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Nitric oxide and other gas interactions with mitochondria

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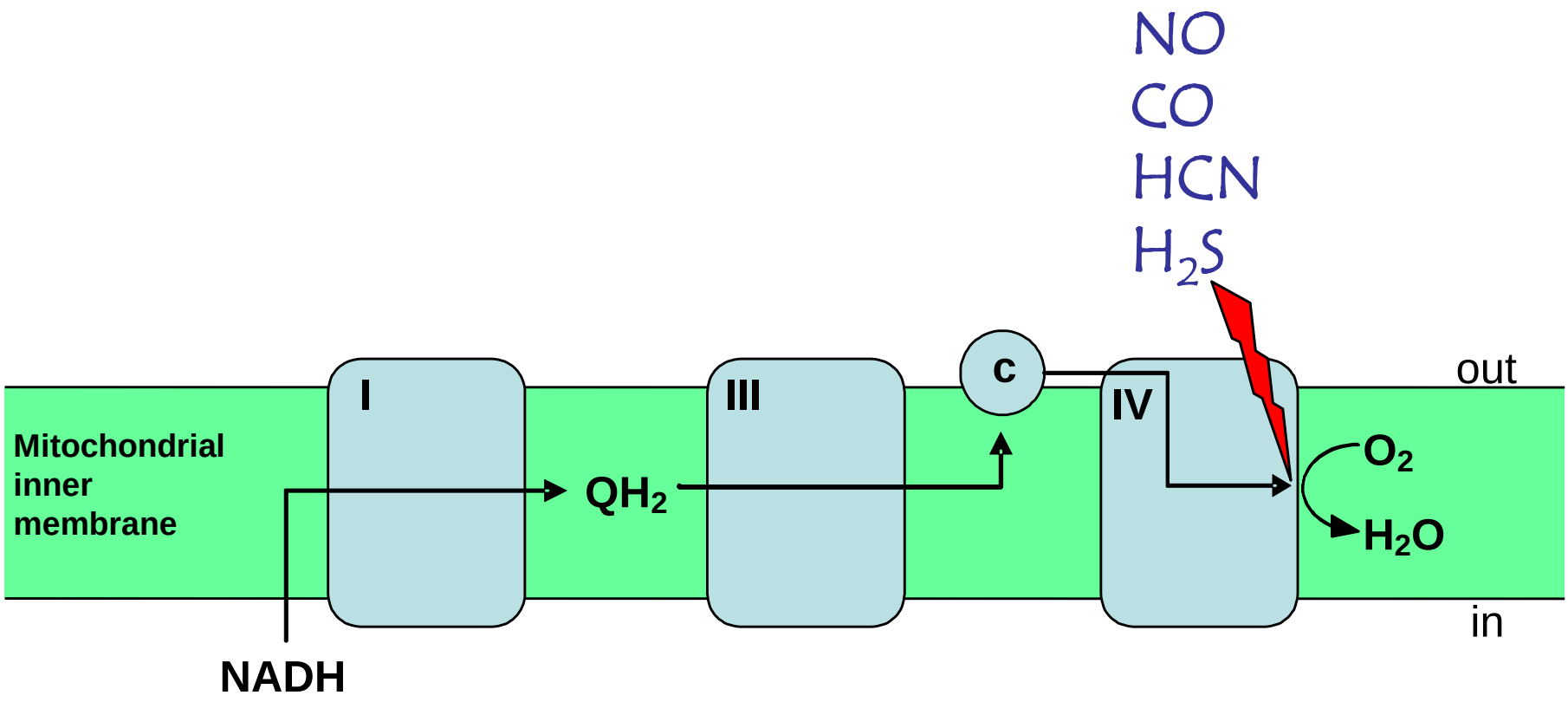
Gases bind to the oxygen binding site of mitochondrial complex IV, blocking energy production, in the same way as hypoxia.

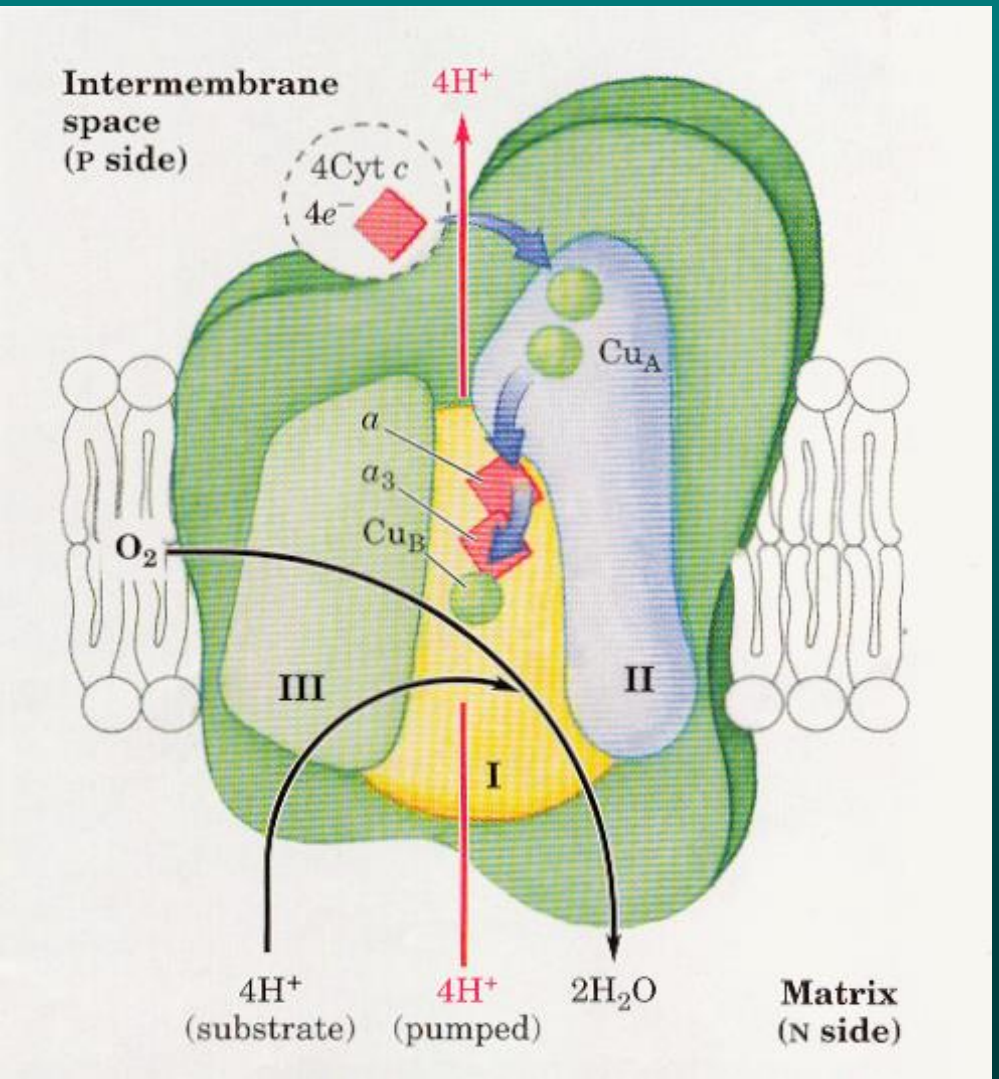
This may be one way in which our cells regulate their energy production.

It may also be one way in which our body kills pathogens.

However, it may also kill our cells in disease.



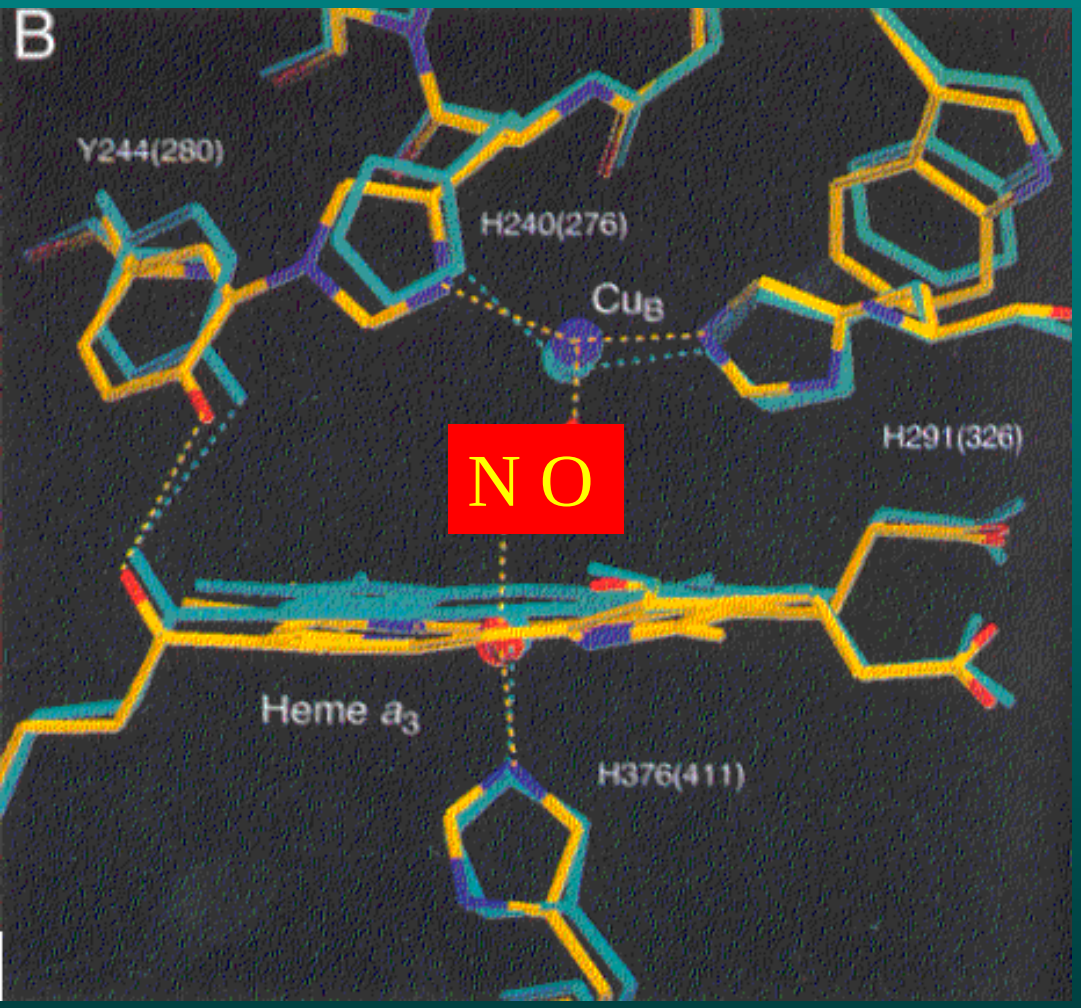
Path of electrons through Complex IV



- Electrons pass from $c > Cu_A > a > a_3/Cu_B$.
- Consumes 90% of our oxygen.
- Major generator of proton motive force.

Figure from Lehninger: Principles of Biochemistry

Gas binding to complex IV



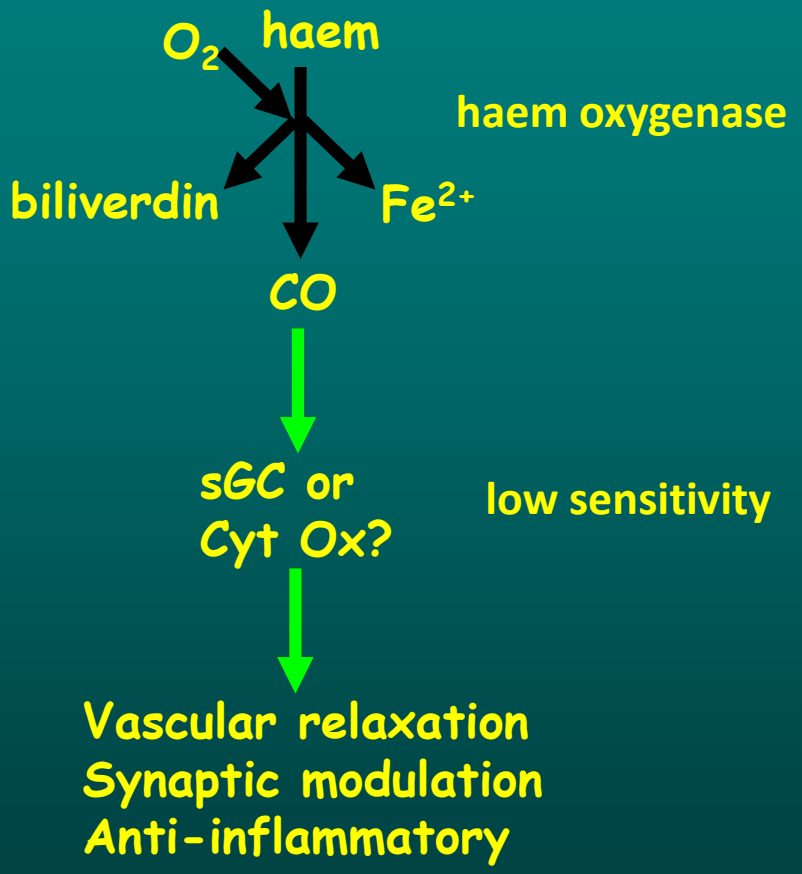
Binuclear centre consists of heme a_3 and Cu_B.

O_2 binds only when both Fe & Cu reduced.

NO & CO bind when Fe reduced (Fe^{2+}).

HCN & H_2S bind when Fe oxidised (Fe^{3+}).

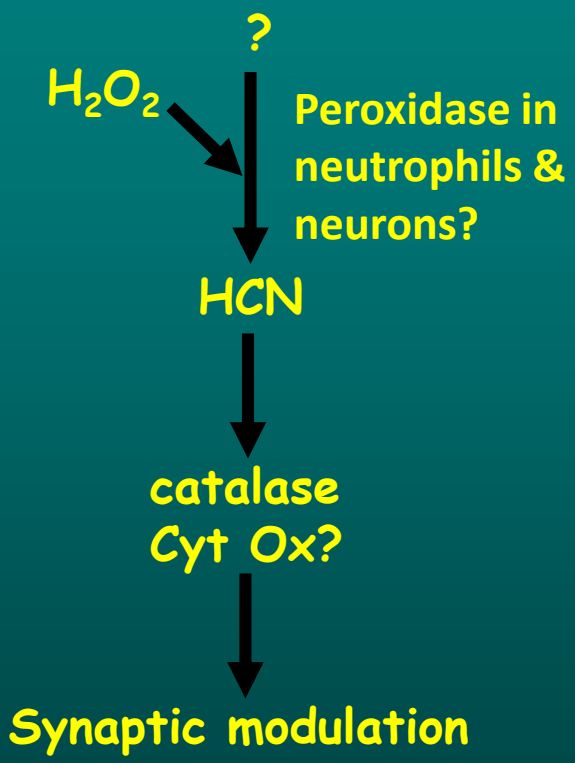
CO – CARBON MONOXIDE



Induction of HO-1 causes small inhibition of cellular respiration at low O_2.

Probably insignificant in vivo due to CO binding to haemoglobin.

HCN – HYDROGEN CYANIDE

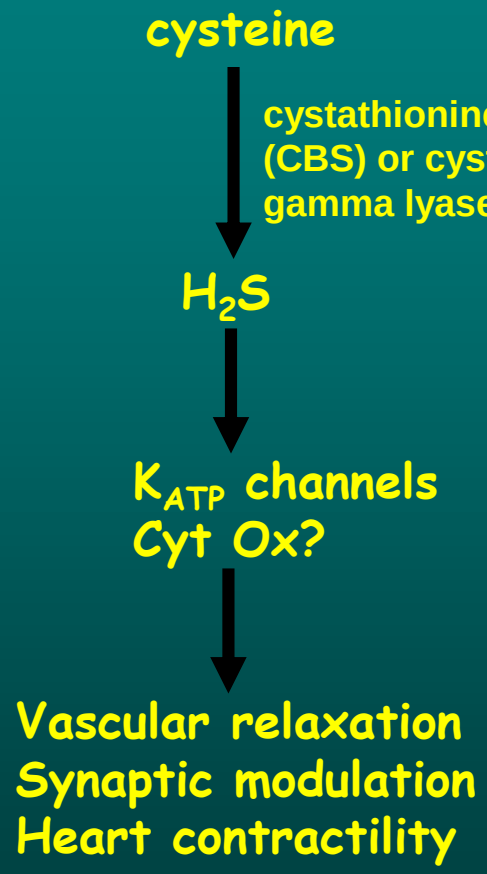


Estimated 5 μM in brain & 1 μM in blood, but may be bound (e.g. to metHb).

Cellular respiration is half inhibited by about 10-50 μM cyanide

Probably insignificant in vivo.

H_2S – HYDROGEN SULPHIDE



Estimated 1-10 μM in aorta.

Cellular respiration is half inhibited by 10-30 μM.

Lower concentrations are rapidly oxidised by mitochondria.

High concentration induce suspended animation state.

Inhibitor	Mechanism	K_i	$k_{on}(M^{-1}\ s^{-1})$	$k_{off}\ (s^{-1})$
HCN	Non-competitive	200 nM	2×10^{3}	5×10^{-4}
H_2S	Non-competitive	200 nM	10^{4}	10^{-3}
CO	Competitive with O_2	200 nM	10^{5}	2×10^{-2}
NO	Competitive with O_2	0.2 nM	10^{8}	2×10^{-2}

Cooper C.E. & Brown G.C. (2008) The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: J. Bioenergetics & Biomembranes

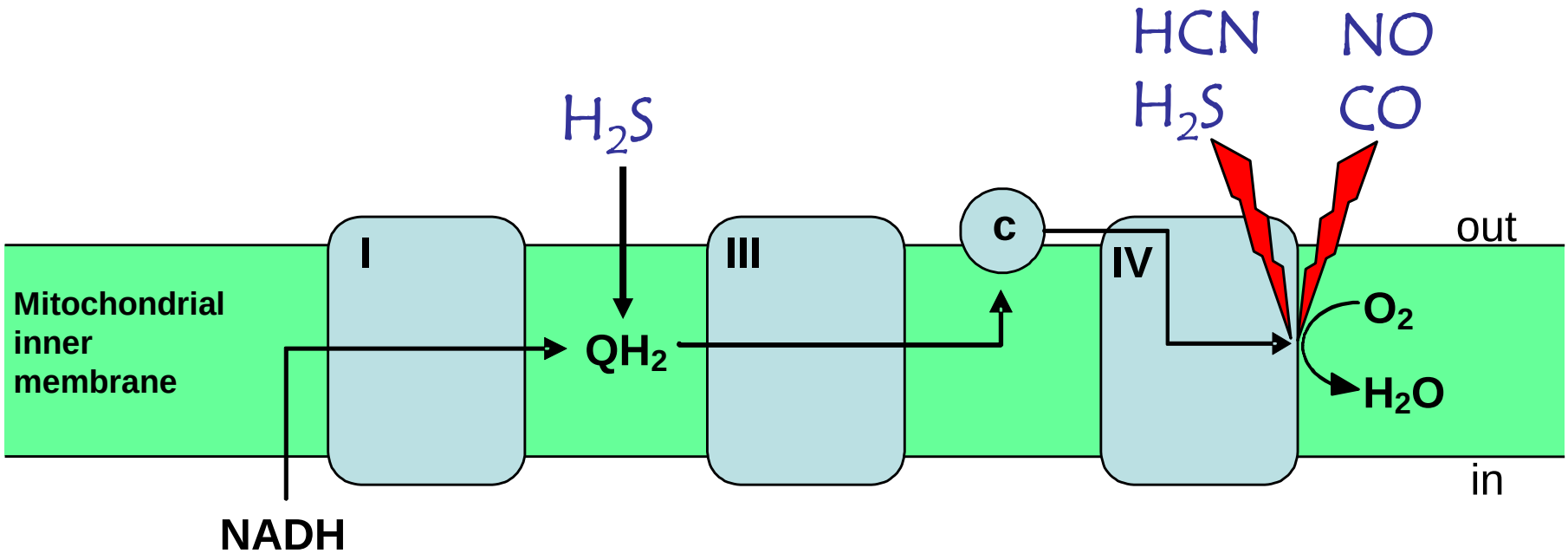
Inhibitor	Mechanism	K_i	Light sensitive	In vivo nM
HCN	Non-competitive	200 nM	No	< 1000
H_2S	Non-competitive	200 nM	No	< 1000
CO	Competitive with O_2	200 nM	Yes	< 1000
NO	Competitive with O_2	0.2 nM	Yes	< 100

Summary

NO & CO inhibition is rapidly reversed by O_2, light or Hb.

