reductase, and the energy-linked transhydrogenase. Other prooxidants such as menadione, allylthiol, and divinyl also stimulate the specific Ca<sup>2+</sup> release because they furnish NAD<sup>+</sup>. The specific Ca<sup>2+</sup> release requires for its activation the hydrolysis of intramitochondrial NAD<sup>+</sup> to ADP-ribose and nicotinamide and is prevented by inhibitors of NAD<sup>+</sup> hydrolysis and protein mono(ADP-ribosyl)ation. Recent experiments reveal that NAD<sup>+</sup> hydrolysis and therefore Ca<sup>2+</sup> release is regulated by vici-

**NO Inhibits Cytochrome Oxidase and Causes Ca<sup>2+</sup> Release from Mitochondria**

"NO at submicromolar, physiologically relevant concentrations potently deenergizes isolated mitochondria (32). Deenergization is observed when mitochondria use respiratory substrates such as pyruvate plus malate, succinate, or ascorbate plus tetramethyl-phenylenediamine, but not when mitochondria are energized with ATP, and is due to a transient inhibition of cytochrome oxidase. The extent and duration of deenergization are determined by the concentration of ‘NO and oxygen and the type of respiratory substrate. The ‘NO-induced changes of the mitochondrial energy state are transient and are paralleled by release and reuptake of mitochondrial Ca<sup>2+</sup>. Importantly, cytochrome oxidase is particularly sensitive to ‘NO at oxygen concentrations below 30 μM (59), i.e., at intracellular oxygen tensions. These findings reveal a direct action of ‘NO on the mitochondrial respiratory chain and suggest that ‘NO exerts some of its physiologic and pathologic effects by deenergizing mitochondria.

In freshly prepared hepatocytes, ‘NO also deenergizes mitochondria (33). Deenergization is reversible at low concentrations but longer lasting at higher ‘NO concentrations. The drop and the recovery of DY are accompanied by a rise and fall of cytosolic Ca<sup>2+</sup> levels. NO at higher concentrations, provided by nitrosoglutathione in combination with dithiothreitol (GSNO/DTT), kills hepatocytes. Killing is reduced when the cytosolic Ca<sup>2+</sup> is chelated or when Ca<sup>2+</sup> cycling by mitochondria is prevented by CSA. Apparently NO can kill cells by releasing Ca<sup>2+</sup> from mitochondria and thereby flooding the cytosol with Ca<sup>2+</sup>.

**bcl-2 Links Oxidative Stress, Ca<sup>2+</sup>, and the Mitochondrial Membrane Potential to Apoptosis**

Given that bcl-2 elicits an antioxidant response in cells, what are the biochemical mechanism(s) by which bcl-2 prevents apoptosis? It was shown (60,61) with the aid of ruthenium red, an inhibitor of the mitochondrial Ca<sup>2+</sup> uptake, that one mechanism is the prevention of ROS-induced mitochondrial Ca<sup>2+</sup> cycling, a process that results in a collapse of DY and in cellular ATP depletion. Thus, bcl-2 prevents disturbances of the cellular Ca<sup>2+</sup> homeostasis and ROS production at the mitochondrial level. On the basis of these and other findings, it was suggested (6)
that a prooxidant-induced Ca\textsuperscript{2+} release from mitochondria, followed by Ca\textsuperscript{2+} cycling and ATP depletion, is a common cause of apoptosis. Accordingly, maintenance of DY stabilizes mitochondria and thereby prevents apoptosis. bel-2 thus provides the link between the antioxidant defense system, Ca\textsuperscript{2+}, and DY [reviewed by Bornkamm and Richter (62)]. In this context it is interesting to recall that many carcinoma cells have an increased DY (63). As prevention of apoptosis seems to contribute to carcinogenesis, it is conceivable that DY contributes to the decision between the life and death of a cell.

