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The intricate interplay among the gasotransmitters NO, CO, H_2s and mitochondrial complex IV

Abstract

The gasotransmitters NO, CO and H_2S, have been considered foryears toxic pollutants in the air, to be carefully avoided. Over the last 25years, however, based on solid experimental evidence obtained in a variety of biomedical contexts, they have rather gained the position of leader molecules in cell signaling. GAST are actively synthesized in our body by specific enzymatic systems, and all of them react with similar targets and metalloproteins. NO, particularly, is characterized by a complex, apparently unique mitochondrial reactivity likely suggesting a peculiar physiological interaction with Complex IV.

Keywords: cell respiration, gasotransmitters, nitric oxide, carbon monoxide, hydrogen sulfide, enzyme inhibition, mitochondria, bioenergetics

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Abbreviations: ATP, adenosine triphosphate; CcOX, cytochrome c oxidase; cGMP, cyclic guanosine-monophosphate; $\Delta\mu H^+$, mitochondrial H^+ electrochemical potential gradient; EDRF, endothelium derived relaxing factor; GAST, gasotransmitters; GSH, reduced glutathione; HaCaT, human keratinocyte (Cell Line); HUVEC, human umbilical vein endothelial cells; Km,O_2, michaelis menten constant for O_2(The Michaelis constant is an inverse measure of the substrate's affinity for the enzyme, i.e. O_2 for CcOX, a small Km indicates a higher affinity.); M1, reaction mechanism 1 of NO reaction with cytochrome c oxidase fully reduced; M2, reaction mechanism 2 of NO reaction with cytochrome c oxidase in turnover; mRNA, mitochondrial ribonucleic acid; OXPHOS, oxidative phosphorylation; PKG, protein kinase G; sGC, soluble guanylate cyclase

Introduction

About 25years work enabled the biomedical science to show that nitric oxide (NO) is only occasionally a poison. All evidence rather suggests that NO is a molecule crucial for life, sharing this feature with CO and H_2S. These gaseous molecules target cell mitochondria. Mitochondria are the sites where the electron and proton transport takes place with free energy changes allowing ATP synthesis.[1,2] The redox chemistry and translocation of H^+ are both carried out by the respiratory chain complexes. The terminal electron acceptor of the chain, i.e. the complex IV, cytochrome c oxidase (CcOX) performs the O_2 reduction to water, meanwhile pumping $1H^+/e^-$ in the mitochondrial inter-membrane space. In doing so, it contributes to built up and maintenance of the mitochondrial H^+ electrochemical potential gradient, $\Delta\mu H^+$. Most if not all the classical mitochondrial effectors/inhibitors bind to CcOX.[3] Mitochondrial O_2 consumption normally occurs in the presence of physiological (small) amounts of highly reactive species, including nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S). Once simply considered as toxic air pollutants, these gases are rather endogenously produced by the cells, being therefore recognized as bio-essential.[4,5] Altogether, they are named *gasotransmitters* (GAST) which are able to trigger and maintain cell-signaling cascades involved in a variety of physiological and pathological events.[6–8] GAST contribute, among others to regulation of blood pressure, energy metabolism, neoangiogenesis and vascular inflammation, taking part to neurotransmission, cell death and the host immune response.[9] All GAST have in common the reactivity towards mitochondrial CcOX, although any gasotransmitter shows its own functional features; these depend on the molecular system(s) targeted and on the reaction mechanism(s) involved.[10]

Table 1 summarizes some relevant biochemical information on GAST. At body temperature, nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H_2S), similarly to O_2 are freely permeable to phospholipid membranes. From their specific cellular production sites, GAST promptly diffuse in mitochondria and tissues. At mitochondrial level they all react, particularly though not exclusively, with the respiratory chain Complex IV whose oxygen consumption becomes depressed to some variable extent.[4] Inhibition of respiration and bioenergetic consequences can be physiological or pathological depending on GAST, on its concentration as well as on the functional state of the target.[4] Although GAST may act in a concerted manner, the knowledge of their reciprocal cross control mechanisms is still poor. Data have been collected strongly suggesting a synergistic action between the NO and H_2S. It was possible to conclude that the hydrogen sulfide, similarly to nitric oxide causes vasorelaxation[11] and more recently the H_2S was shown to optimize the NO→cGMP→sGC→PKG pathway, from which the eNOS function depends.[12]