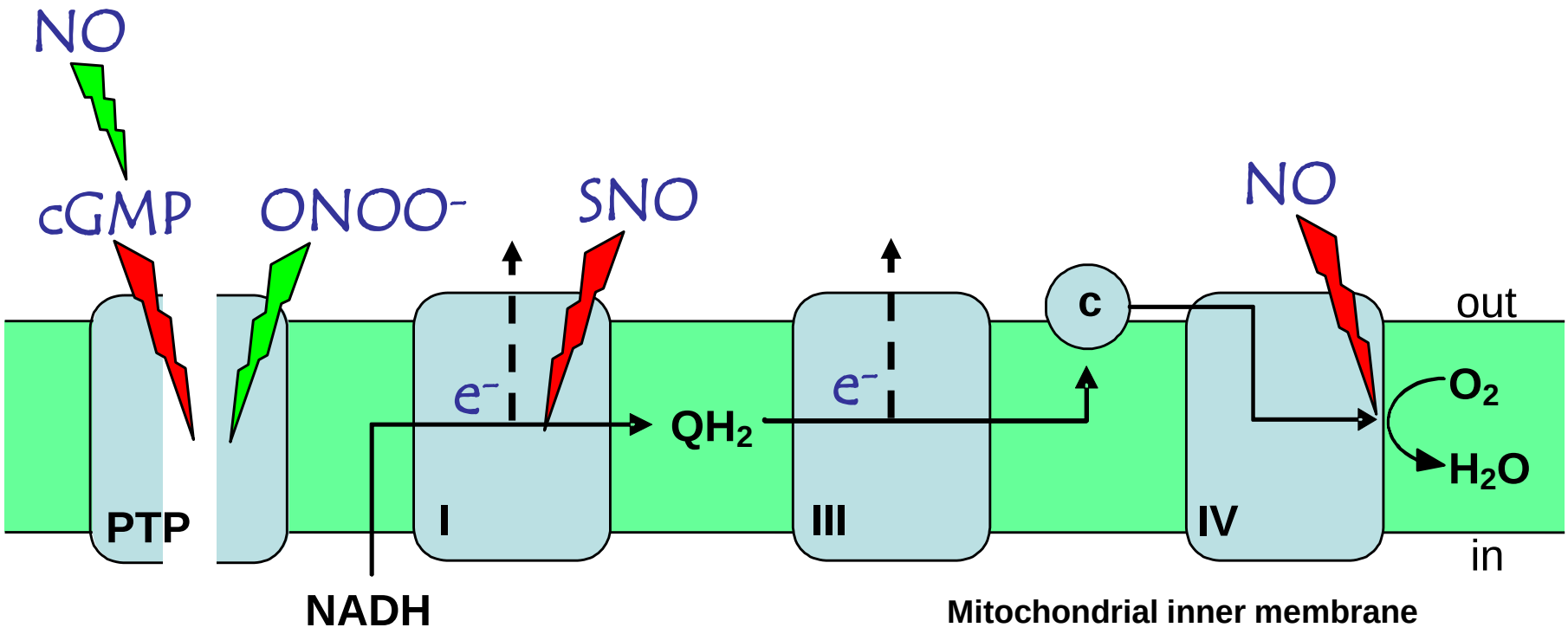
HCN & H_2S inhibition is slow & insensitive to O_2, light or Hb.

It is unclear whether gas inhibition is significant in vivo.

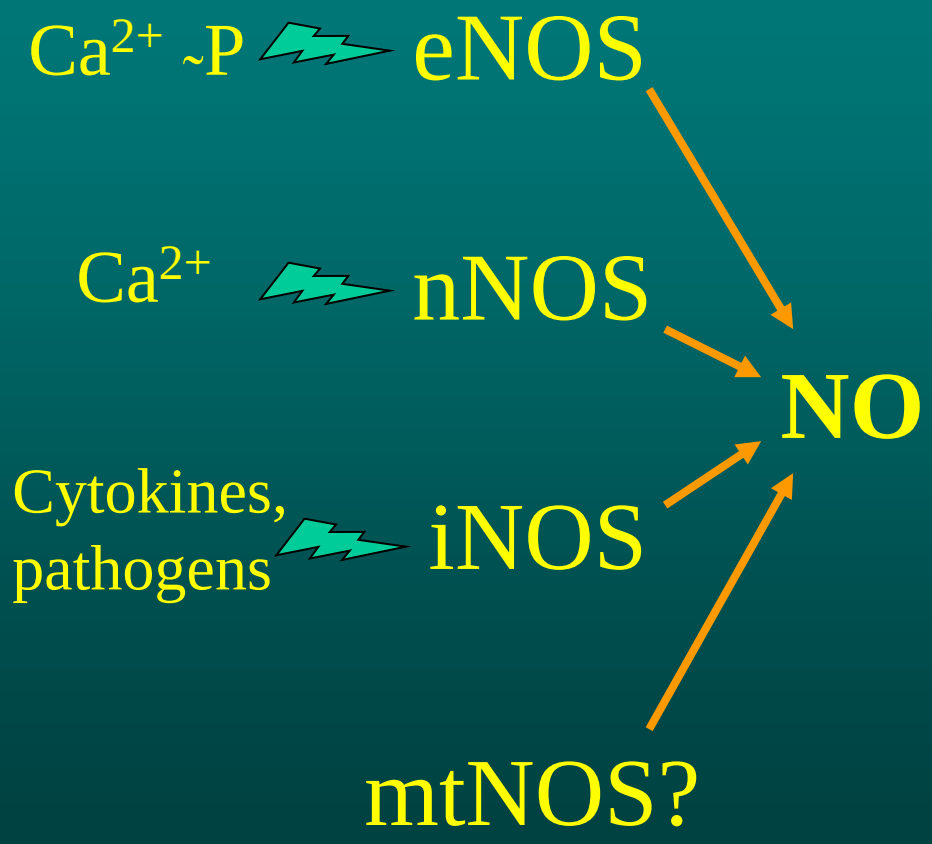


Nitric oxide (NO) and its derivatives have 3 major effects on mitochondria:

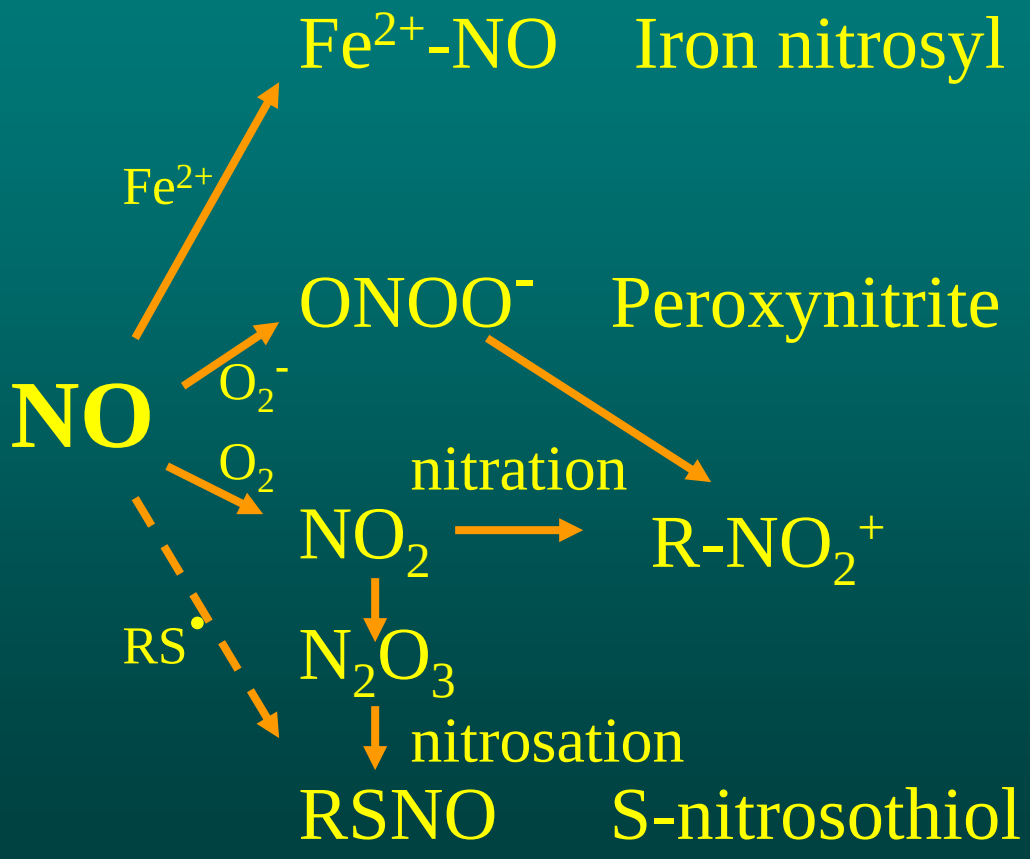
1. NO inhibition of cytochrome oxidase
2. SNO inactivation of complex I
3. $ONOO^-$ activation of permeability transition.



Sources of nitric oxide (NO)



Targets/reactions of nitric oxide (NO)



Function of NO:
"The double-edged sword"

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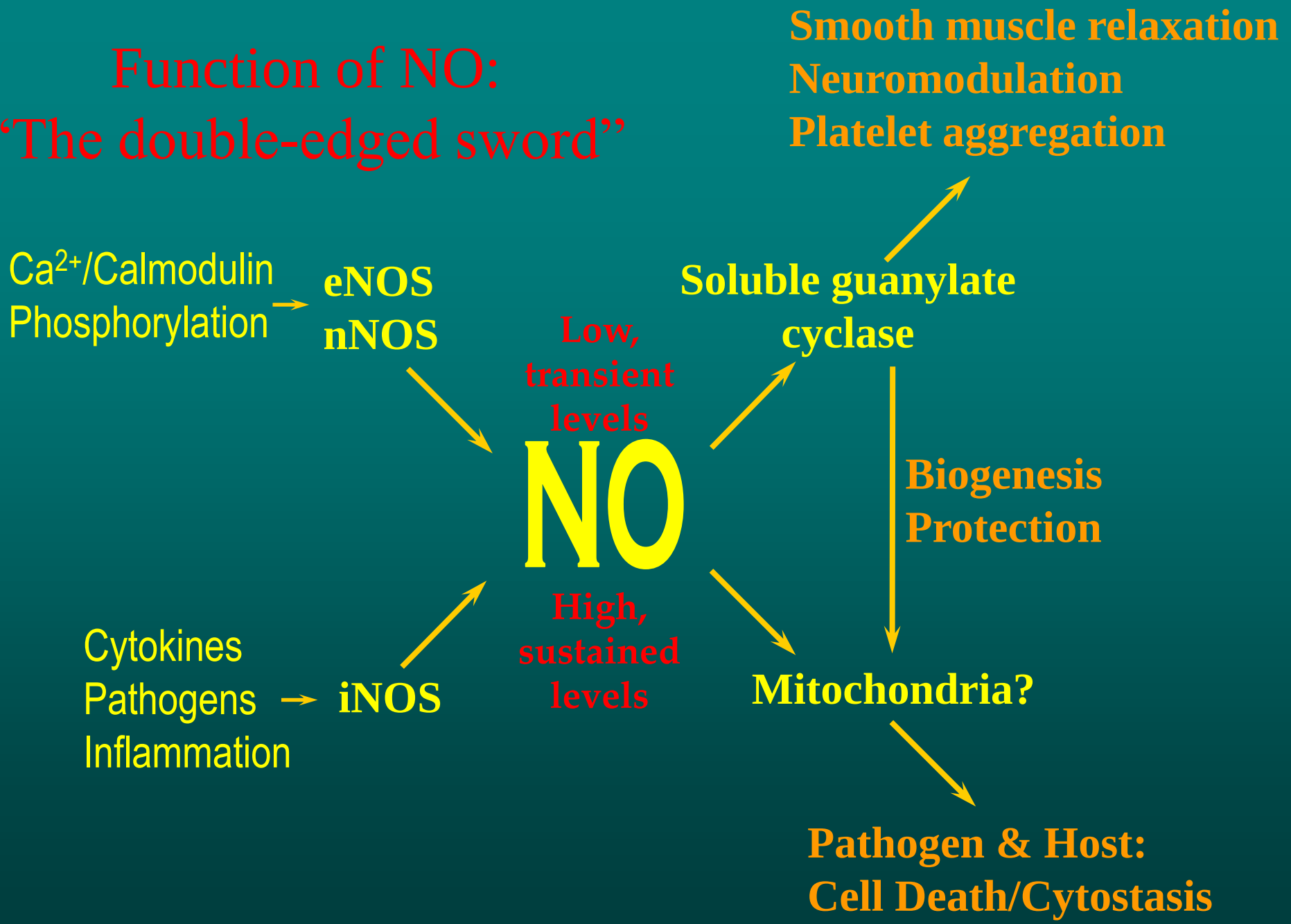
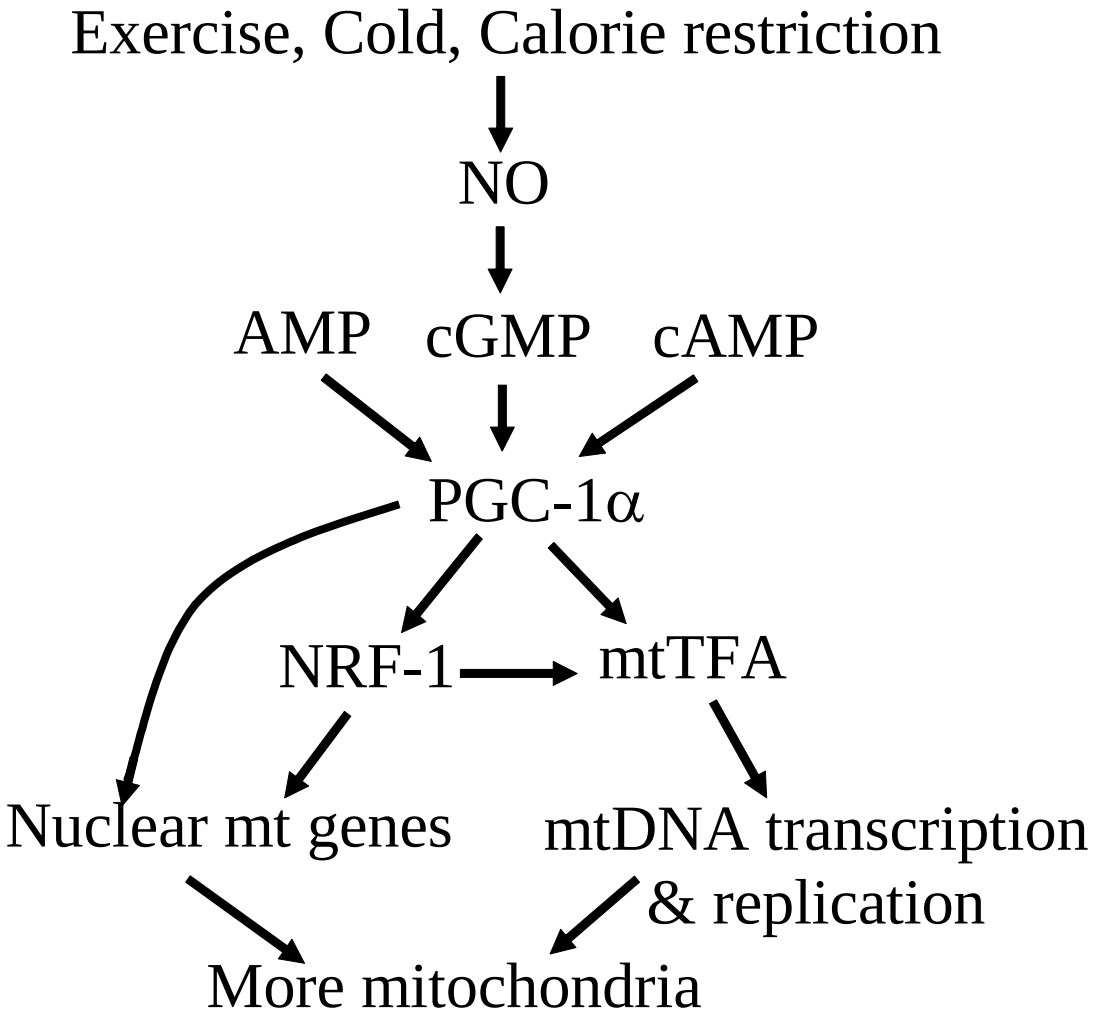


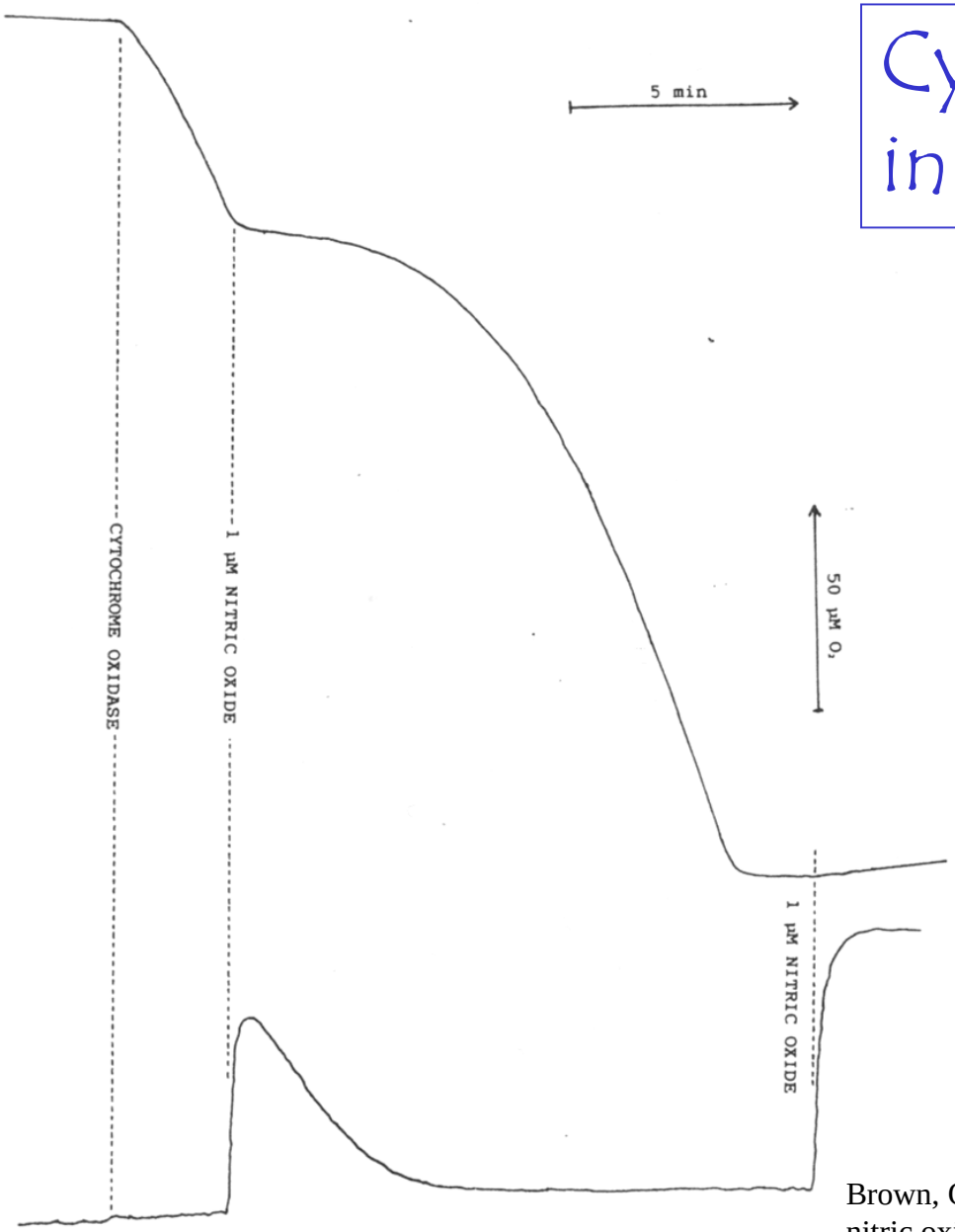


Brown, G. C. (2007) Nitric oxide and mitochondria. Front. Biosci. 12, 1024-33.