NO and ONOO\textsuperscript{•} in Apoptosis

Early studies had indicated that NO can cause (64–67) or inhibit (68) apoptosis. It had also been found that ONOO\textsuperscript{•} induces apoptosis in a time- and concentration-dependent manner (26) and that depending on the concentration of ONOO\textsuperscript{•}, cells die either by apoptosis or necrosis (69). Recent studies have now made it clear that, depending on the cell type and trigger of apoptosis, NO and its congeners indeed either stimulate or abrogate apoptotic cell death (70), and S-nitrosylation regulates the balance between apoptosis and necrosis (71). The mechanisms by which the reactive nitrogen species act is not always evident, but they comprise gene activation, DNA damage, poly(ADP-ribose) polymerase and protease modulation. Apoptosis is inhibited by NO and its congeners in neuroblastoma cells (72), human embryonic kidney HEK-293 cells (73), hepatocytes (74–76), endothelial cells (77–79), and human leukocytes (80). Apoptosis is stimulated by NO and its congeners in lung epithelial cells (81), intestinal epithelial cells (82), endothelial cells (83), fibroblasts (84), hematopoietic cells (85), vascular smooth muscle cells (86), HL-60 cells (87–89), mesangial cells (90,91), T lymphocytes (92,93), macrophages (94–96), melanoma cells (97), colon cancer cells (98), hypothalamic cells (99), and neurons (100–103) but not in glial cells (103).

Whether the NO congeners cause apoptosis because of interference with mitochondrial respiration or Ca\textsuperscript{2+} handling is unclear, but it should be noted that the GSNO/DTT-induced killing of hepatocytes (33) appears to engage mitochondrial Ca\textsuperscript{2+} cycling. Other investigators have proposed that NO induces apoptosis via triggering the mitochondrial permeability transition (104).

Conclusion

An exciting new aspect in biology is the discovery that NO congeners have an enormous impact on mitochondria. NOS is active inside mitochondria. Physiologic concentrations of NO at physiologic cellular oxygen pressure inhibit cytochrome oxidase and thereby respiration. A transient inhibition of cytochrome oxidase by NO appears to be used in some forms of cell signaling. ONOO\textsuperscript{•}, the product of the reaction between superoxide anion radicals and NO, can stimulate the specific calcium release pathway from mitochondria by oxidizing some vicinal thiols in mitochondria. Mounting evidence indicates that mitochondrial calcium handling and its modulation by reactive nitrogen species is important for apoptotic cell death. It appears that NO and its congeners can induce apoptosis by destabilizing mitochondria via deenergization and/or by inducing a specific Ca\textsuperscript{2+} release followed by Ca\textsuperscript{2+} cycling.