The peculiar mitochondrial reactivity of NO

NO has been the first GAST to be intensively studied after the amazing discovery that it was the endothelium-derived relaxing factor (EDRF).[13,14] Meanwhile, in the early 90's the involvement of NO in mitochondrial bioenergetics was shown,[15,16] mostly due to its high reactivity towards CcOX, comparable to that of O_2.[17,18] The reaction with NO leads to inhibition of mitochondrial respiration,[19] with depression of the oxidative phosphorylation (OXPHOS) and ATP production. The degree of inhibition and its bioenergetic consequences may be different depending on the cells/tissues exposed to NO. In the brain, for instance, neurons compared to astrocytes, have been proposed to be characterized by a limited glycolytic metabolism.[20] Although controversial,[21] according to the original finding only astrocytes should be able to compensate for OXPHOS ATP loss with glycolytic ATP.[20] Experiments carried out using purified CcOX or mitochondria, as well as intact cells proved that the most severe inhibition of respiration by NO is observed at low O_2, as

during hypoxia, and at high concentrations of reduced cytochrome c.[18,22–25] The comparison between the experimental data allowed one to conclude that the extent of OXPHOS inhibition is bound to:

i. The actual concentration of NO whether low (nM) or high (μM), thus its time persistence in the cell environment,

ii. The O_2 concentration in the system

iii. The level of electron flux within the respiratory chain, and particularly at the Complex IV site.[22]

One may predict that under rather extreme metabolic conditions, the cell oxidative phosphorylation becomes severely impaired, and NO may become lethal. This likely occurs when a substantial amount of exogenous NO accidentally pollutes the environment. Alternatively, endogenous NO, could be produced in hypoxic tissues by the NOSs, particularly the iNOS.2[6,27] Under these conditions NO likely persists in the cellular environment at concentrations >>μM, i.e. to be either efficiently oxidised to nitrite by CcoX in the mitochondrion, and thereafter disposed/recycled, or to be scavenged, e.g. by reduced glutathione (GSH). Such extreme conditions may occur in vivo only during pathological states, such as inflammatory diseases or sepsis,[26,27] when also the concentration of other reactive oxygen species (ROS), particularly the superoxide ion, O_2^-, rises. It is worth to point out that in the presence of both NO and O_2^- the highly toxic peroxynitrite species, $ONOO^-$, is formed very rapidly at diffusion limited rate.[26] Evidence is growing that over and above its own (high) concentration level, the dark side of NO resides on the production of peroxynitrite. In this view it is the $ONOO^-$ that damages membranes, proteins, enzymes and nucleic acids.[28] It is worth recalling that CcOX can be inhibited by NO following two different reaction mechanisms,[18] respectively leading to formation of

i. A stable, CcOX NO bound derivative, mechanism 1(M1):

$[a_3^{2+}Cu_B^{+}] + NO \rightarrow [a_3^{2+}Cu_B^{+}NO]$ (NO bound, derivative)

ii. A labile, NO_2^- bound, CcOX-derivative, mechanism 2 (M2):

$[a_3^{3+}Cu_B^{2+}] + NO \rightarrow [a_3^{3+}Cu_B^{+}NO^{+}] \rightarrow [a_3^{3+}Cu_B^{2+}\ NO_2^{-}]$ (NO_2^- bound, derivative)

When the metabolic cell conditions favour M1, the respiratory chain is severely inhibited,[17–23] and the cells may rapidly undergo apoptosis and irreversible damage, unless alternative ATP-producing pathways, such as glycolysis, become activated. In addition, when M1 prevails, the nitrosylated CcOX does not degrade NO to nitrite, rather releasing the gas back to the environment at the thermal dissociation rate k'=0.01^{s-1} at 37°C.[23]

Table 1 Cell site(s) where gasotransmitters are produced, by specific enzymatic systems & alternative endogenous mechanisms; Physiological and pathological bioavailability of gasotransmitters (average values)

	NO	**H_2S**	**CO**
Cell location/ compartment	Tissue specific Cell cytosol, mitochondria[34]	Cell cytosol, mitochondria	Multiple subcellular compartments, ER, PM, N[35]
	eNOS	CBS	HO-1
Cell enzymatic systems	nNOS	CSE	HO-2
	i NOS	3MST	HO-3
Alternative endogenous mechanisms	NO_2^-, NO-buffers (GSNO, PtNO)	Reduction of: Thiosulfate, Polysulfurated intermediates[36,37]	not described
Physiological cell concentration	$10^{-3} \div 10^{-1}$ μM	$10^{-3} \div 10^{-1}$ μM	~ 2 nM
Pathological cell concentration	> μM	> μM	> μM
Mobility in tissues (D) Cm^2s^{-1}	4.8×10^{-5}[38,39]	3.0×10^{-6}[40]	33.0×10^{-6}[41]