NO effects on mitochondria:

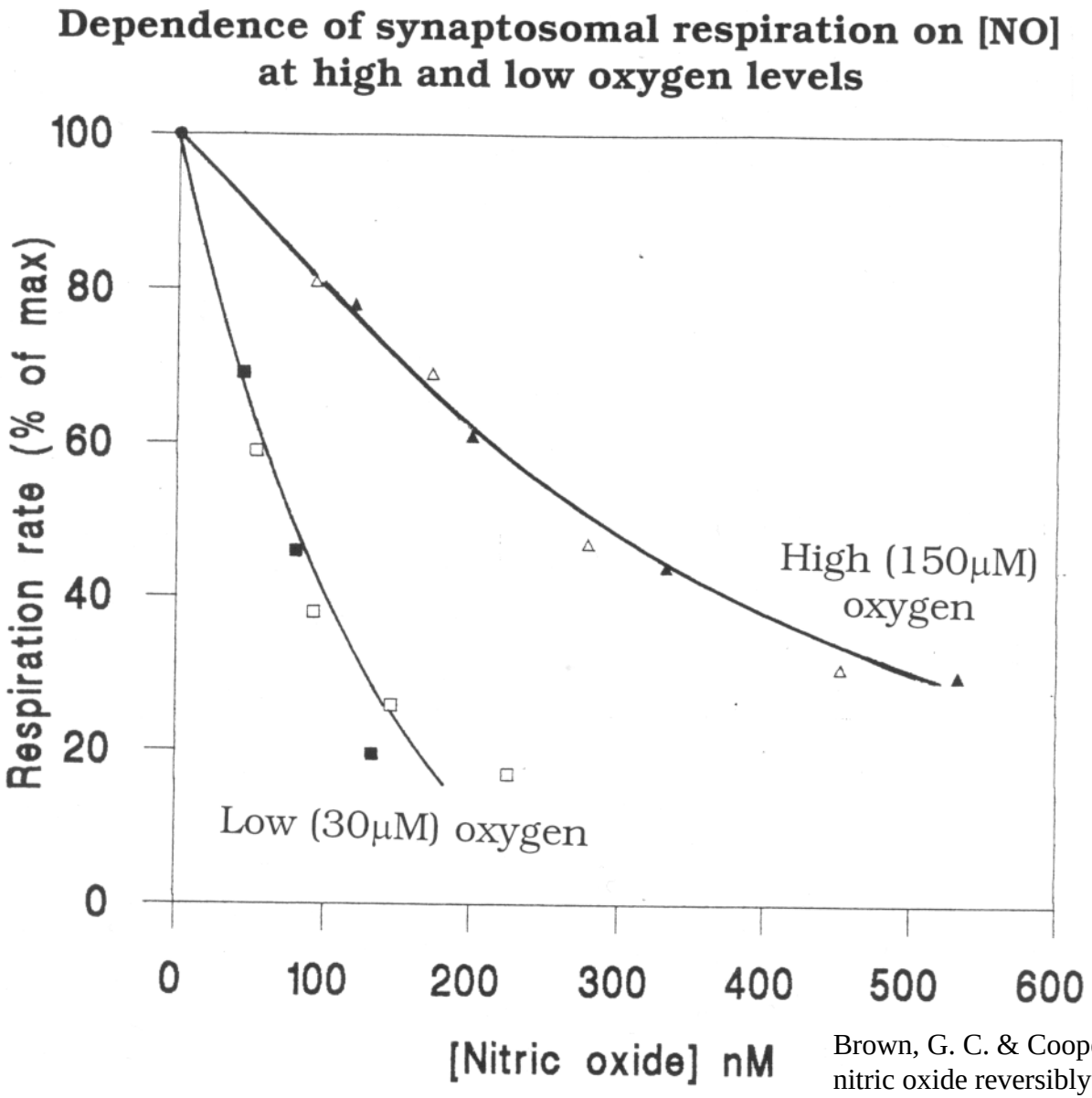
- Inhibition of respiration
- O_2^-, H_2O_2 & $ONOO^-$ production
- Mitochondrial permeability transition

Cytochrome oxidase is inhibited by NO



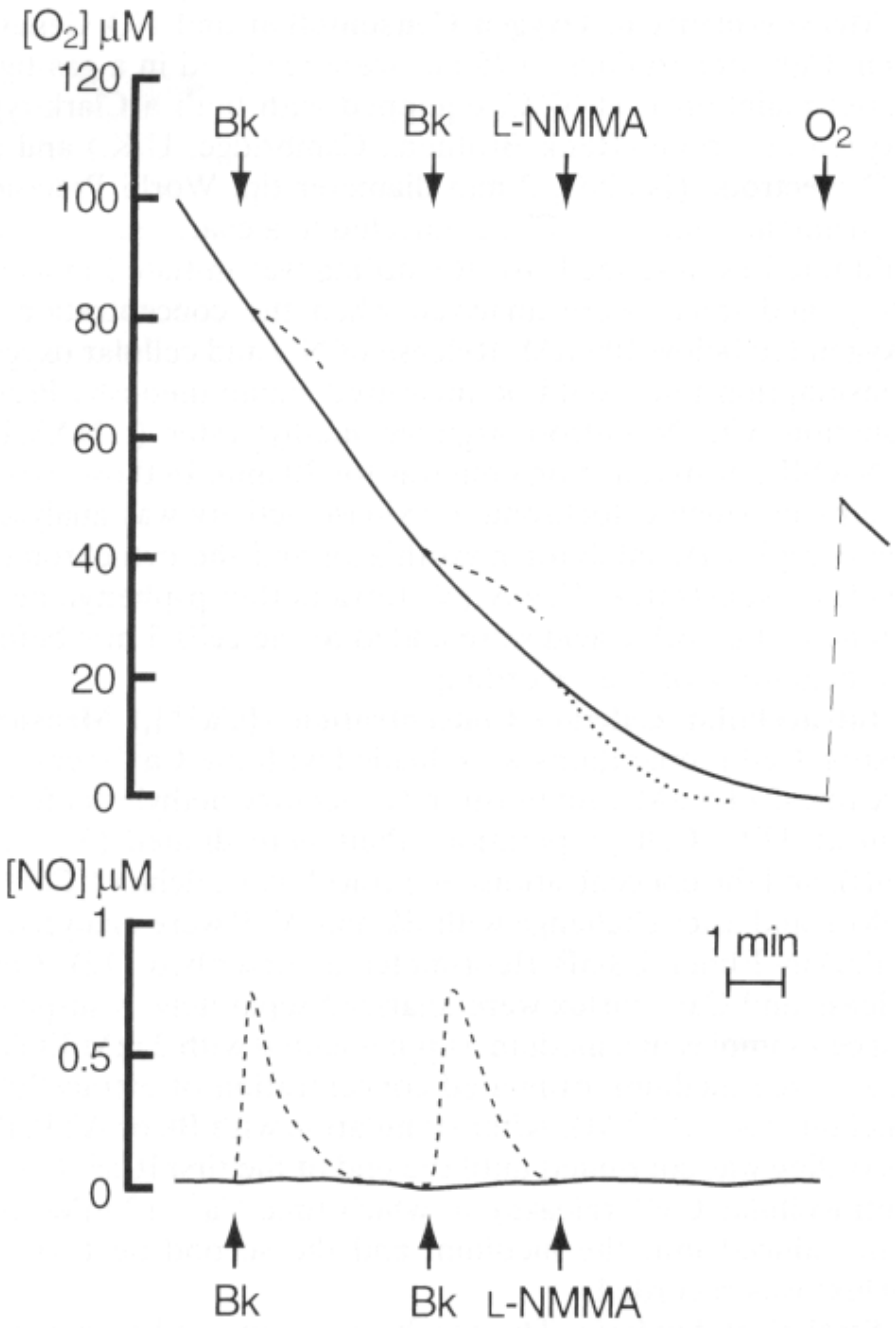
- Inhibition is rapid.
- Inhibition is potent.
- Inhibition is reversible when NO gone.
- Inhibition reversible by light.
- Inhibition at oxygen binding site in competition with O_2.

Brown, G. C. & Cooper, C. E. (1994) Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. FEBS Lett. 356, 295-298.



NO inhibition of cytochrome oxidase is competitive with O_2, raising the K_M of respiration into the physiological range.

Brown, G. C. & Cooper, C. E. (1994) Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. FEBS Lett. 356, 295-298.

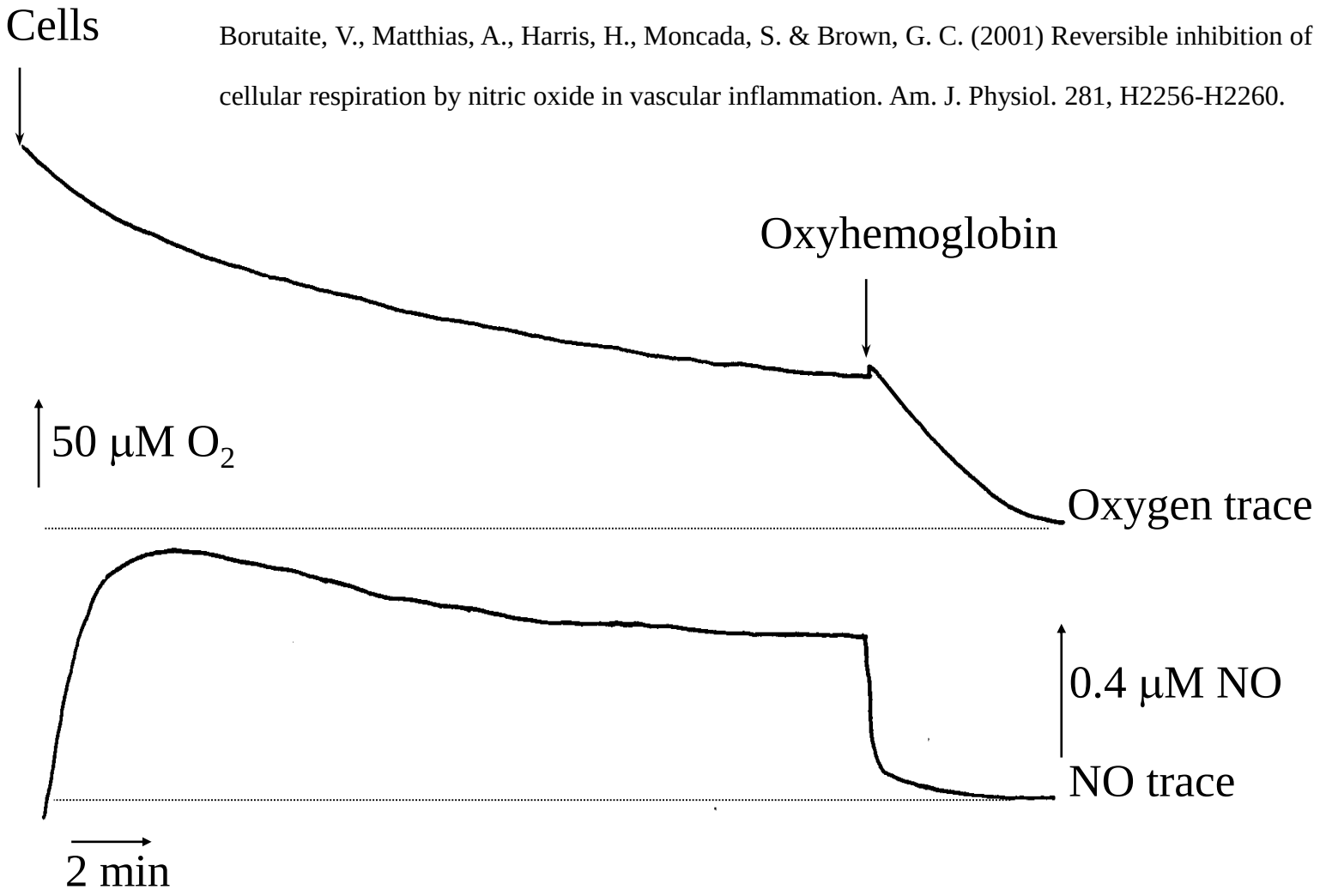


eNOS regulates cellular respiration and its sensitivity to oxygen in cultured endothelial cells

NO may be a physiological regulator of respiration and its affinity for oxygen

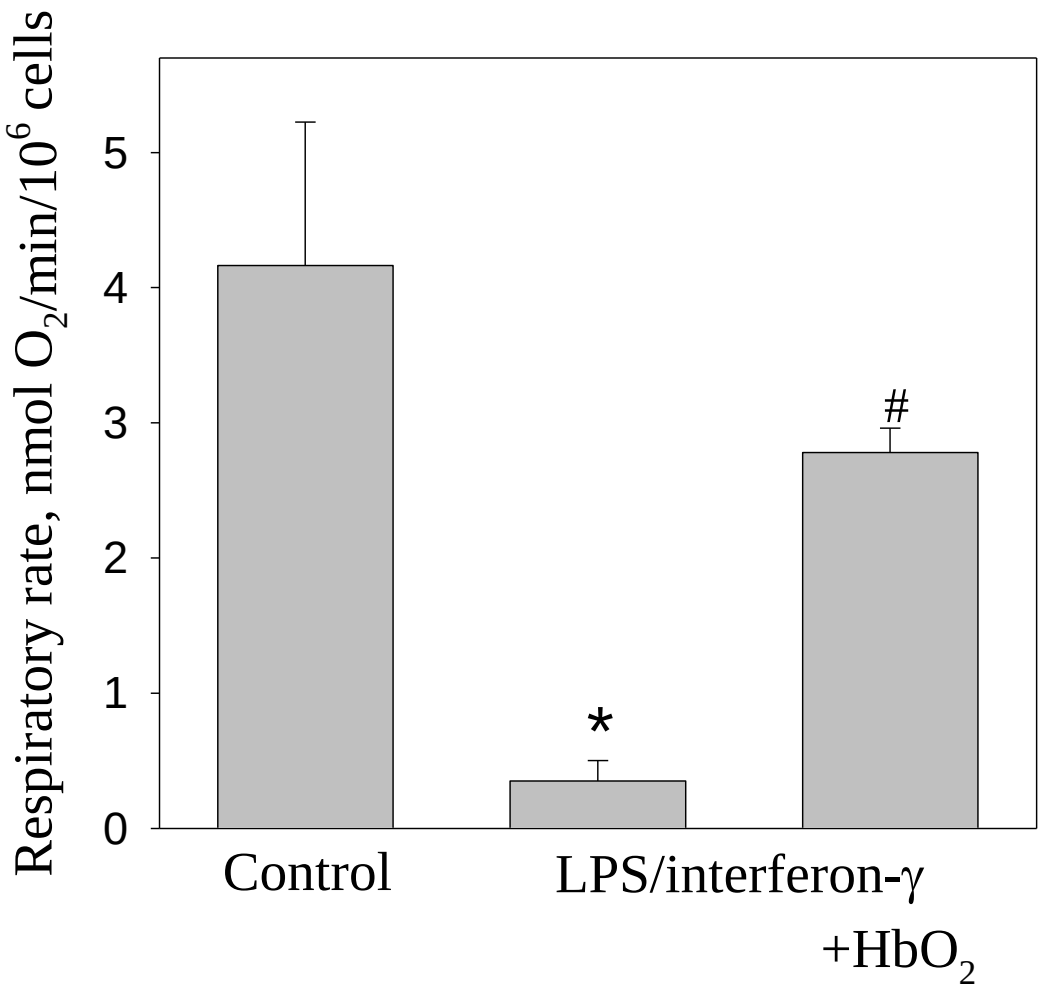
Clementi, E., Brown, G. C., Foxwell, N. & Moncada, S. (1999) On the mechanism by which vascular endothelial cells regulate their oxygen consumption. Proc. Natl. Acad. Sci. USA 96, 1559-1562.

Aortic endothelial cells activated with LPS+IFNγ produce NO from iNOS that continuously inhibits respiration until reversed by oxyhaemoglobin.



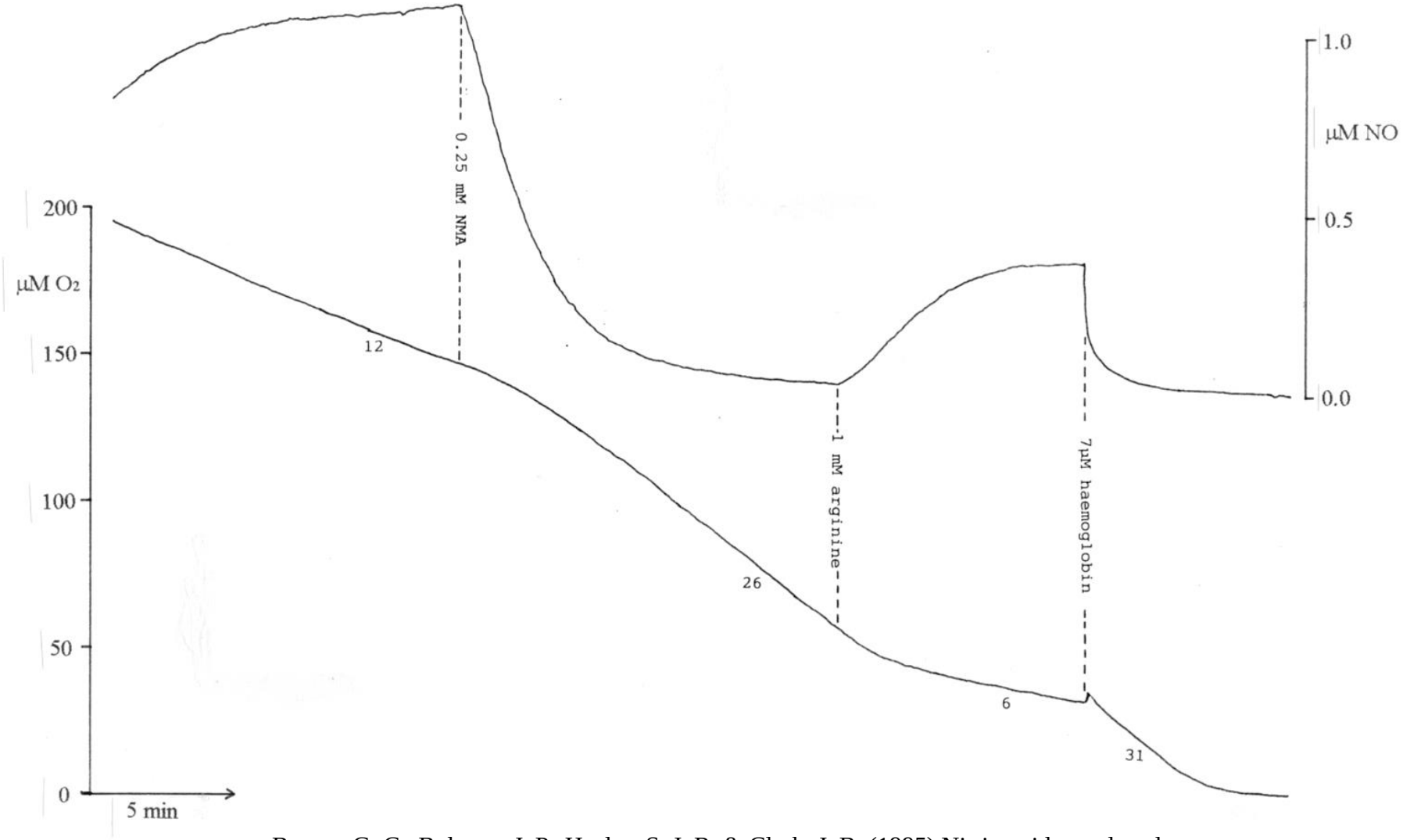
Borutaite, V., Matthias, A., Harris, H., Moncada, S. & Brown, G. C. (2001) Reversible inhibition of cellular respiration by nitric oxide in vascular inflammation. Am. J. Physiol. 281, H2256-H2260.

The oxygen consumption of aortic endothelial cells is inhibited by LPS/IFNγ-induced iNOS induction, and reversed by the NO scavenger haemoglobin



Borutaite, V., Matthias, A., Harris, H., Moncada, S. & Brown, G. C. (2001) Reversible inhibition of cellular respiration by nitric oxide in vascular inflammation. Am. J. Physiol. 281, H2256-H2260.