REFERENCES AND NOTES

1. Thompson CB. Apoptosis in the pathogenesis and treatment of diseases. Science 267:1456–1462 (1995).
2. Steller H. Mechanisms and genes of cellular suicide. Science 267:1445–1449 (1995).
3. Schanne FA, Kane A, Young E, Farber J. Calcium dependence of toxic cell death: a final common pathway. Science 206:699–700 (1979).
4. Trump BF, Berezesky IK. Calcium-mediated cell injury and cell death. FASEB J 9:219–228 (1995).
5. Nicotera P, Bellomo G, Orrenius S. Calcium-mediated mechanisms in chemically induced cell death. Annu Rev Pharmacol Toxicol 32:449–470 (1992).
6. Richter C. Prooxidants and mitochondrial Ca\textsuperscript{2+}: their relationship to oncogenesis. FEBBS Lett 325:104–107 (1993).
7. Carafoli E. Intracellular calcium homeostasis. Annu Rev Biochem 56:395–433 (1987).
8. Richter C, Kass GEN. Oxidative stress in mitochondria; its relationship to cellular Ca\textsuperscript{2+} homeostasis, cell death, proliferation, and differentiation. Chem-Biol Interact 77:1–23 (1991).
9. Buttké TM, Sandstrom PA. Oxidative stress as a mediator of apoptosis. ImmunoL Today 15:7–10 (1994).
10. Steinman HM. The Bcl-2 oncprotein functions as a pro-oxidant. J Biol Chem 270:3487–3490 (1995).
11. Garcia I, Martinou I, Tsujimoto Y, Martinou J-C. Prevention of programmed cell death of sympathetic neurons by the bel-2 proto-oncogene. Science 258:302–304 (1992).
12. Beaver JP, Waring P. Lack of correlation between early intracellular calcium ion rises and the onset of apoptosis in thymocytes. Immunol Cell Biol 72:489–499 (1994).
13. Chacon E, Acosta D. Mitochondrial regulation of superoxide by Ca\textsuperscript{2+}: an alternate mechanism for the cardiotoxicity of doxorubicin. Toxicol Appl Pharmacol 107:117–128 (1991).
14. Kroemer G, Perret P, Zamzami N, Vayssiere JL, Mignotte B. The biochemistry of programmed cell death. FASEB J 9:1277–1287 (1995).
15. Richter C, Schweizer M, Cossarizza A, Franceschi C. Hypothesis. Control of apoptosis by the cellular ATP level. FEBBS Lett 378:107–110 (1996).
16. Ankarcrona M, Dybukht JM, Bonfoco E, Zhirovovsky B, Orrenius S, Lipton SA, Nicotera P. Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function. Neuron 15:961–973 (1995).
17. Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell 86:147–157 (1996).
18. Susa SA, Zamzami N, Castedo M, Hirsch T, Marchetti P, Macho A, Daugas E, Geuskens M, Kroemer G. bel-2 inhibits the mitochondrial release of an apoptotic protease. J Exp Med 184:1331–1342 (1996).
19. Susa SA, Zamzami N, Castedo M, Daugas E, Wang H-G, Geley S, Fassy F, Reed JC, Kroemer G. The central executioner of apoptosis: multiple connections between protease activation and mitochondria in Fas/APO-1/CD95- and ceramide-induced apoptosis. J Exp Med 186:25–37 (1997).
20. Schlegel J, Schweizer M, Richter C. "Pore" formation is not required for the hydroperoxide-induced Ca\textsuperscript{2+} release from rat liver mitochondria. Biochem J 285:65–69 (1992).
21. Stanler JS, Singel D, Locolzio J. Biochemistry of nitric oxide and its redox-activated forms. Science 258:1898–1902 (1992).
22. Stanler JS. Redox signalling: nitrosylation and related target interactions of nitric oxide. Cell 78:931–936 (1994).
Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 87:1620–1624 (1990).

24. Radd R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. J Biol Chem 266:4244–4250 (1991).

25. Mulders RB, Folkerts G, Henricke PA, Sadeghi Hashjin G, Nijkamp FP, Peroxynitrite, a two-faced metabolite of nitric oxide. Life Sci 60:1833–1845 (1997).

26. Lin K-T, Xue J-Y, Nomen M, Spur B, Wong PY-K, Peroxynitrite-induced apoptosis. J Biol Chem 270:16487–16490 (1995).

27. Murphy ME, Sies H. Reversible conversion of nitrosyl anion to nitric oxide by superoxide dismutase. Proc Natl Acad Sci USA 88:10680–10684 (1991).

28. Lipton SA, Singel DJ, Stamler JS. Redox-activated states of nitric oxide determine neuronal protection versus neuronal injury. In: Nitric Oxide: Roles in Neuronal Communication and Neurotoxicity (Takagi H, Toda N, Hawkins RD, eds). (Tokyo) Japan Scientific Societies Press, 1994;183–189.

29. Lipton SA, W-K Kim, PV Rayudu W, Asaad, DR Arnelle, JS Stamler. Siniglet and triplet nitrosyl anion (NO•) lead to NMDA receptor downregulation and neuroprotection [Abstract]. In: Fourth International Meeting of Nitric Oxide, 17–21 September 1995, Amelia Island, Florida. Endothelium 3:344 (1995).

30. Brown GC, Cooper CE. Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. FEBS Lett 356:295–298 (1995).

31. Carr GJ, Ferguson SJ. Nitric oxide formed by nitrite reductase of Paracoccus denitirificans is sufficiently stable to inhibit cytochrome oxidase activity and is reduced by its reductase under aerobic conditions. Biochim Biophys Acta 1017:57–62 (1994).