CBS, cystathione-β-synthase; CO, carbon monoxide; CSE, cystathione-γ-lyase; 3MST, 3-mercaptopyruvate-sulfurtransferase; D, diffusion rate (cm^2s^{-1}); ER, endothelium reticulum; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; iNOS, inducible nitric oxide synthase; N, cell nucleus; NO, nitric oxide; NO_2^-, nitrite; O_2, molecular oxygen; PM, plasma membrane; GSNO, nitro sated glutathione; PtNO, nitrosated/nitrosylated endogenous proteins

Table 2 Degradation of NO by cytochrome c oxidase, in turnover at room temperature (20°C)

	NO	**H_2S**	**CO**
Overall reaction^	$NO+CuB^{2+}+OH^- \rightarrow \boldsymbol{NO_2^-}+e^-+H^++CuB^+$	$H_2S+½O_2 \rightarrow \boldsymbol{S_0}+H_2O$	$CO+½O_2 \rightarrow \boldsymbol{CO_2}$
Reaction rate	~ 6×10^{-2} (s^{-1})[42]	~ 1.0 (min^{-1})[43,44]	~ 0.02 (s^{-1})[3]

^All reactions occur at the CcOX, cytochrome a_3-Cu_B binuclear site where the chemistry, with O_2 and other gaseous ligands, occurs. Reactions are multistep, and have been simplified to outline the final degraded, non toxic, reaction end product (italic, bold)

S_0, metallic sulphur

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At this point it might be worth to consider that the accumulation of the inhibited NO-bound CcOX is favoured by low O_2 and high reducing substrates. It should be also considered, however, that when in cells and tissues the O_2 tension decreases to μM or less, also the NOSs dependent production of NO may become compromised, owing to the lack of O_2, one of the NOSs substrates. Upon lowering O_2, the inactivation of the NOSs occurs, indeed, consistently with their Km,O_2. Thus, sliding towards anoxia, only the endothelial NOS (eNOS), whose Km,O_2 is about 5-6Mm[26,29] may remain active, ensuring some blood vessel dilation. When tissues run out of O_2, however, alternative NO releasing sources may become active; nitroso glutathione (GS-NO) and other NO-donors may act as NO buffers. Moreover, under anoxic conditions, the presence of metal ions (Fe^{2+} and Cu^{+}), protein bound or free in solution, promotes the environmental acidification, and at low pH the reduction of NO_2^- to NO is favoured,[30] with positive effects on blood flow and oxygenation.[31] According to this schematic view, a rapid recovery of tissues oxygenation appears to be a priority that prevails on the CcOX recovery (NO would maintain the enzyme inhibited): as a matter of fact, at this point glycolysis should already have taken place!

When M2 prevails, CcOX oxidizes NO to nitrite, thus contributing to NO degradation and detoxification.[22,23] It is worth to point out that under normal physiological conditions, e.g. when NO is endogenously produced at minute concentrations (nM or less) by the constitutive NO synthesis (eNOS and nNOS), M2 likely prevails and the chemistry, responsible for the physiological NO cell signaling predominates over the highly detrimental severe inhibition of mitochondrial oxidative phosphorylation, apparently bound to M1. The physiological relevance of M2 has been clearly shown by the experiments on keratinocytes-derived cells in culture (HaCaT) that proved useful to shed light on the putative physiological bioenergetic role of NO in living cells.[32] The experimental design was based on the use of N-acetyl-5-methoxy tryptamine, melatonin, at hormonal concentrations (≤nM) far below the pharmacological ones (μM÷mM). In those experiments the cells, incubated with nanomolar or sub-nanomolar melatonin and under conditions compatible with a circadian rhythm, showed a significant increase of the expression level of both the neuronal NO synthase-mRNA, and the corresponding encoded protein.[32,33] Almost synchronously, the concentration of the NO oxidation products, NOx, i.e. nitrite (NO_2^-) and nitrate (NO_3^-) was found increased in the cell culture medium.

Meanwhile a significant (~30%) depression of both the mitochondrial membrane potential and the oxidative phosphorylation was observed. Interestingly, the depression of the OXPHOS ATP production was compensated by an increased efficiency of glycolysis, directly measured.[33] The melatonin concentration and the time dependence of the onset of the bioenergetic changes both suggest that the NO-dependent nitrite-releasing M2 involving CcOX might play a physiological role in the circadian melatonin chemistry. It is tempting to speculate that the M2 reaction pathway originally observed using CcOX in solution,[23] and confirmed using purified mitochondria and cells in culture[24,25] might contribute to explain the cell bioenergetic changes induced by different pathophysiological stimuli, under those conditions in which a slowdown of the respiratory chain is observed: these conditions should have in common a transient increase of NO leading to a temporary down regulation of the respiratory chain activity, possibly with glycolytic compensation.

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Conflict of interest

Author declares that there is no conflict of interest.

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