Activated astrocytes reversibly inhibit cellular respiration via NO

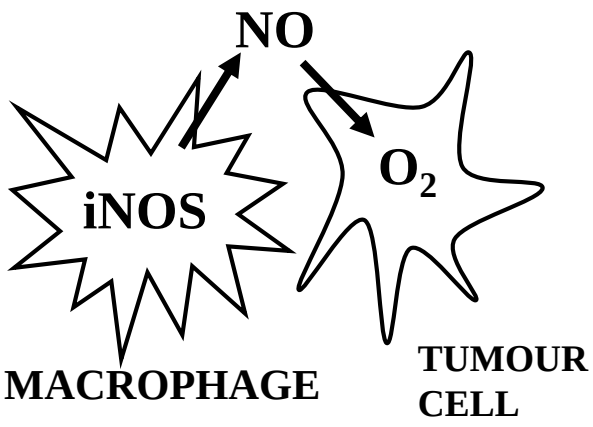


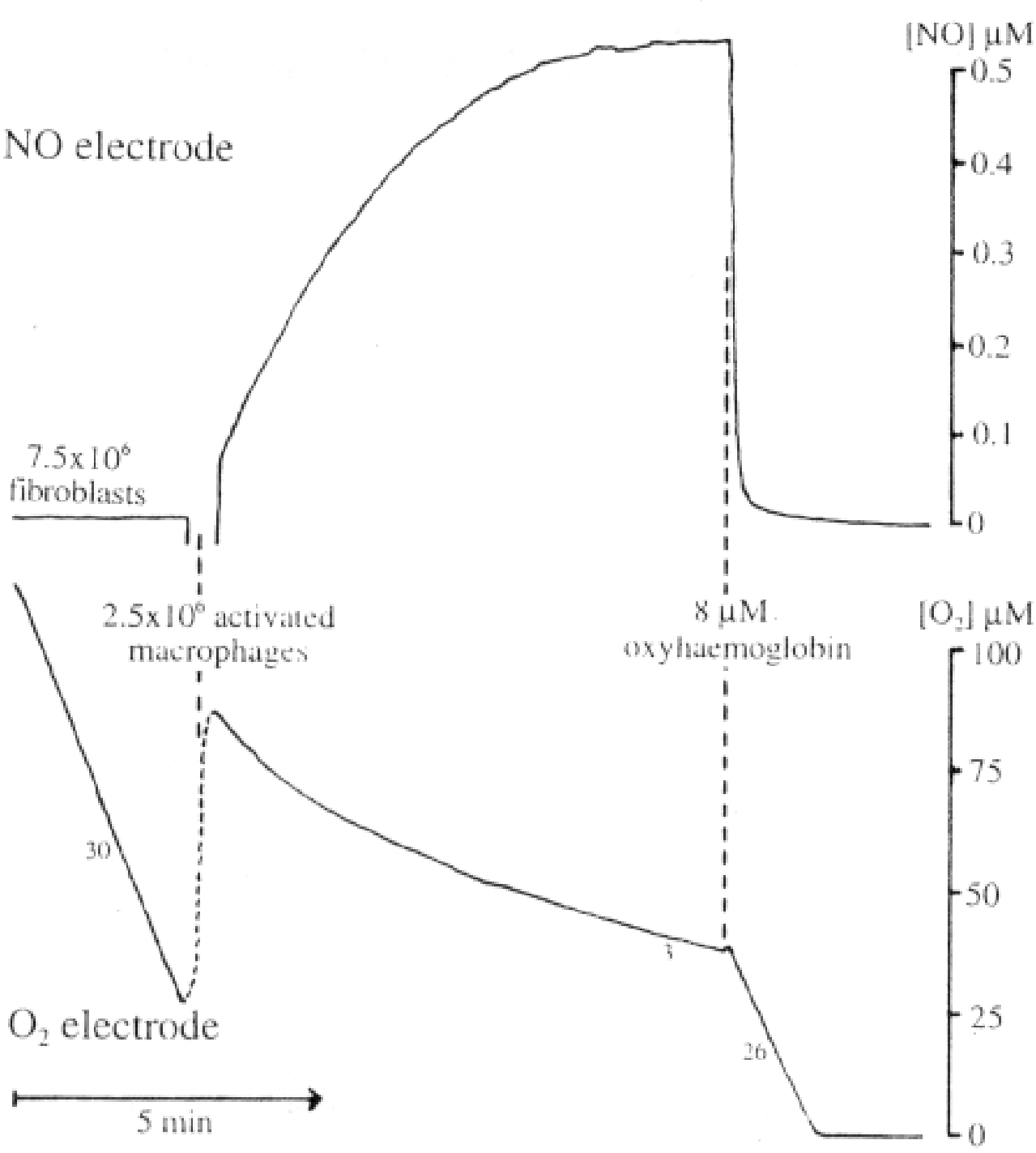
Brown, G. C., Bolanos, J. P., Heales, S. J. R. & Clark, J. B. (1995) Nitric oxide produced by activated astrocytes rapidly and reversibly inhibits cellular respiration. Neuroscience Lett. 193, 201-204.

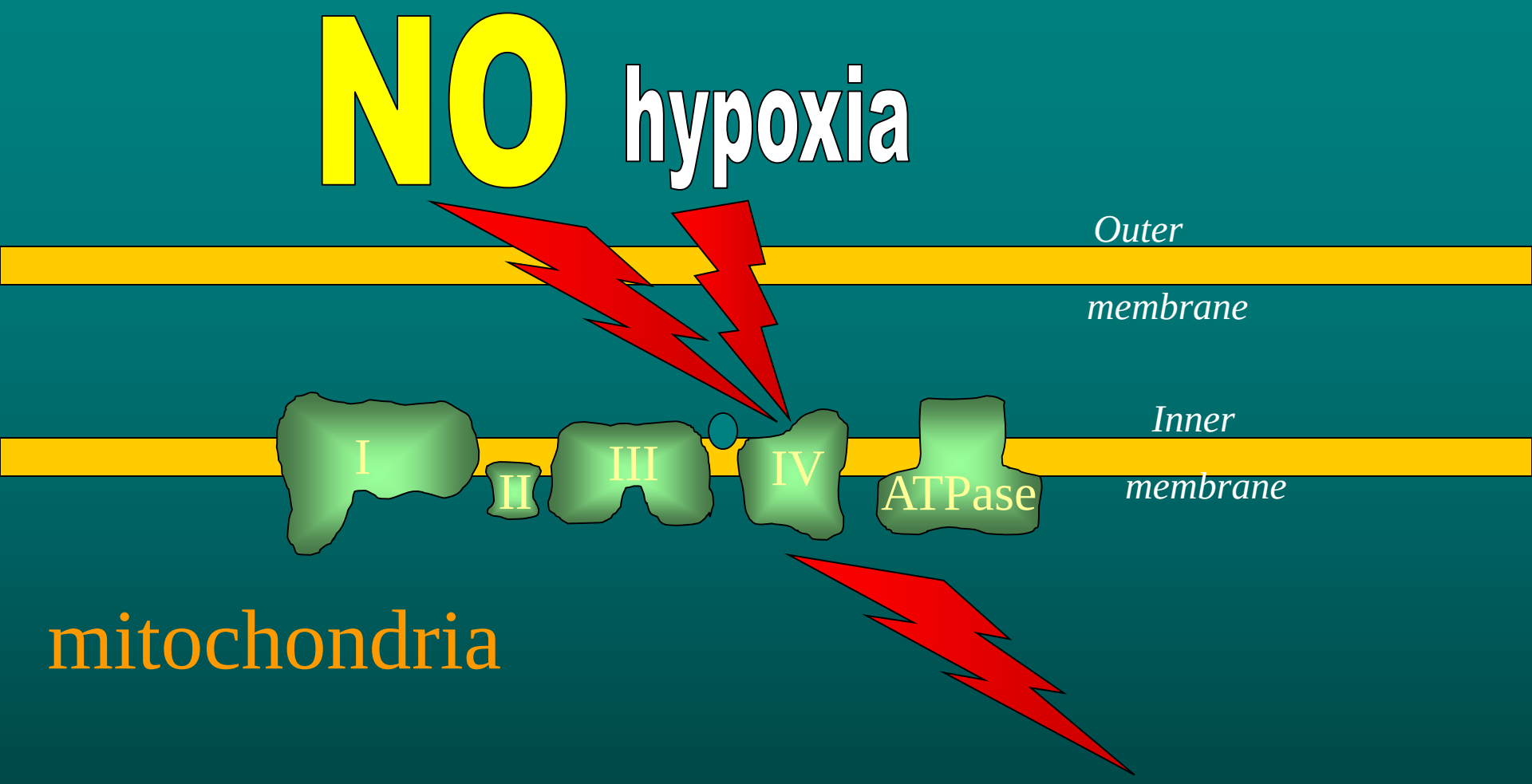
Cytokine-activated macrophages express iNOS and reversibly inhibit the respiration of co-incubated cells.

Brown, G. C., Foxwell, N. & Moncada, S. (1998) Transcellular regulation of cell respiration by nitric oxide generated by activated macrophages. FEBS Lett.439,

321-324.



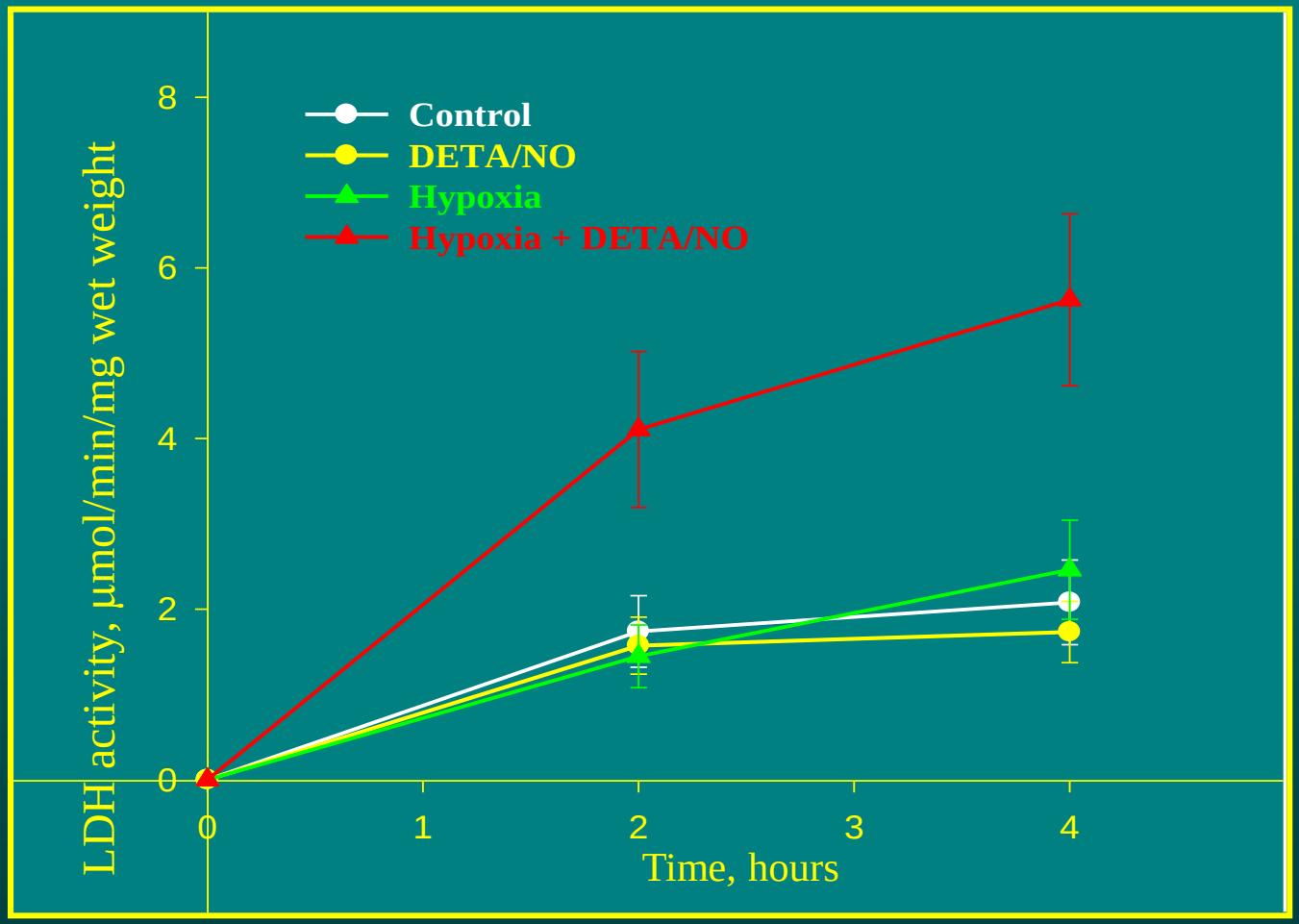




mitochondria

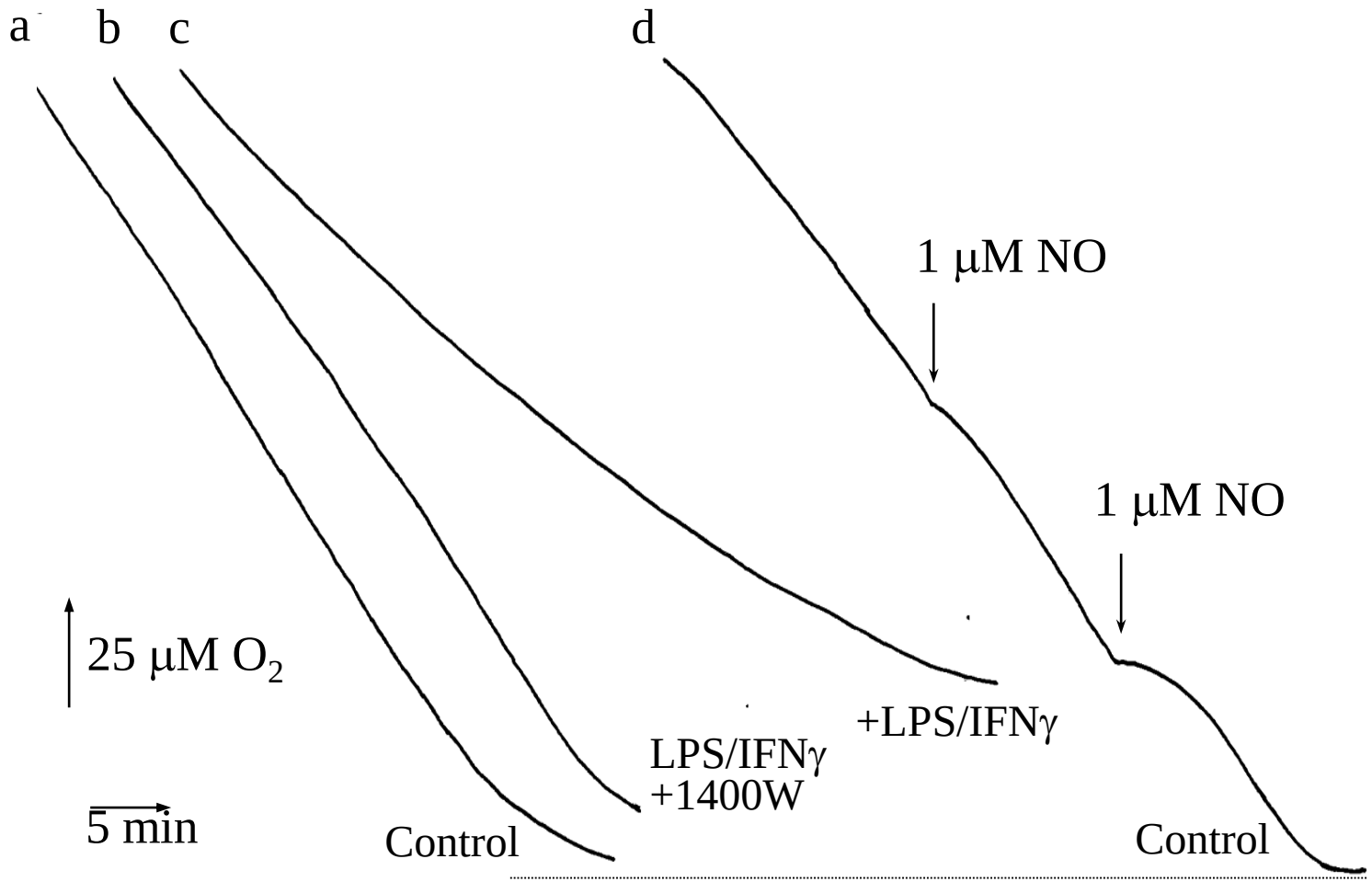
Death?
Apoptosis or
Necrosis?

NO sensitizes isolated aorta to hypoxia-induced necrosis



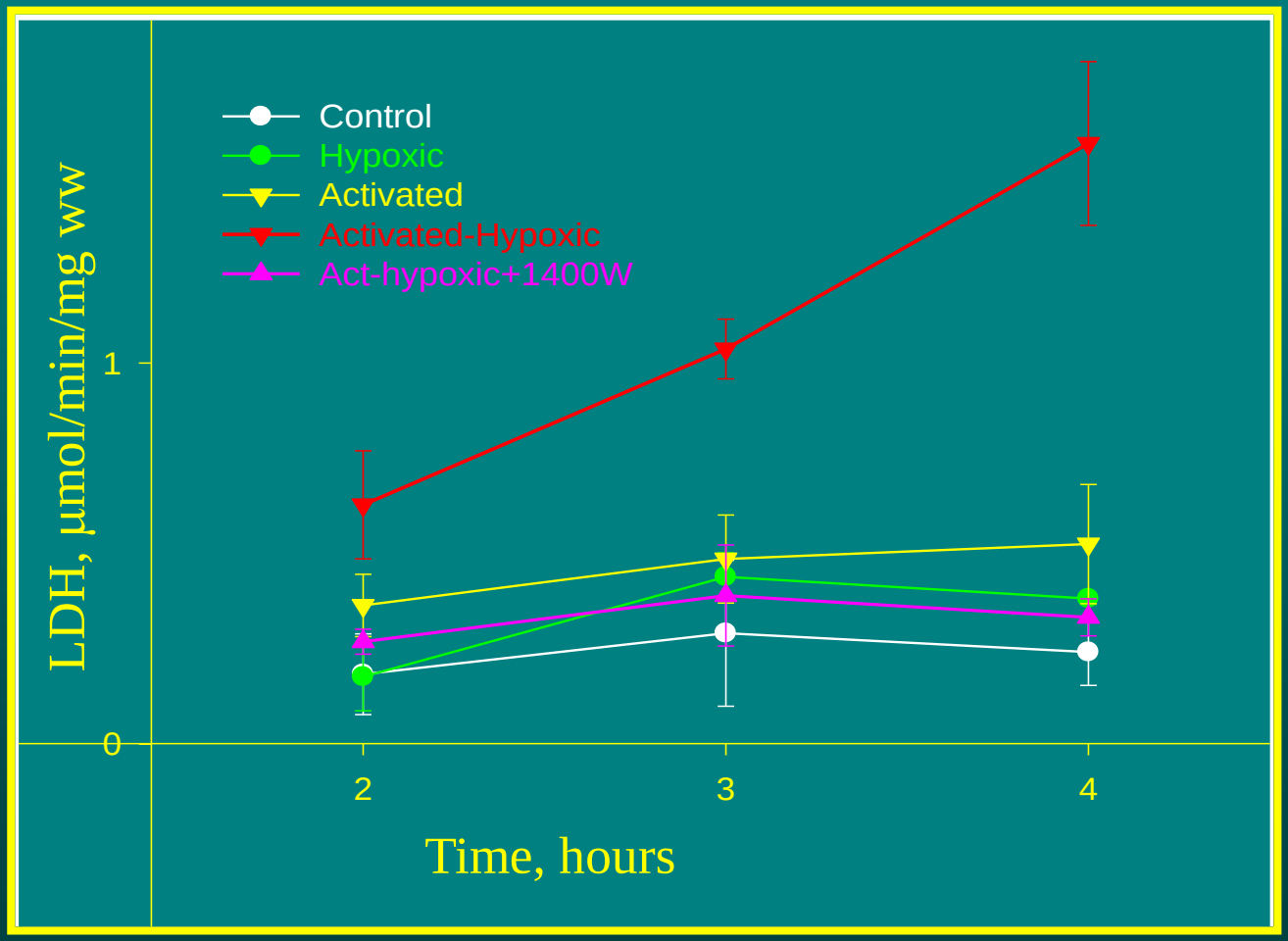
Borutaite, V., Moncada, S. & Brown, G. C. (2005) Nitric oxide from inducible nitric oxide synthase sensitizes the inflamed aorta to hypoxic damage via respiratory inhibition. Shock 23, 319-323.

The oxygen consumption of aortic rings is inhibited and oxygen-dependent after iNOS induction by LPS+IFNγ



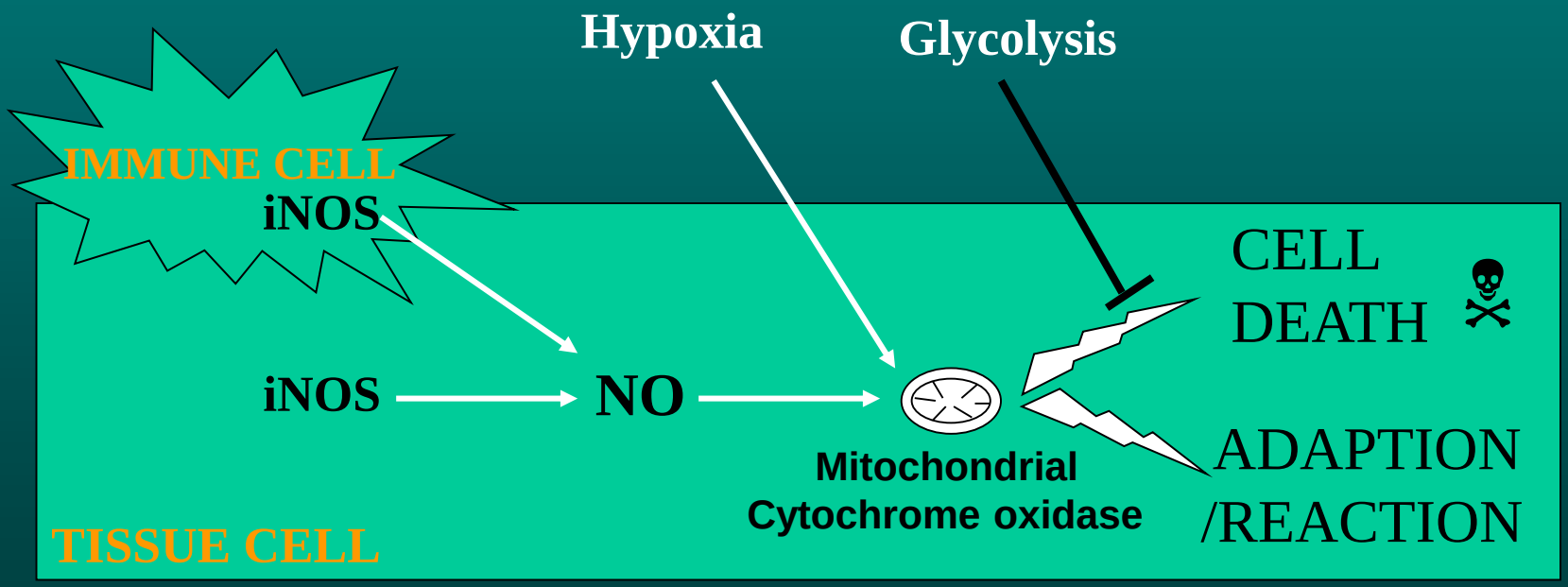
Borutaite, V., Moncada, S. & Brown, G. C. (2005) Nitric oxide from inducible nitric oxide synthase sensitizes the inflamed aorta to hypoxic damage via respiratory inhibition. Shock 23, 319-323.