32. Schweizer M, Richter C. Nitric oxide potently and reversibly deenergizes mitochondria at low oxygen tension. Biochem Biophys Res Commun 204:169–175 (1994a).

33. Richter C, Gogvdaze V, Schlapbach R, Schweizer M, Schlegel J. Nitric oxide kills hepatocytes by mobilizing mitochondrial calcium. Biochem Biophys Res Commun 205:1143–1150 (1994).

34. Cleeter MWJ, Cooper JM, Darley-Usmar VM, Moncada S, Schapira AHV. Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. FEBS Lett 345:50–54 (1994).

35. Brown GC, Bolanos JP, Heales SJR, Clark JB. Nitric oxide produced by activated astrocytes rapidly and reversibly inhibits cellular respiration. Neurosci Lett 193:102–204 (1995).

36. Takehara Y, Kanno T, Yoshioka T, Inoue M, Utsumi K. Oxygen-dependent regulation of mitochondrial energy metabolism by nitric oxide. Arch Biochem Biophys 323:27–32 (1995).

37. Brudvig OW, Stevens OH, Chan OL. Reactions of nitric oxide with cytochrome oxidase. Biochemistry 19:5275–5285 (1980).

38. Torres J, Darley-Usmar V, Wilson MT. Inhibition of cytochrome c oxidase in turnover by nitric oxide: mechanism and implications for control of respiration. Biochem J 312:169–173 (1995).

39. Clarkson RB, Norby SW, Smirnov A, Boyer S, Vahidi N, Nims RW, Wink DA. Direct measurement of the accumulation and mitochondrial conversion of nitric oxide within Chinese hamster ovary cells using an intracellular electron paramagnetic resonance technique. Biochim Biophys Acta 1243:496–502 (1995).

40. Zhao XJ, Sampath V, Caughey WS. Cytochrome c oxidase catalysis of the reduction of nitric oxide to nitrous oxide. Biochim Biophys Res Commun 212:1054–1060 (1994).

41. Brown GC. Nitric oxide regulates mitochondrial respiration and cell functions by inhibiting cytochrome oxidase. FEBS Lett 369:136–139 (1995).

42. Ghafoorifar P, Richter C. Nitric oxide synthase activity in mitochondria. FEBS Lett 418:291–296 (1997).

43. Castro L, Rodriguez M, Radi R. Aconitate is readily inactivated by peroxynitrite, but not by its precursor, nitric oxide. J Biol Chem 269:29405–29415 (1994).

44. Hausladen, A, Fridovich I. Superoxide and peroxynitrite inactivate aconitases, but nitric oxide does not. J Biol Chem 269:29405–29408 (1994).

45. McDonald LS, Moss J. Stimulation by nitric oxide of an NAD linkage to glyceraldehyde-3-phosphate dehydrogenase. Proc Natl Acad Sci USA 90:6238–6241 (1993).

46. Brüne B, Dimmeler S, Vedia LMY, Lapetina EG. Nitric oxide—a signal for ADP-ribosylation of proteins. Life Sci 54:61–70 (1994).

47. Richter C. Mitochondrial calcium transport. In: New Comprehensive Biochemistry (Neuberger A, Van Deenen LLM, eds). Amsterdam:Elsevier, 1992;349–358.

48. Rizzuto R, Bastianutto C, Brini M, Murgia M, Pozzan T. Mitochondrial Ca2+ homeostasis in intact cells. J Cell Biol 126:1183–1194 (1994).

49. Hainoeczy G, Robb-Gaspers LD, Seitz M, Thomas AP. Desensitizing the pyroelectric calcium oscillations in the mitochondria. Cell 82:415–424 (1994).

50. Loew LM, Carrington W, Tuft RA, Fay FS. Physiological cytosolic Ca2+ transients evoke concurrent mitochondrial depolarization. Proc Natl Acad Sci USA 91:4340–4344 (1994).

51. Sparagna GC, Gunter KK, Sheu S-S, Gunter TE. Mitochondrial calcium uptake from physiological-type pulses of calcium. A description of the rapid uptake mode. J Biol Chem 270:27510–27515 (1995).

52. Jouaville LS, Ichas F, Holmuhamedov EL, Camacho P, Lechleiter JD. Synchronization of calcium waves by mitochondrial substrates in Xenopus laevis oocytes. Nature 377:438–441 (1995).

53. Crompton M. The regulation of mitochondrial calcium transport in heart. Curr Top Membr Transp 25:231–278 (1988).

54. Richter C, Frei B. Ca2+ release from mitochondria induced by prooxidants. Free Radical Biol Med 4:365–375 (1988).

55. Richter C. Control of the pro-oxidant-dependent calcium release from intact liver mitochondria. Redox Report 2:217–221 (1996).

56. Schweizer M, Durrer P, Richter C. Phenylarsine oxide stimulates the pyridine nucleotide-linked Ca2+ release from rat liver mitochondria. Biochem Pharmacol 48:967–973 (1994).

57. Schweizer M, Richter C. Glutoxin stimulates Ca2+ release from intact rat liver mitochondria. Biochemistry 33:13401–13405 (1994b).

58. Schweizer M, Richter C. Peroxynitrite stimulates the pyridine nucleotide-linked Ca2+ release from intact rat liver mitochondria. Biochemistry 35:4524–4528 (1996).

59. Richter C., Gogvdaze V, Laffranchi R, Schlapbach R, Schweizer M, Suter M, Walter P, Yaffe M. Oxidants in mitochondria: from physiology to diseases. Biochim Biophys Acta 1271:67–74 (1995).

60. Hennet T, Richter C, Petersen H. Tumour necrosis factor-α induces superoxide anion generation in mitochondria of 1.929 cells. Biochem J 289:587–592 (1993).

61. Hennet T, Petersen H, Richter C, Bertoni G. Expression of BCL-2 protein enhances the survival of mouse fibrosarcoma cells in tumour necrosis factor-mediated cytotoxicity. Cancer Res 53:1456–1460 (1993).

62. Bornkamm GW, Richter C. A link between the antioxidant defense system and calcium: a proposal for the biochemical function of Bcl-2. In: Current Topics in Microbiology and Immunology, Vol 194: Mechanisms in B-Cell Neoplasia (Porter M, Melchers F, eds). Berlin:Springer Verlag, 1994;323–330.

63. Chen L-B. Mitochondrial membrane potential in living cells. Annu Rev Cell Biol 4:155–181 (1988).

64. Albina JE, Cui S, Mateo RB, Reichen J. Nitric oxide-mediated apoptosis in murine peritoneal macrophages. J Immunol 150:5080–5085 (1993).

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65. Cui S, Reinehr JS, Mateo RB, Albina JE. Activated murine macrophages induce apoptosis in tumor cells through nitric oxide-dependent and -independent mechanisms. Cancer Res 54:2462–2467 (1994).

66. Ankarcrona M, Dypbukt JM, Brüne B, Nicotera P. Interleukin 1β-induced nitric oxide production activates apoptosis in pancreatic RINm5F cells. Exp Cell Res. 213:172–177 (1994).

67. Messer UK, Ankarcrona M, Nicotera P, Brüne B. p53 expression in nitric oxide-induced apoptosis. FEBS Lett 355:23–26 (1994).

68. Mannik JB, Asano K, Izumi K, Kieff E, Stampler JS. Nitric oxide produced by human B lymphocytes inhibits apoptosis and Epstein-Barr virus reactivation. Cell 79:1137–1146 (1994).

69. Bonfoco E, Krawiec D, Ankarcrona M, Nicotera P, Lipton SA. Apoptosis and necrosis: Two distinct events induced, respectively, by mild and intense insults with N-methyl-L-aspartate or nitric oxide/superoxide in cortical cell neurons. Proc Natl Acad Sci USA 92:7162–7166 (1995).