NO produced by iNOS sensitizes aorta to hypoxia

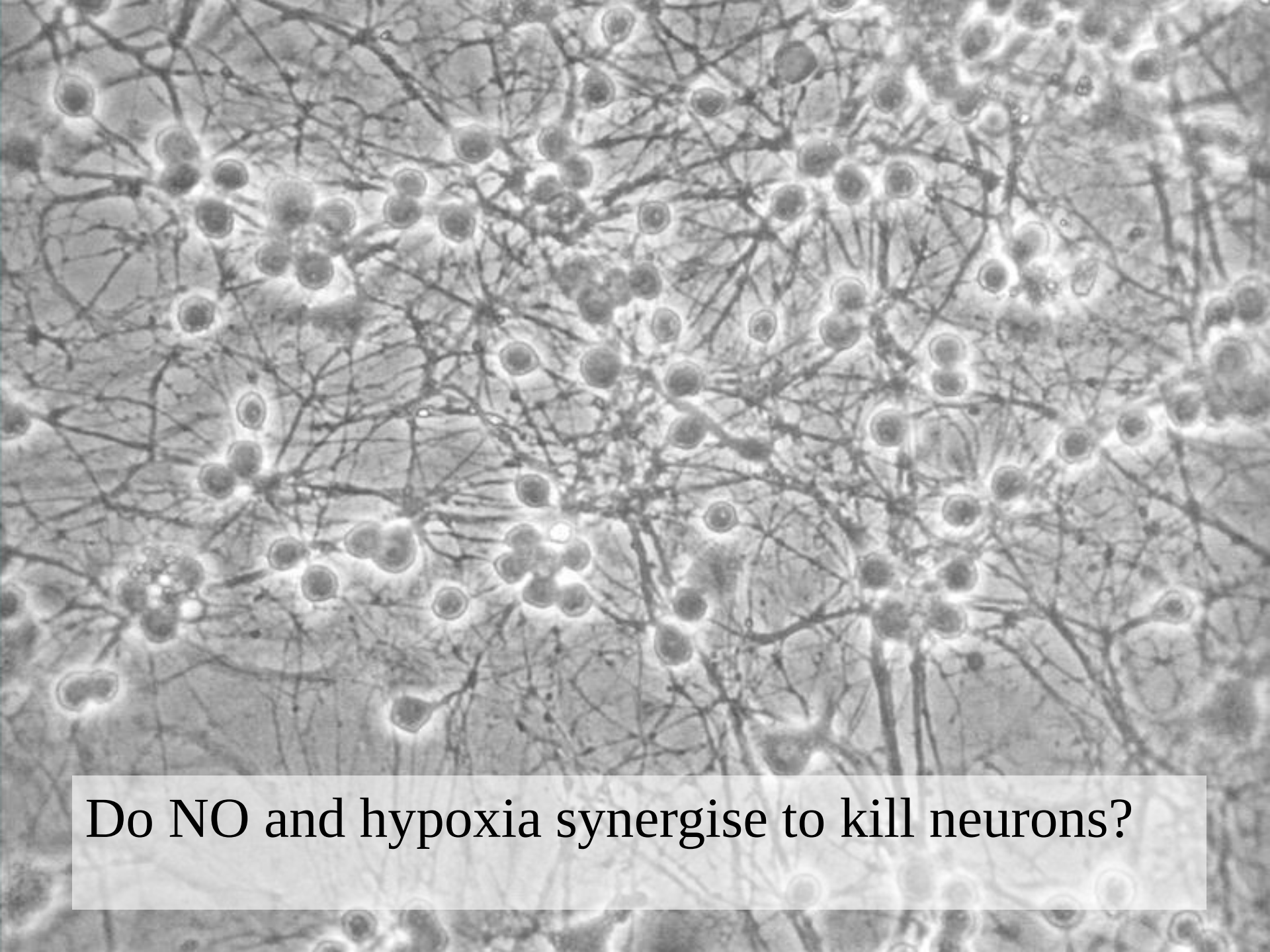


Borutaite, V., Moncada, S. & Brown, G. C. (2005) Nitric oxide from inducible nitric oxide synthase sensitizes the inflamed aorta to hypoxic damage via respiratory inhibition. Shock 23, 319-323.

- NO reversibly inhibits mitochondrial respiration at cytochrome oxidase.
- NO inhibition is competitive with O_2, raising the K_m of respiration into physiological range.
- NO from iNOS may sensitise tissues to hypoxia.

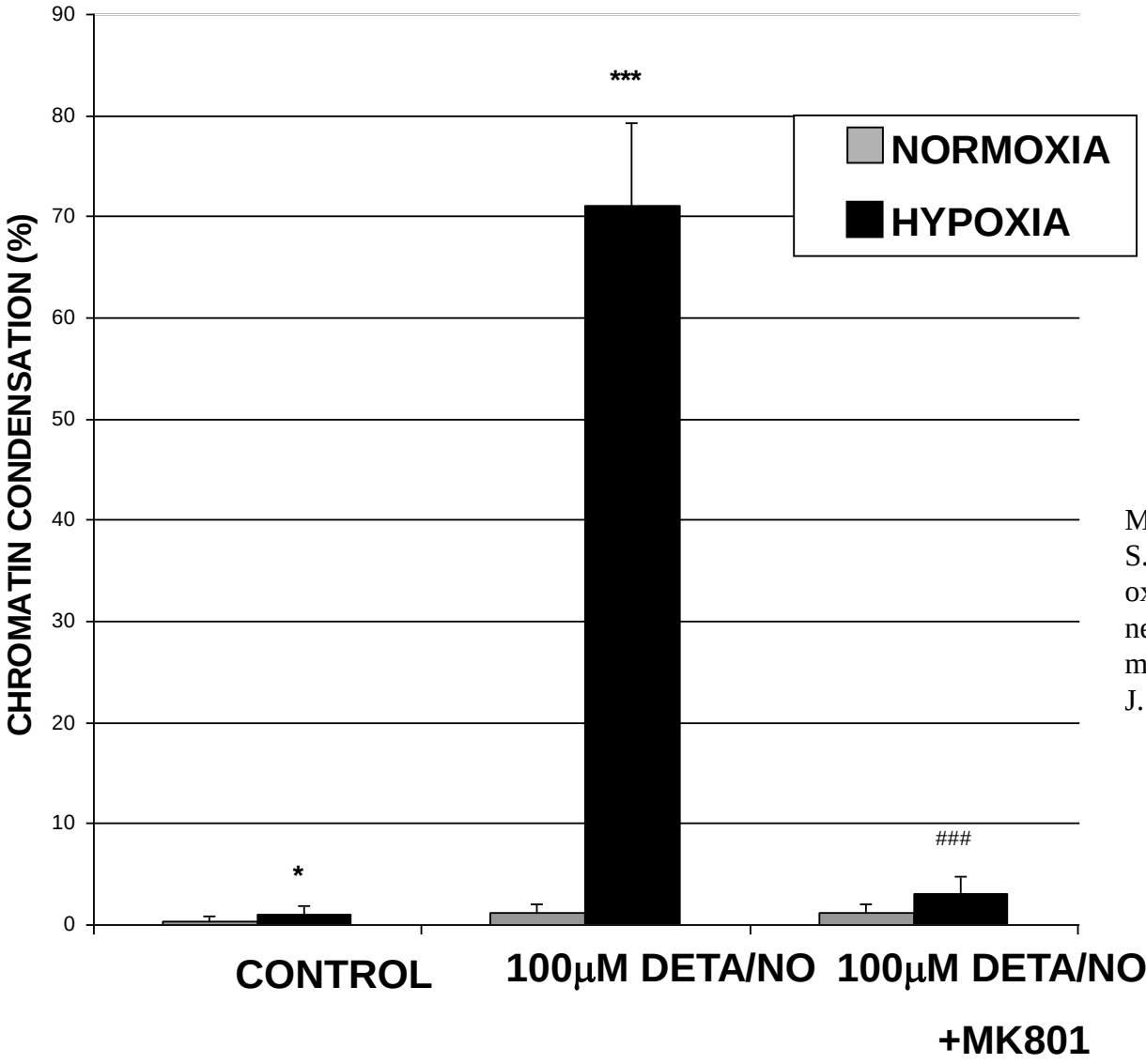


Relevant to: inflammation, sepsis, ischaemia, cancer, atheroschlerosis, neurodegeneration?



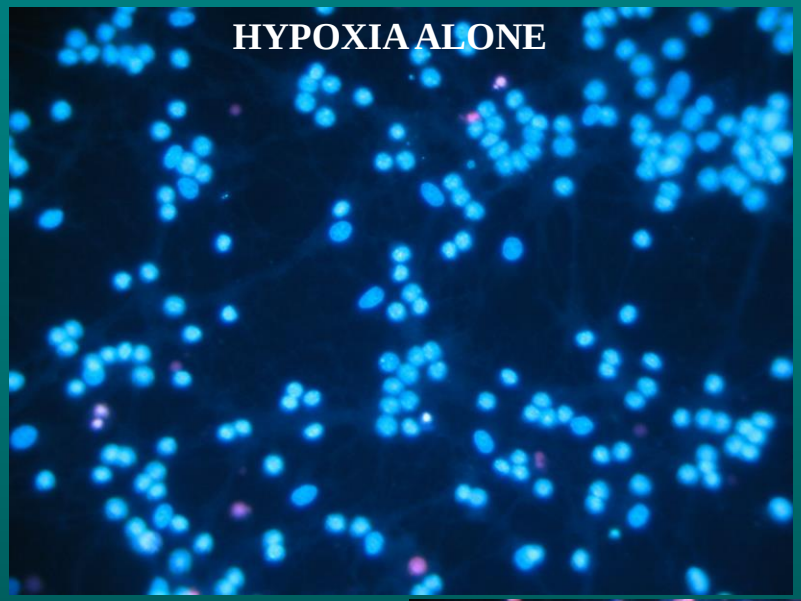
Do NO and hypoxia synergise to kill neurons?

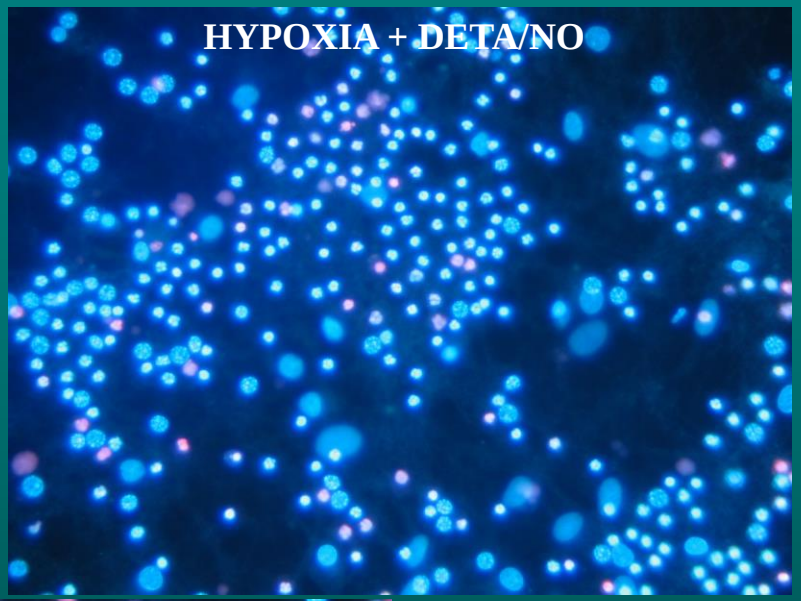
NO donor DETA/NO synergises with hypoxia (2% O_2) to induce 'apoptosis' in CGC neurons

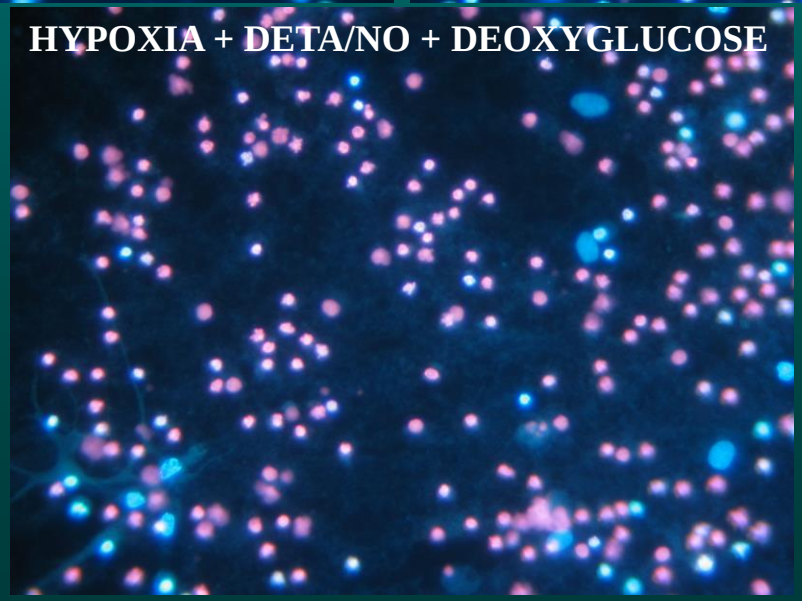


Mander, P., Borutaite, V., Moncada, S. & Brown G. C. (2005) Nitric oxide from glial iNOS sensitizes neurons to hypoxic death via mitochondrial respiratory inhibition. J. Neurosci. Res. 79, 208-215.