70. Nicotera P, Brüne B, Bagetta G. Nitric oxide: inducer or suppressor of apoptosis? Trends Pharmacol Sci 18:189–190 (1997).

71. Melino G, Knight RA, Corasaniti MT, Nistico G, Finazzi-Agro A. S-nitrosylation regulates apoptosis Nature 388:432–433 (1997).

72. Ogura T, Tatemichi M, Esumi H. Nitric oxide inhibits CPP32-like activity under redox regulation. Biochem Biophys Res Commun 236:365–369 (1997).

73. Tenneti L, D’Emilia DM, Lipton SA. Suppression of apoptosis by S-nitrosylation of caspases. Neuron Sci Lett 236:139–142 (1997).

74. Kim YM, Talanian RV, Billiar TR. Nitric oxide inhibits apoptosis by preventing increases in caspase-3-like activity via two distinct mechanisms. J Biol Chem 272:31138–31148 (1997).

75. Li J, Billiar TR, Talanian RV, Kim YM. Nitric oxide reversibly inhibits seven members of the caspase family via S-nitrosylation. Biochem Biophys Res Commun 240:419–424 (1997).

76. Kim YM, de Vera ME, Watkins SC, Billiar TR. Nitric oxide protects cultured rat hepatocytes from tumor necrosis factor-α-induced apoptosis by inducing heat shock protein 70 expression. J Biol Chem 272:1402–1411 (1997).

77. Dimmelser S, Ripmann W, Welland U, Haendeler J, Zeiher AM. Angiogenin II induces apoptosis in human endothelial cells. Protective effect of nitric oxide. Circ Res 89:970–976 (1997).

78. Dimmelser S, Haendeler J, Nehls M, Zeiher AM. Suppression of apoptosis by nitric oxide via inhibition of interleukin-1β-converting enzyme (ICE)-like and cysteine protease protein (CPP32)-like activities. J Exp Med 185:601–607 (1997).

79. Tseng E, Kim YM, Pitt BR, Lizonova A, Kovesdi I, Billiar TR. Adenoviral transfer of the inducible nitric oxide synthase block endothelial cell apoptosis. Surgery 122:255–263 (1997).

80. Mannik JB, Miao XQ, Stampler JS. Nitric oxide inhibits Fas-mediated apoptosis. J Biol Chem 272:24125–24128 (1997).

81. Jansen YM, Matalon S, Mossman BT. Differential induction of c-fos, c-myc, and apoptosis in lung epithelial cells exposed to ROS and RNS. Am J Physiol 273:L789–796 (1997).

82. Sandoval M, Liu X, Mannik EE, Clark DA, Miller MJ. Peroxynitrite-induced apoptosis in human intestinal epithelial cells is attenuated by mesalamine. Gastroenterology 113:1480–1488 (1997).

83. Lopez-Collazo E, Mateo J, Miras-Portugal MT, Bosca L. Requirement of nitric oxide and calcium mobilization for the induction of apoptosis in adrenal vascular endothelial cells. FEBS Lett 413:124–128 (1997).

84. Khan S, Kayahara M, Joashi U, Mazarakis ND, Sarraf C, Edwards AD. Differential induction of apoptosis in Swiss3T3 cells by nitric oxide and the nitrosylation cation. J Cell Sci 110 Part B:2315–2322 (1997).

85. Selleri C, Sato T, Raiola AM, Rotoli B, Young NS, Maciejewski JP. Induction of nitric oxide synthase is involved in the mechanism of Fas-mediated apoptosis in haemopoietic cells. Br J Haematol 99:481–489 (1997).

86. Zhao Z, Francis CE, Welch G, Loscalzo J, Ravid K. Reduced glutathione prevents nitric oxide-induced apoptosis in vascular smooth muscle cells. Biochim Biophys Acta 1359:143–152 (1997).

87. Lin KT, Xue JY, Wong PY. Peroxynitrite. An apoptotic agent in HL-60 cells. Adv Exp Med Biol 407:413–419 (1997).

88. Lin KT, Xue JY, Sun FF, Wong PY. Reactive oxygen species participate in peroxynitrite-induced apoptosis in HL-60 cells. Biochem Biophys Res Commun 230:115–119 (1997).