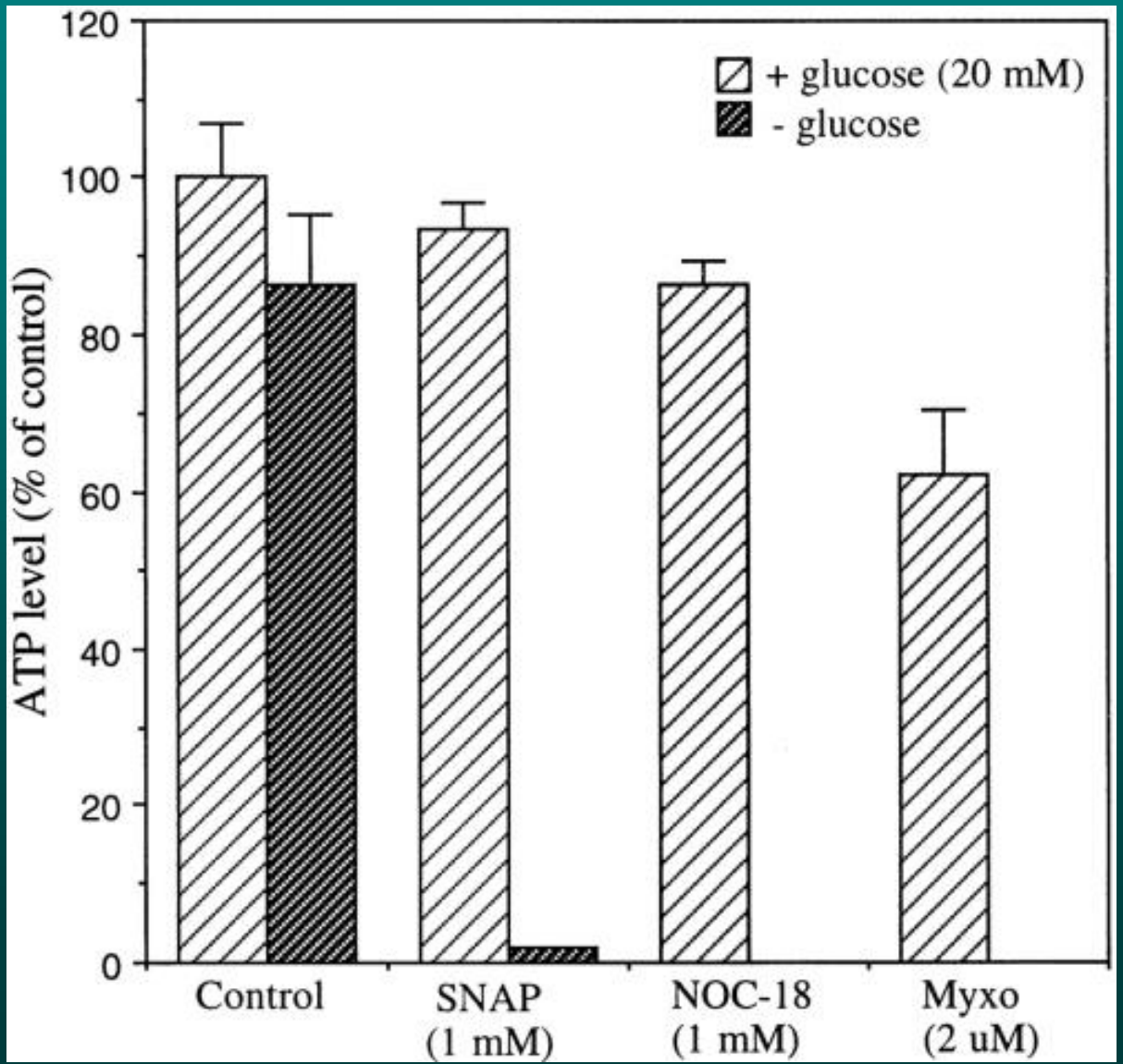
NO/HYPOXIA INDUCE NEURONAL DEATH



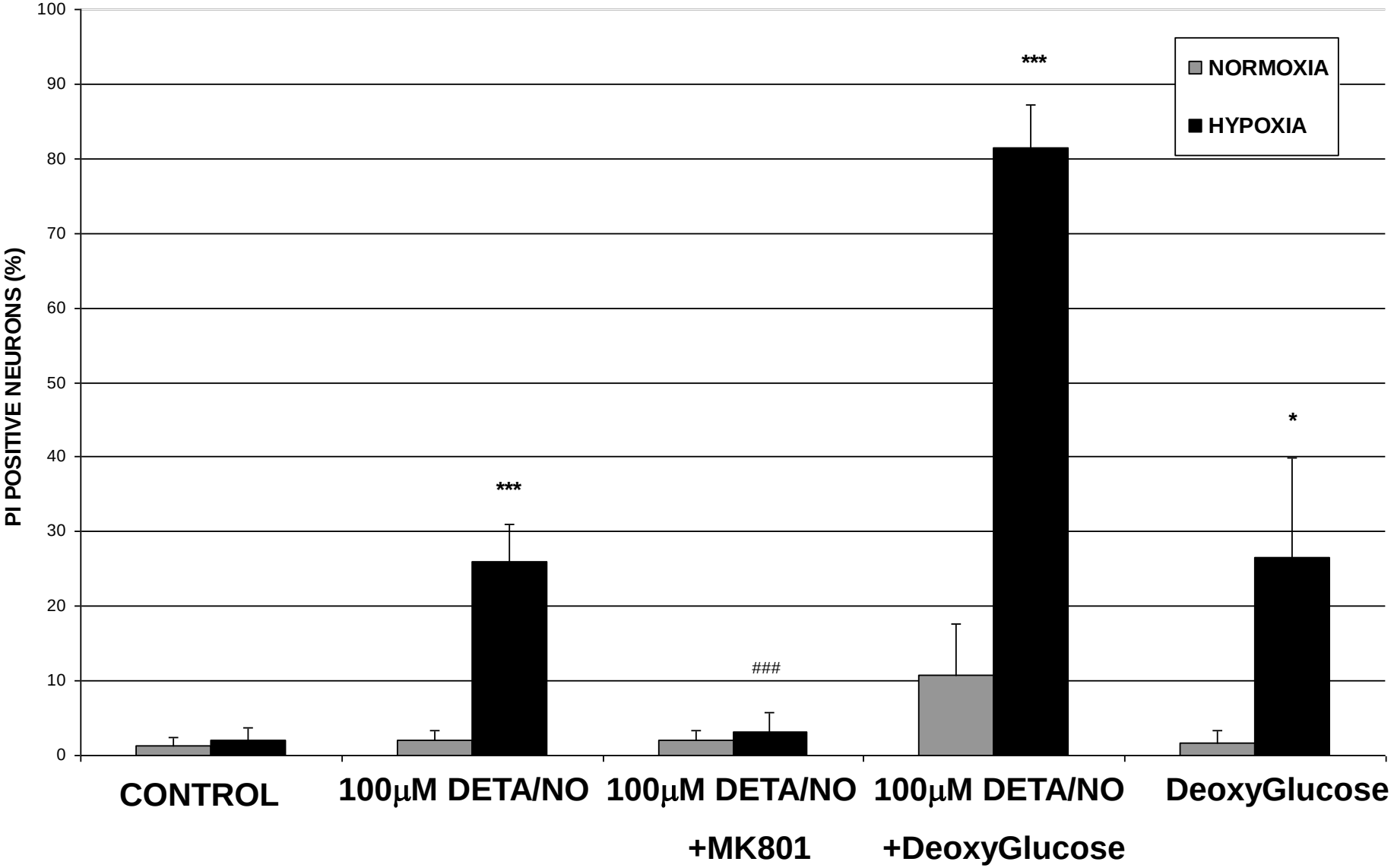




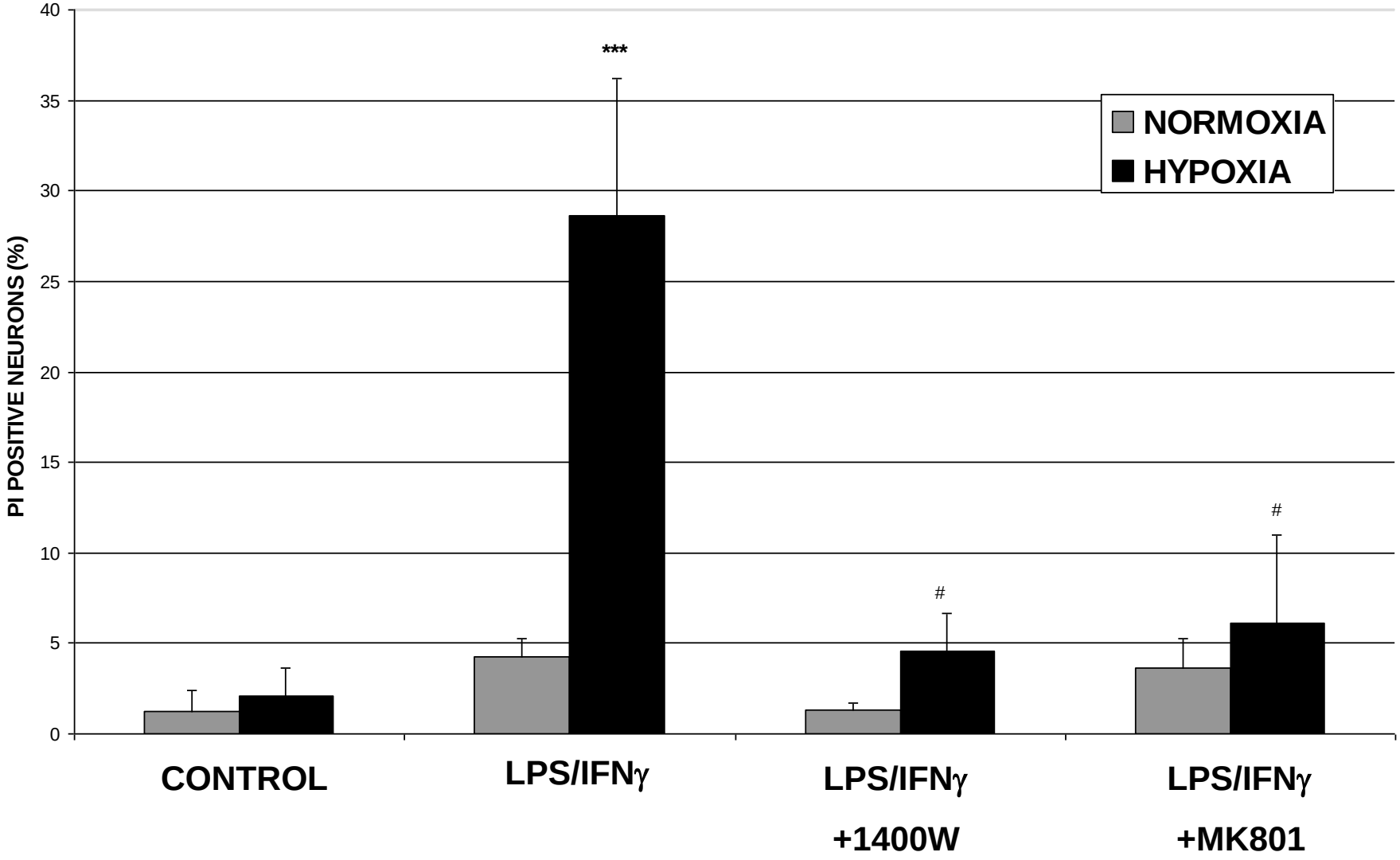
NO donors lower cellular ATP in presence of glucose, but completely deplete ATP in absence of glucose.



NO donor DETA/NO synergises with hypoxia (2% O_2) and deoxyglucose to induce necrosis in CGC neurons

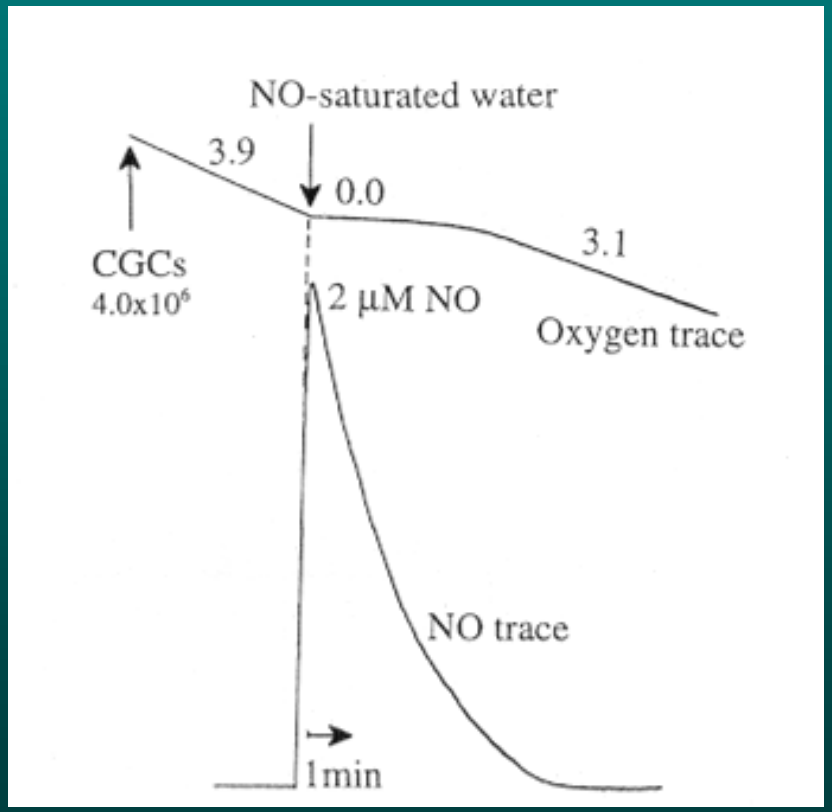


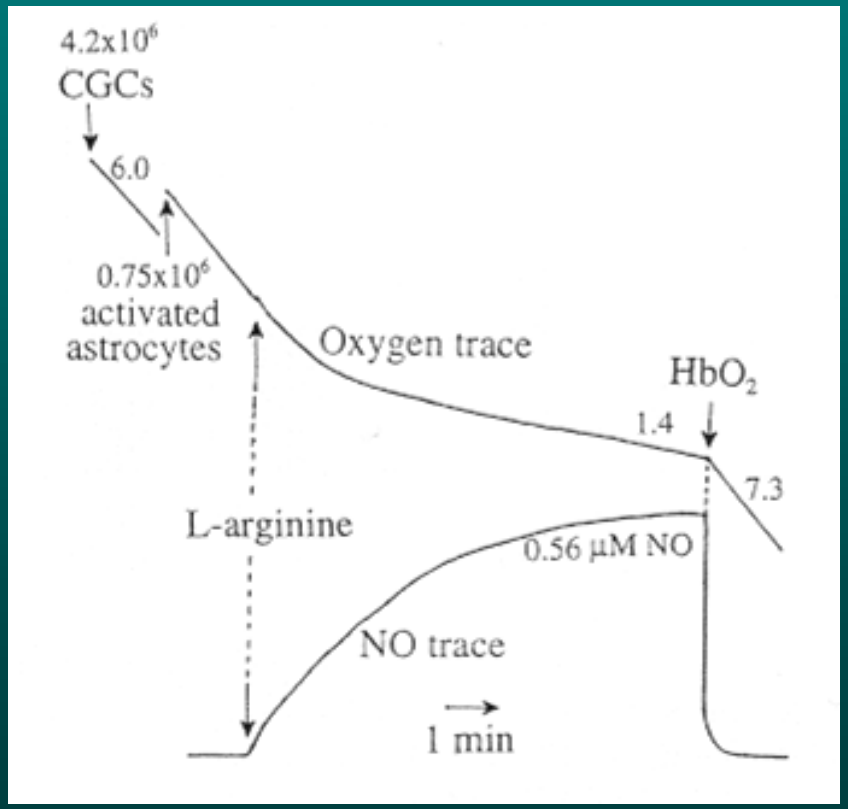
NO from iNOS (induced by LPS/IFNγ) synergises with hypoxia (2% O_2) to induce necrosis in CGC neurons



NO completely but reversibly inhibits neuronal respiration at cytochrome oxidase

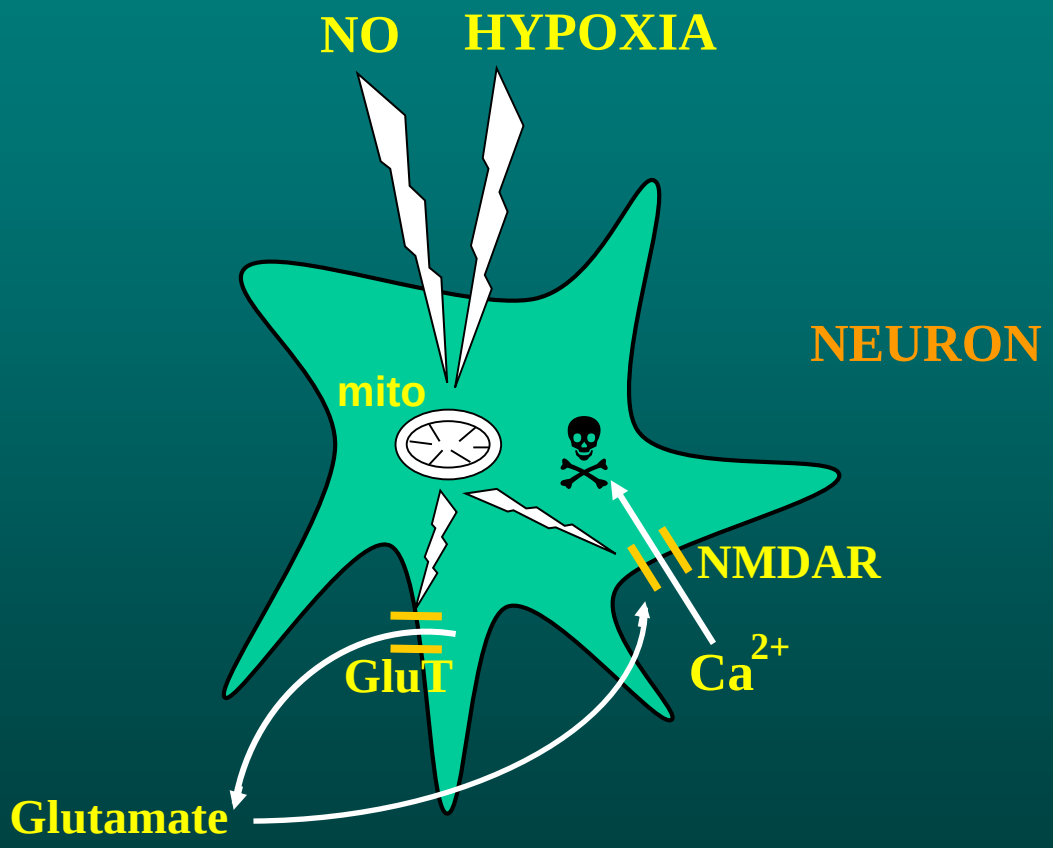
NO from activated astrocytes reversibly inhibits neuronal respiration





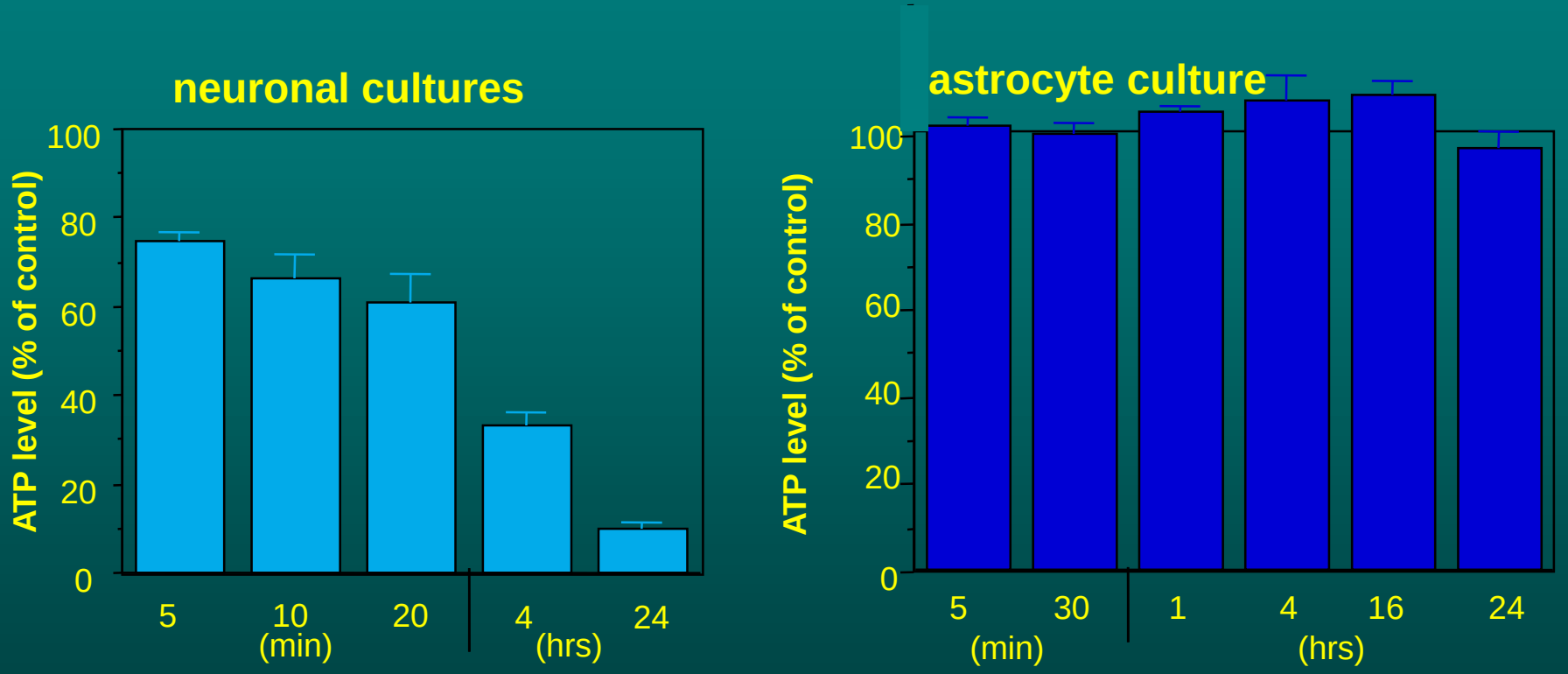
Bal-Price, A. & Brown, G. C. (2001) Inflammatory neurodegeneration mediated by nitric oxide from activated glia, inhibiting neuronal respiration, causing glutamate release and excitoxicity. J. Neuroscience 21, 6480-6491.

Hypoxia induces neuronal death via inhibiting cytochrome oxidase resulting in excitotoxity



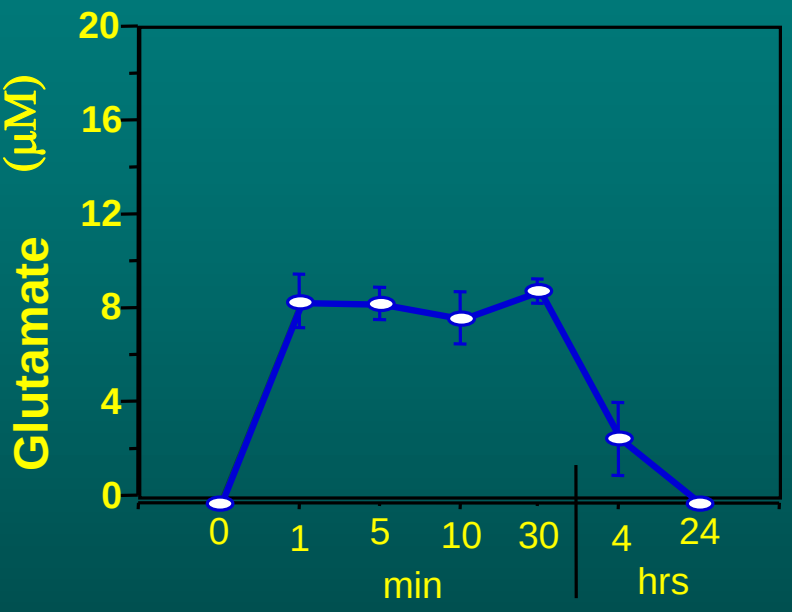
Important in stroke and dementia?

NO causes rapid depletion of ATP in neuronal but not in astrocytic cultures

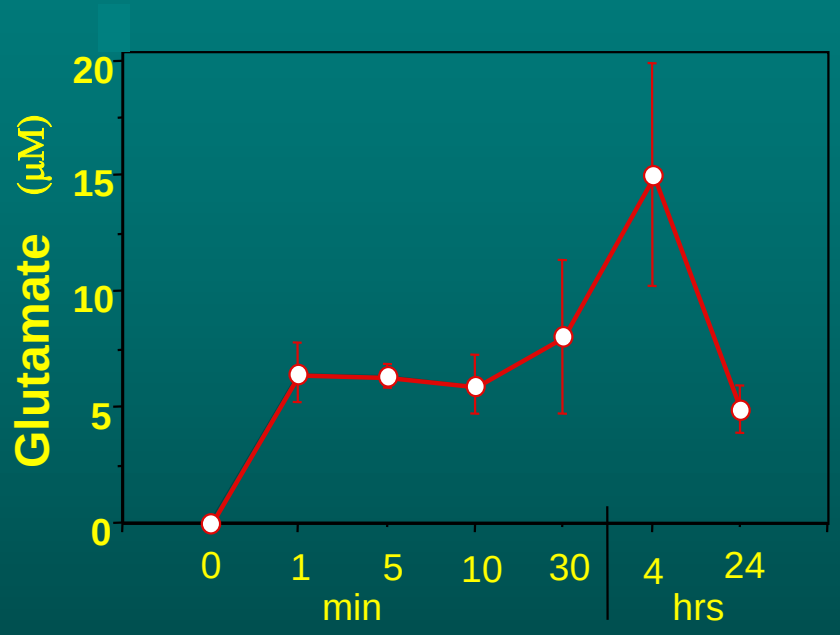


Time of exposure to NO donor DETA/NO (500 μM)

Rapid release of glutamate from neuronal cultures induced by an NO donor DETA/NO and respiratory inhibitor myxothiazol



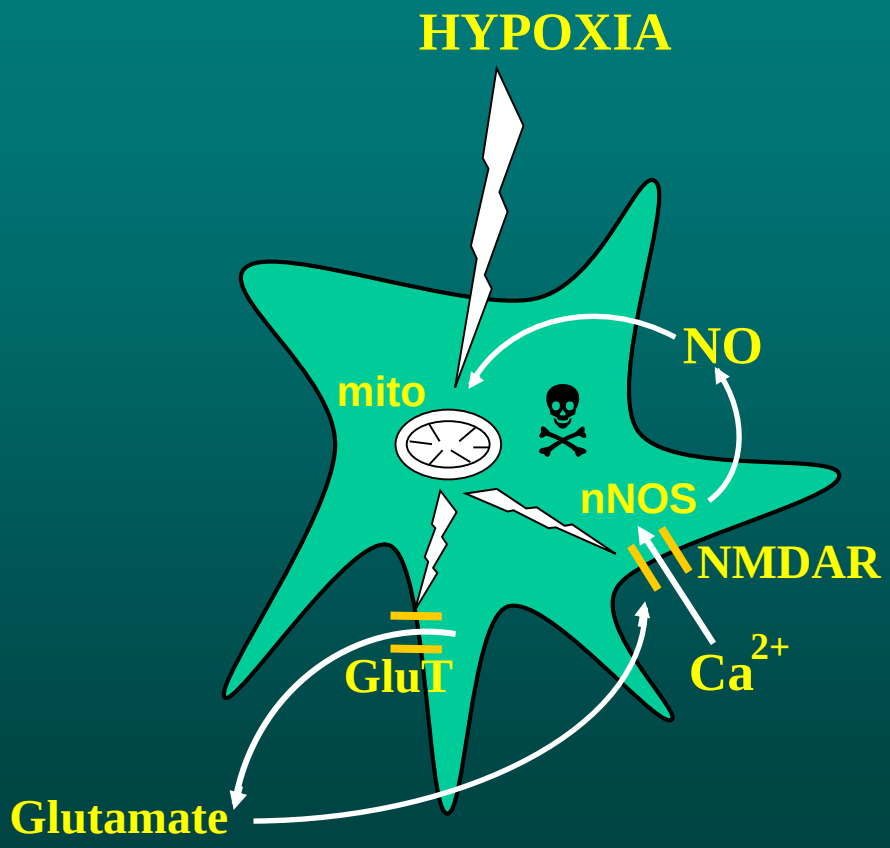
Time of exposure to DETA/NO (500 μM)



Time of exposure to myxothiazol (2 μM)

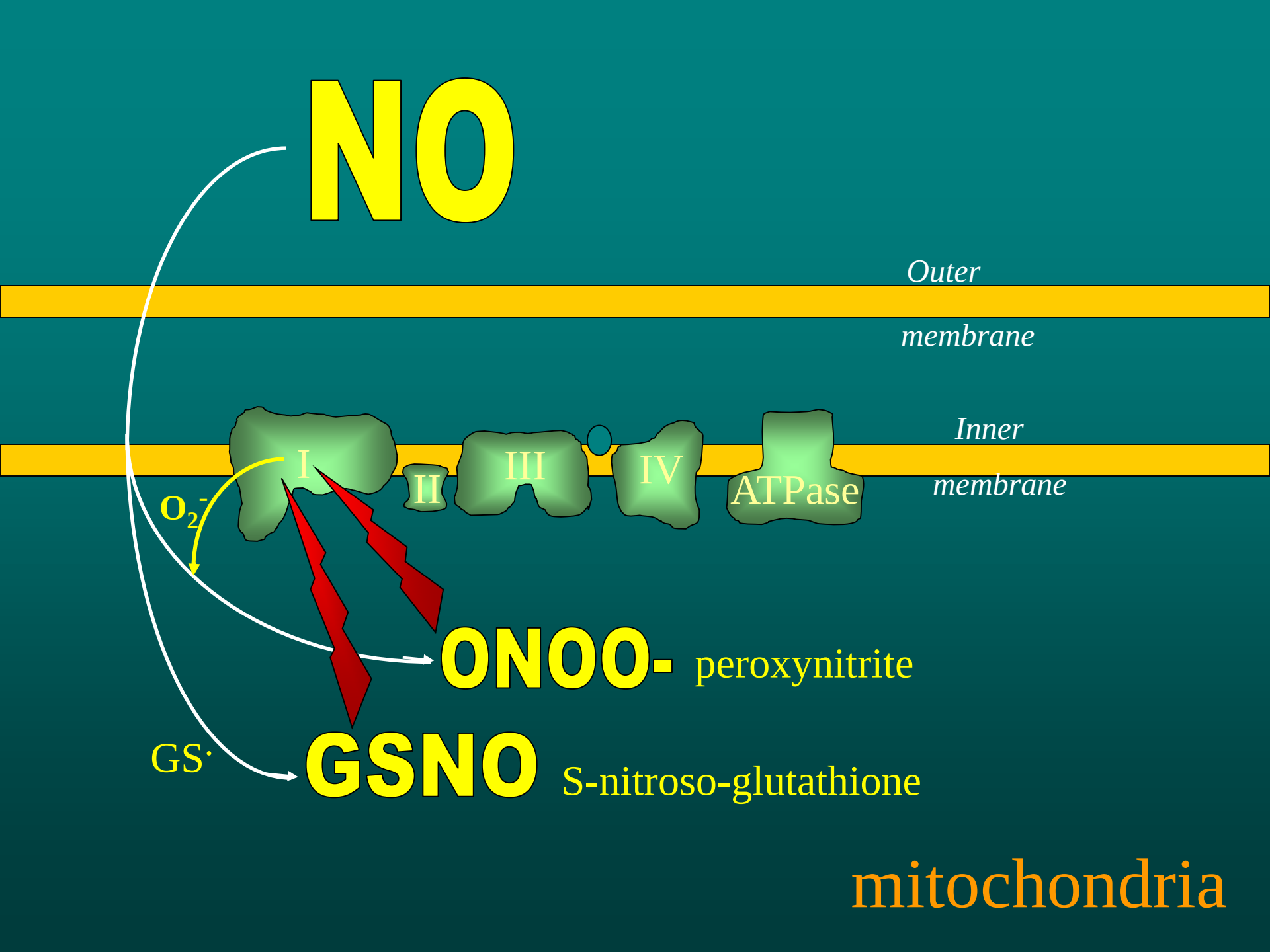
- Release is rapid. Over concentration range as inhibits respiration.
- Other respiratory inhibitors (e.g. cyanide) cause release.
- Release greater at low O_2. Calcium and cGMP independent.

nNOS is activated by NMDA receptor and might contribute to hypoxic death by sensitising cytochrome oxidase

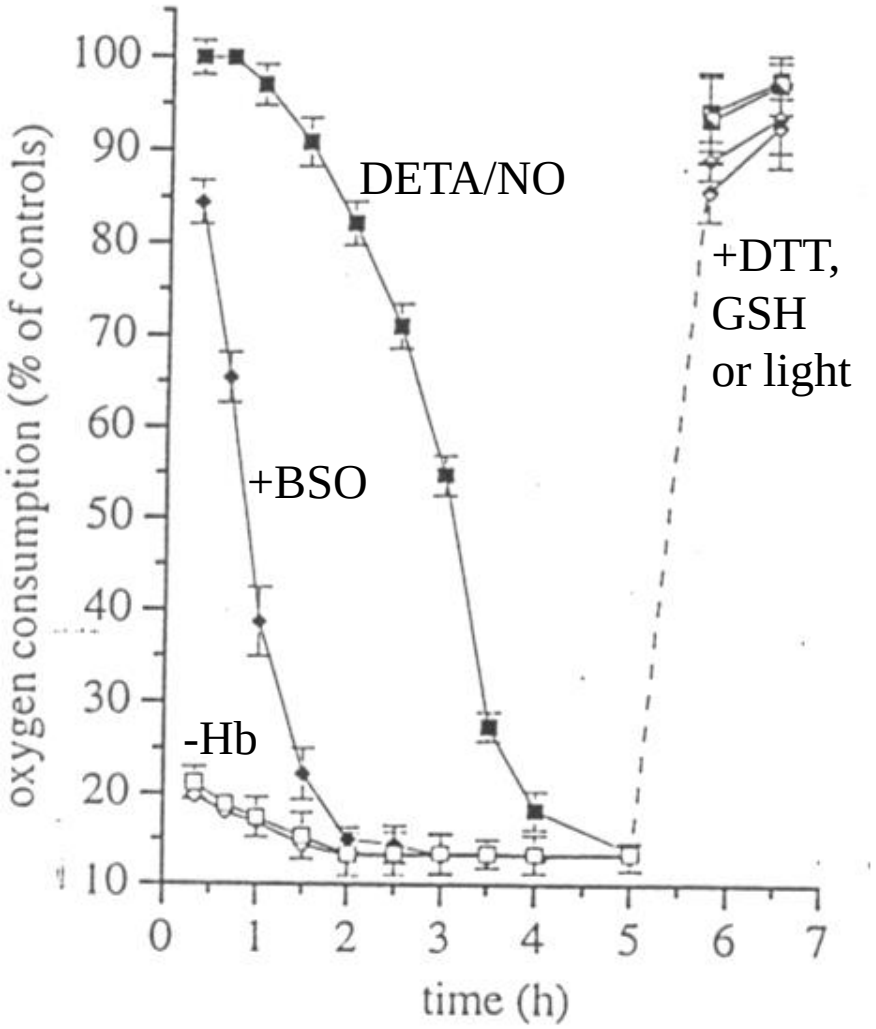


Summary:

- NO reversibly inhibits mitochondrial respiration at cytochrome oxidase.
- NO inhibition is competitive with O_2, raising the K_m of respiration into physiological range.
- NO from iNOS may sensitise cells to hypoxic/ischaemic death.
- Glycolytic capacity determines sensitivity and form of cell death.
- Relevant in ischaemic, infectious, inflammatory and neurodegerative diseases.


NO
Outer
membrane
Inner
membrane
I
II
III
IV
ATPase
$O_2^{\cdot-}$
ONOO- peroxynitrite
GS·
GSNO S-nitroso-glutathione
mitochondria

NO inactivates complex I



Incubation of cells with an NO donor (0.5mM DETA/NO) for hours results in inactivation of complex I and respiration.

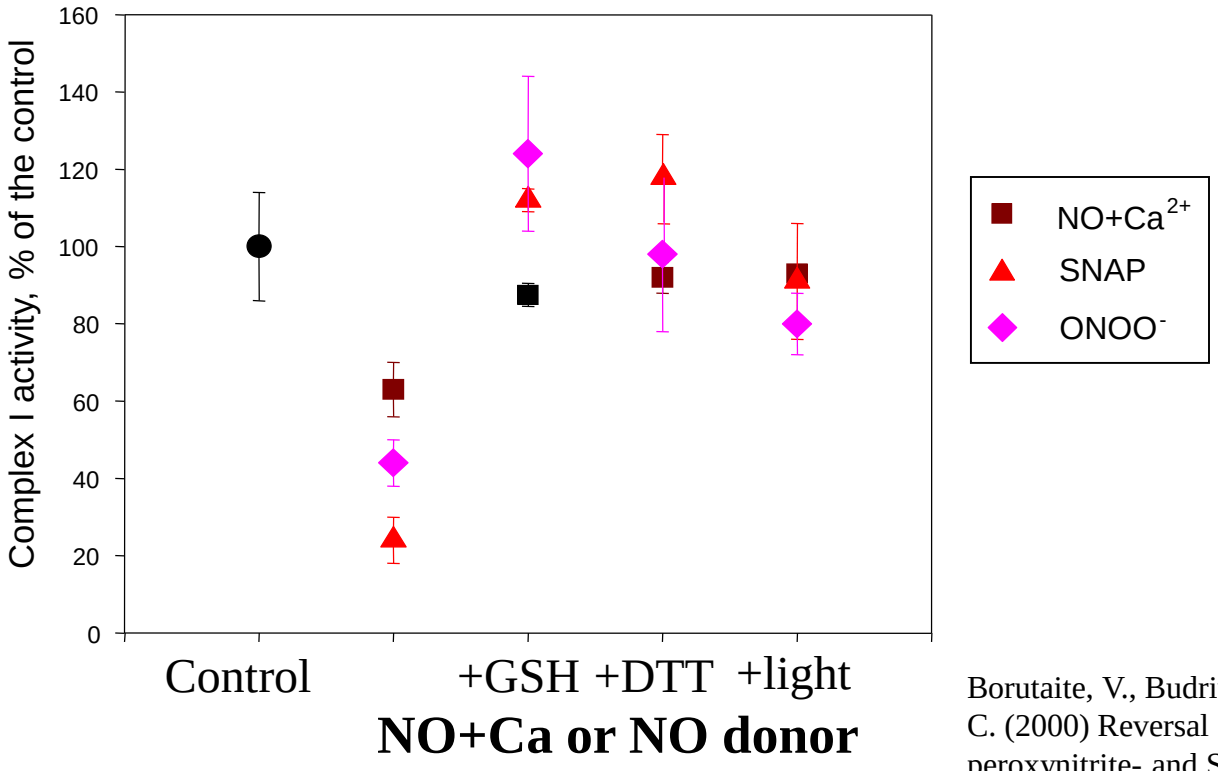
The inactivation is speeded by depleting cellular GSH with BSO.

The inactivation is reversed by DTT, GSH methylester or light.

The inactivation may be due to nitrosation of complex I thiol.

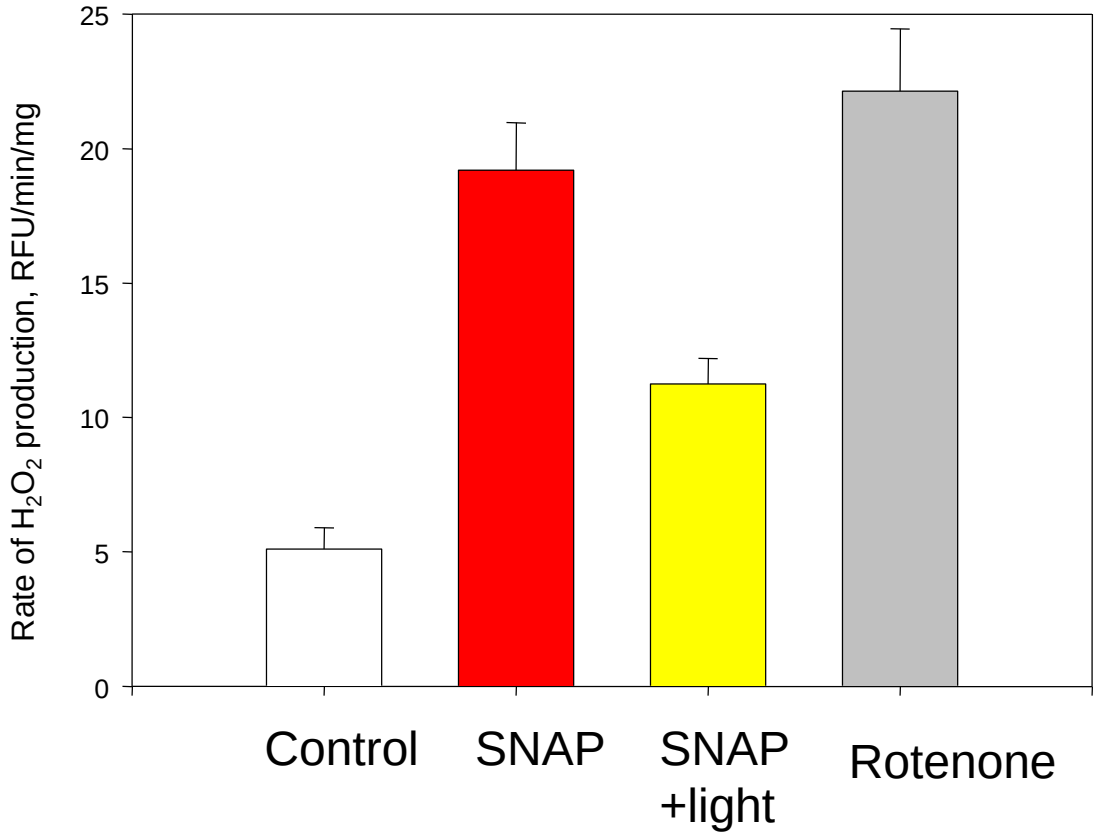
Clementi, E., Brown, G. C., Feelisch, M. & Moncada, S. (1998) Persistent inhibition of cell respiration by nitric oxide: Crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. Proc. Natl. Acad. Sci. 95, 7631-7636.

NO + Ca^{2+}, peroxynitrite or S-nitrosothiols cause inhibition of complex I and this inhibition is reversed by light and thiols

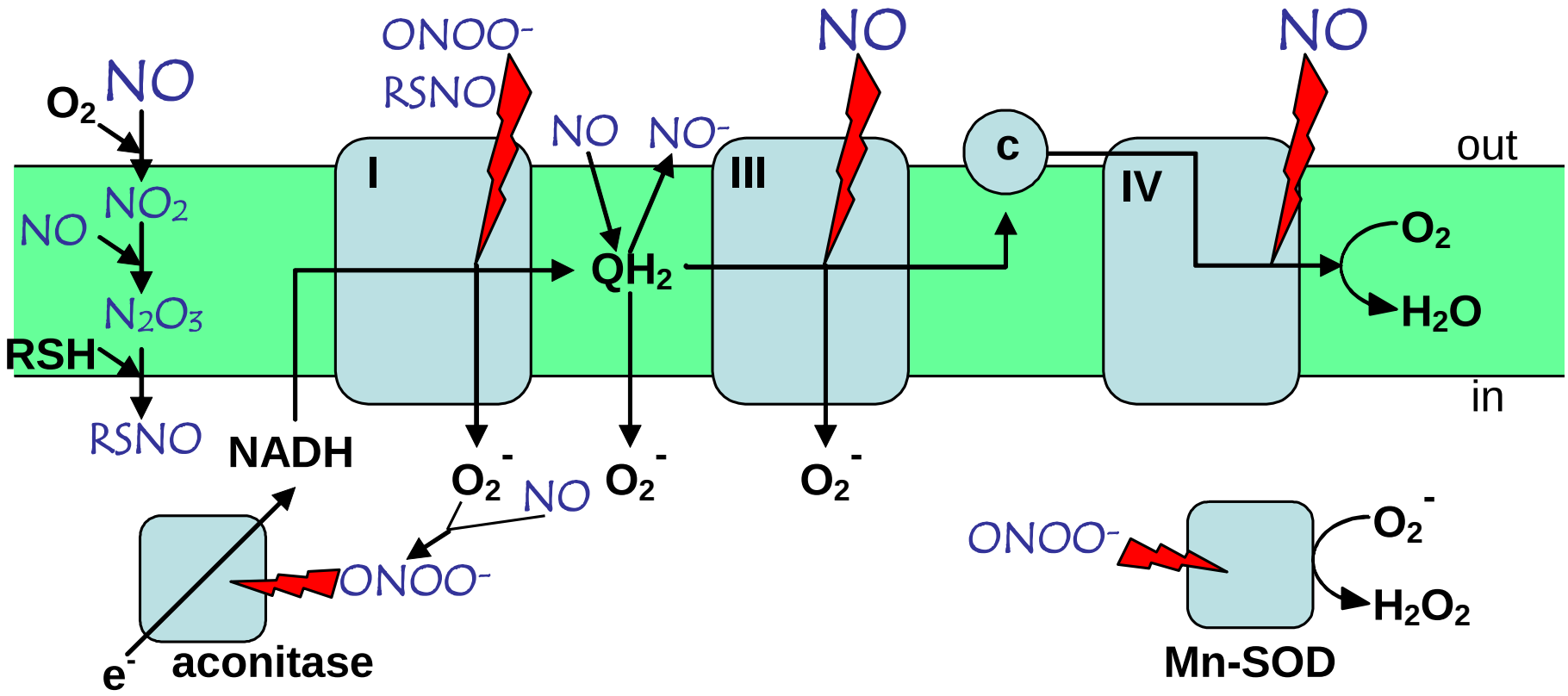


Borutaite, V., Budriunaite, A. & Brown, G. C. (2000) Reversal of nitric oxide-, peroxynitrite- and S-nitrosothiol-induced inhibition of mitochondrial respiration or complex I activity by light and thiols. Biochim. Biophys. Acta 1459,405-412.