89. Yusuki K, Kariya Y, Inai Y, Matozaki K, Yoshioka T, Yasuda T, Horton AA, Utsumi K. Molecular mechanisms of apoptosis in HL-60 cells induced by a nitric oxide-releasing compound. Free Radic Res 27:325–335 (1997).

90. Muhl H, Sandau K, Brüne B, Briner VA, Pfeilschifter J. Nitric oxide donors induce apoptosis in glomerular mesangial cells. Eur J Pharmacol 317:137–149 (1996).

91. Sandau K, Pfeilschifter J, Brüne B. The balance between nitric oxide and superoxide determines apoptotic and necrotic death of rat mesangial cells. J Immunol 158:4938–4946 (1997).

92. Sciorti C, Rovere P, Ferrari M, Heltai S, Manfredi AA, Clementi E. Autocrine nitric oxide modulated CD95-induced apoptosis in gammadelta T lymphocytes. J Biol Chem 272:23211–23215 (1997).

93. Zettl UK, Mix E, Zielke M, Stangel M, Hartung HP, Gold R. Apoptosis of myelin-reactive T cells induced by reactive oxygen and nitrogen intermediates in vitro. Cell Immunol 178:1–8 (1997).

94. Messer UK, Brüne B. Attenuation of p53 expression and Bax down-regulation during phobol ester mediated inhibition of apoptosis. Br J Pharmacol 121:625–634 (1997).

95. von Knechten A, Brüne B. Cyclooxygenase-2: an essential regulator of NO-mediated apoptosis. FASEB J 11:887–895 (1997).

96. Sandoval M, Zhang XJ, Liu X, Mannik EE, Clark DA, Miller MJ. Peroxynitrite-induced apoptosis in T84 and RAW 264.7 cells: attenuation by t-ascorbic acid. Free Radic Biol Med 22:489–495 (1997).

97. Xie K, Wang Y, Huang S, Xu L, Bielenberg D, Salas T, McConkey DJ, Jiang W, Fidler IJ. Nitric oxide-mediated apoptosis of K-1753 melanoma cells is associated with down-regulation of Bel-2. Oncogen 15:771–779 (1997).

98. Ho YS, Lee HM, Mou TC, Wang YJ, Lin JK. Suppression of nitric oxide-induced apoptosis by N-acetyl-L-cysteine through modulation of glutathione, bcl-2, and bax protein levels. Mol Carcinog 19:101–113 (1997).

99. Bonfoco E, Zhitkowsky B, Rossi AD, Aguilar-Santelises M, Orenius S, Lipton SA, Nicotera P. Bel-2 delay apoptosis and PARP cleavage induced by NO donors in G1-7 cells. Neuroreport 8:273–276 (1996).

100. Vincent AM, Mohammad Y, Ahmad I, Greenberg R, Maisie K. Metabolotropic glutamate receptors prevent nitric oxide-induced programmed cell death. J Neurosci Res 50:549–564 (1997).

101. Hu J, Ferreira A, Van Eldik LJ. S100B induces neuronal cell death through nitric oxide release from astrocytes. J Neurochem 69:2294–2301 (1997).

102. Kamoshima W, Kitamura Y, Nomura Y, Taniguchi T. Possible involvement of ADP-ribosylation of particular enzymes in cell death induced by nitric oxide-donors in human neuroblastoma cells. Neurochem Int 30:305–311 (1997).

103. Nomura Y, Uehara T, Nakazawa M. Neuronal apoptosis by gial NO: involvement of glyceraldehyde-3-phosphate dehydrogenase. Hum Cell 9:205–214 (1996).

104. Hortelano S, Dallaporta B, Zamzami N, Hirsch T, Susin SA, Marzo I, Bosca L, Nistico G. Nitric oxide induces apoptosis via triggering mitochondrial permeability transition FEBS Lett 410:373–377 (1997).