S-nitrosothiol inactivation of complex I reversibly increases H_2O_2 production by mitochondria

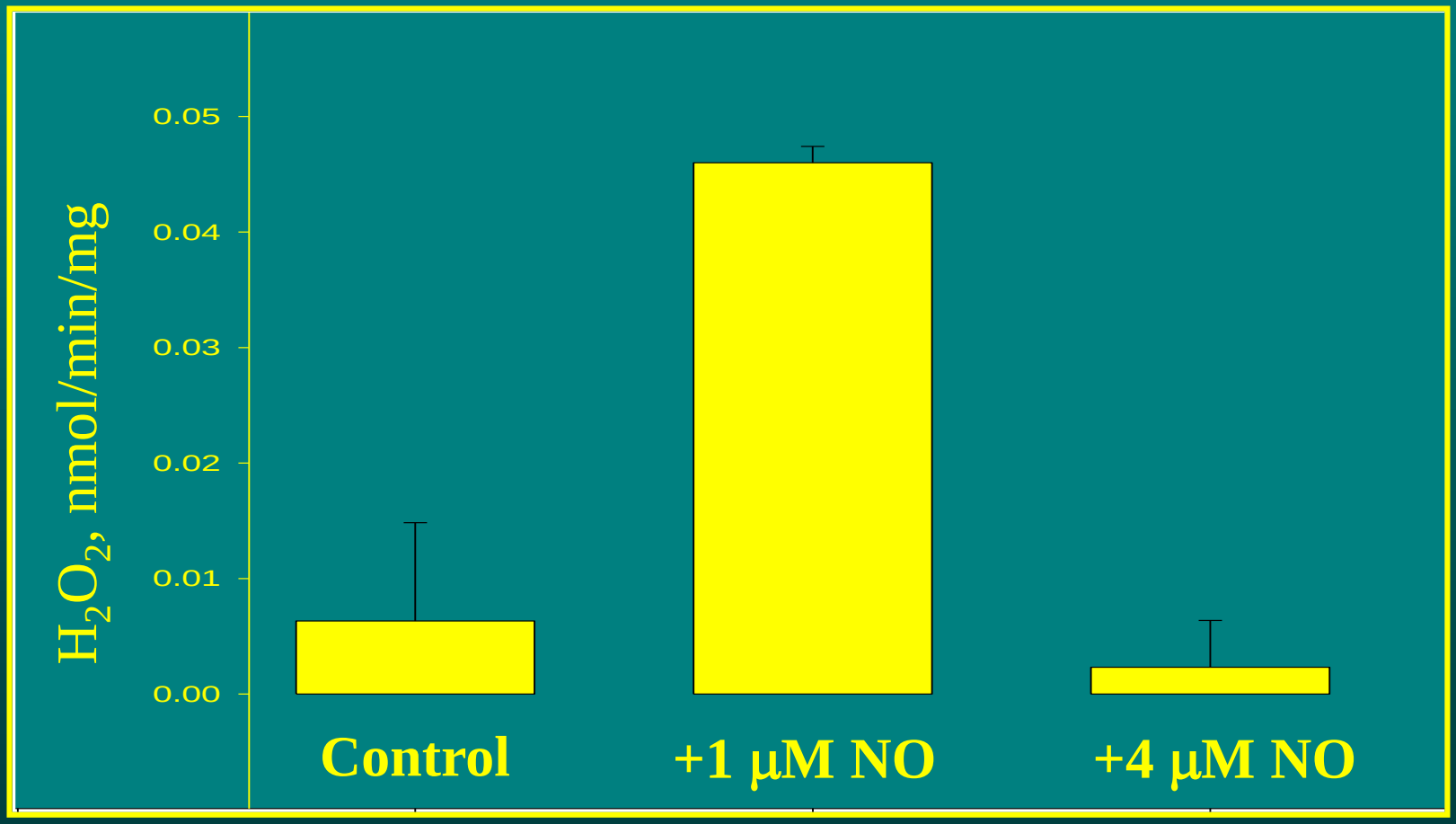


Borutaite, V. & Brown, G. C. (2006) S-nitrosothiol inhibition of mitochondrial complex I causes a reversible increase in mitochondrial hydrogen peroxide production. Biochim. Biophys. Acta 1757, 562-6.



Brown, G. C. (2007) Nitric oxide and mitochondria. Front. Biosci. 12, 1024-33.

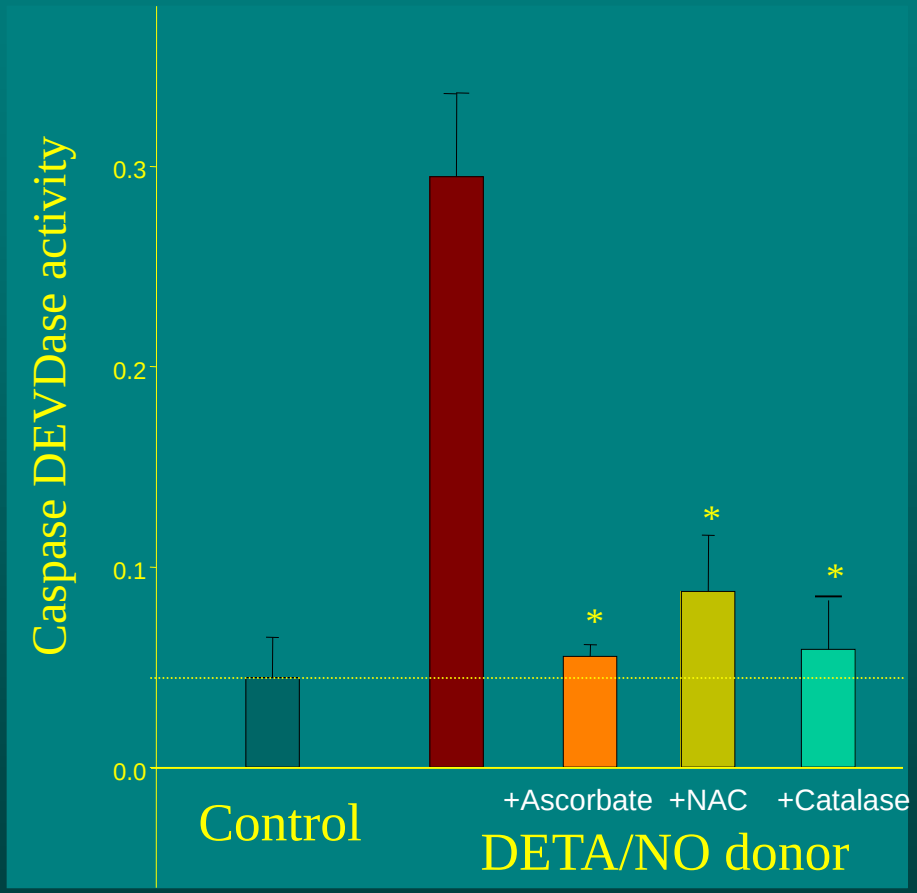
NO causes H_2O_2 production in isolated mitochondria



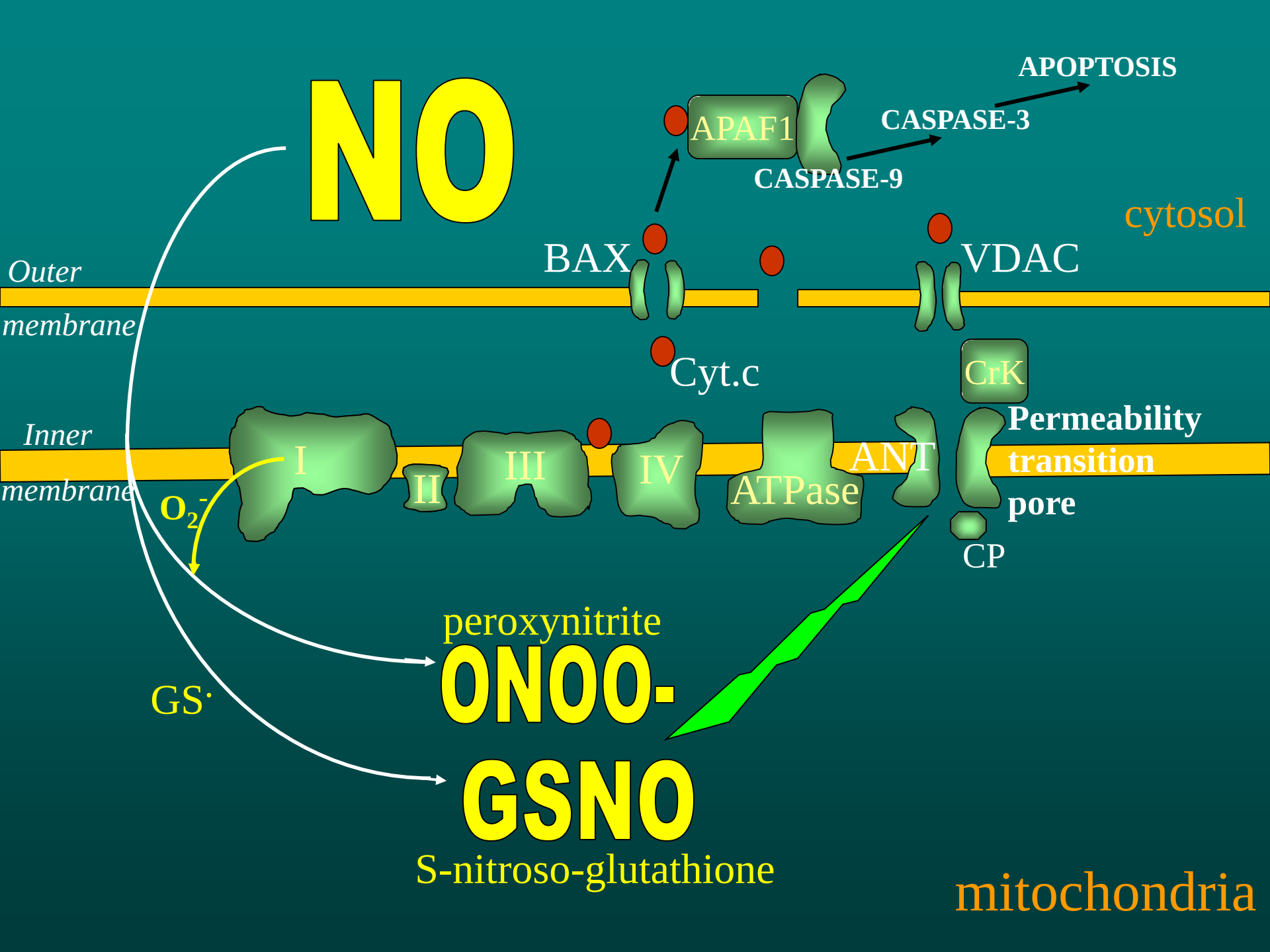
Borutaite, V. & Brown, G. C. (2003) Nitric oxide induces apoptosis via hydrogen peroxide, but necrosis via energy and thiol depletion. Free Rad. Biol. Med. 35, 1457-68.

NO-induced apoptosis is mediated by H_2O_2

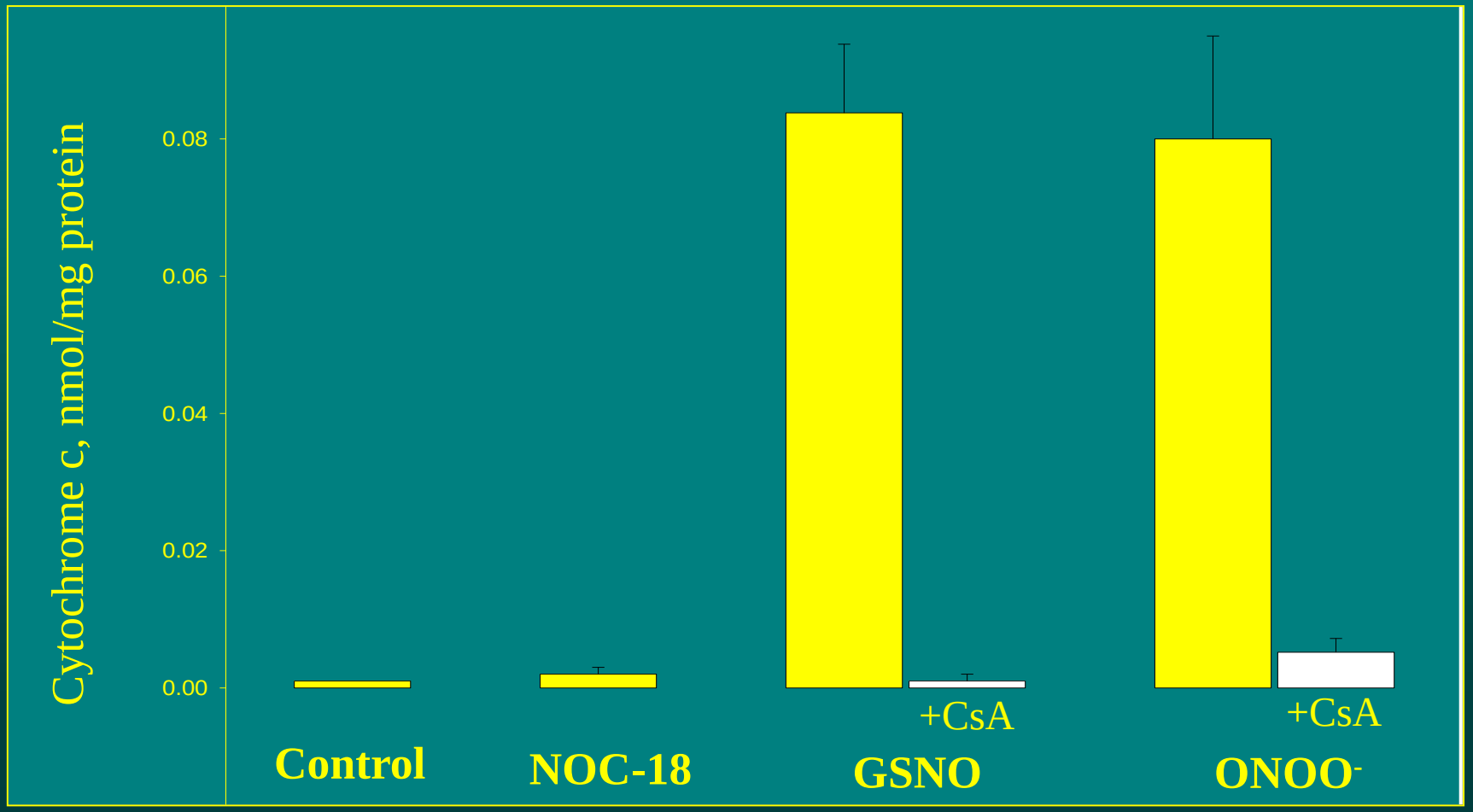
- NO increases oxidative stress in cells (DCF).
- NO can induce apoptosis via H_2O_2.
- Cells subsequently die by necrosis preceded by energy depletion.



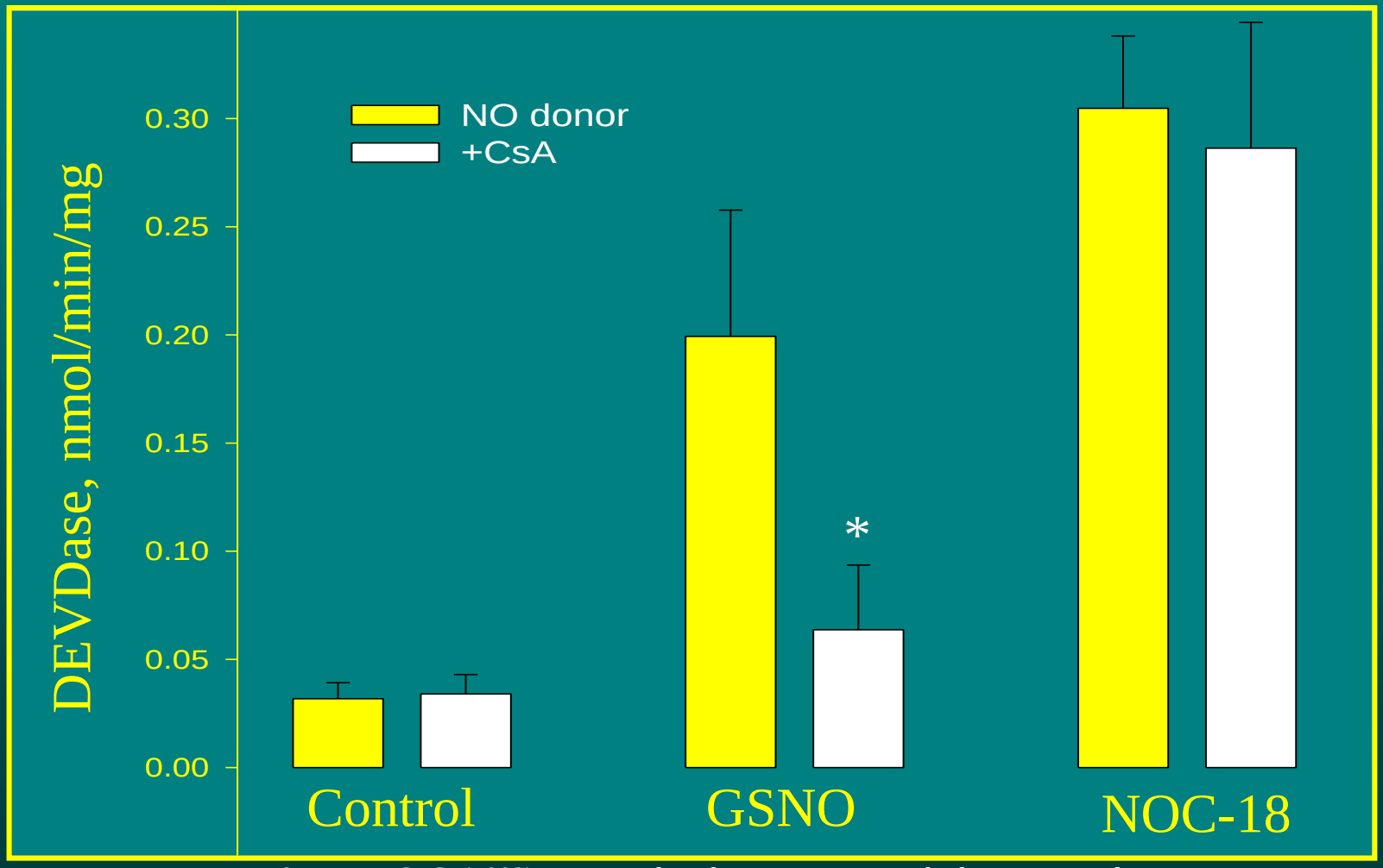
Borutaite, V. & Brown, G. C. (2003) Nitric oxide induces apoptosis via hydrogen peroxide, but necrosis via energy and thiol depletion. Free Rad. Biol. Med. 35, 1457-68.


NO
APOPTOSIS
APAF1
CASPASE-3
CASPASE-9
cytosol
BAX
VDAC
Outer
membrane
Cyt.c
CrK
Permeability
transition
pore
Inner
membrane
I
II
III
IV
ATPase
ANT
CP
O_2^-
peroxynitrite
ONOO-
GS·
GSNO
S-nitroso-glutathione
mitochondria

Nitrosothiols and peroxynitrite induce opening of permeability transition pore and release of cytochrome *c* in isolated mitochondria

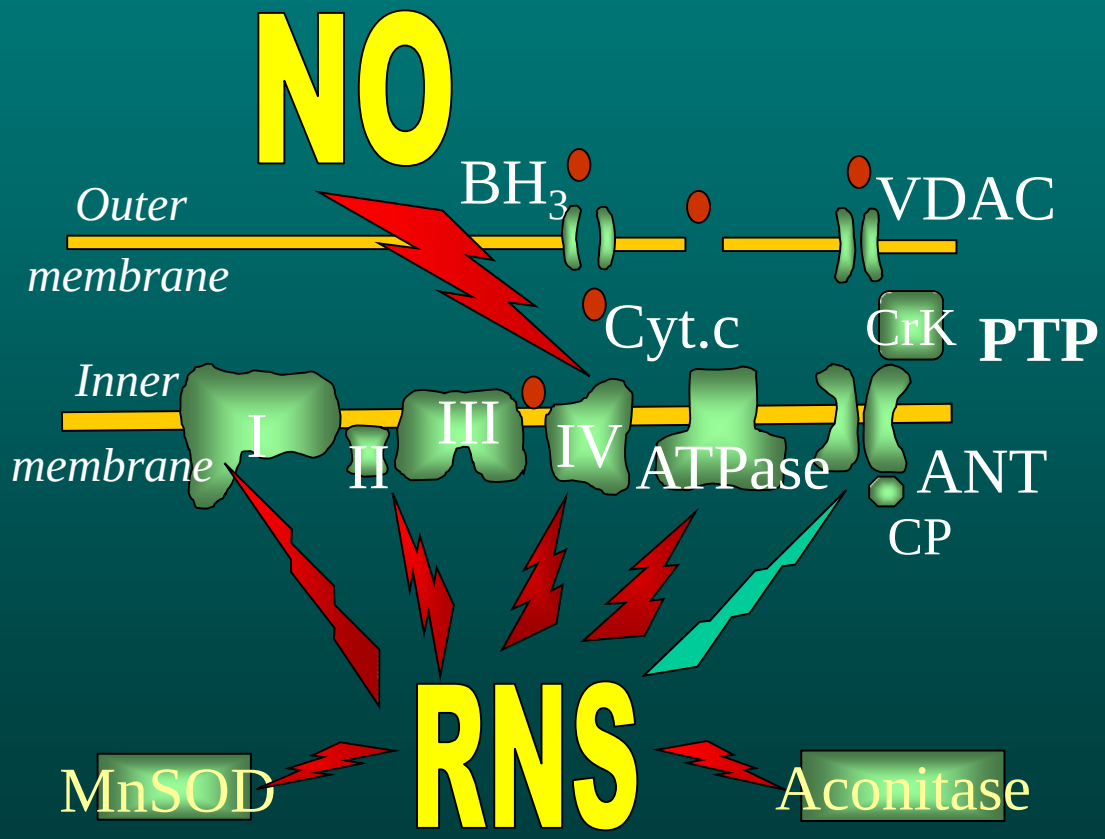


Nitrosothiols-induced activation of caspases is blocked by cyclosporin A



Borutaite, V. & Brown, G. C. (2003) Nitric oxide induces apoptosis via hydrogen peroxide, but necrosis via energy and thiol depletion. Free Rad. Biol. Med. 35, 1457-68.

NO actions on mitochondria relevant to cell death



- Respiratory inhibition at complexes I & IV.
- Stimulation of oxidant production.
- Induction of permeability transition.
- NO can induce cell death by each of these means.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Palwinder Mander