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The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance

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Abstract The four gases, nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H_2S) and hydrogen cyanide (HCN) all readily inhibit oxygen consumption by mitochondrial cytochrome oxidase. This inhibition is responsible for much of their toxicity when they are applied externally to the body. However, recently these gases have all been implicated, to greater or lesser extents, in normal cellular signalling events. In this review we analyse the chemistry of this inhibition, comparing and contrasting mechanism and discussing physiological consequences. The inhibition by NO and CO is dependent on oxygen concentration, but that of HCN and H_2S is not. NO and H_2S are readily metabolised by oxidative processes within cytochrome oxidase. In these cases the enzyme may act as a physiological detoxifier of these gases. CO oxidation is much slower and unlikely to be as physiologically important. The evidence for normal physiological levels of these gases interacting with cytochrome oxidase is equivocal, in part because there is little robust data about their steady state concentrations. A reasonable case can be made for NO, and perhaps CO and H_2S, inhibiting cytochrome oxidase in vivo, but endogenous levels of HCN seem unlikely to be high enough.

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Introduction

Cytochrome *c* oxidase is the terminal electron acceptor of the mitochondrial electron transport chain, catalysing the oxidation of ferrocytochrome *c* by gaseous molecular oxygen. It is therefore not surprising that other gases can also interact with this enzyme. This review will focus on four gases of particular current import: nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H_2S) and hydrogen cyanide (HCN). All of these gases interact with mammalian systems, entering the body via direct enzymatic production, as intermediates in metabolic pathways, as foodstuffs or as a by-product of industrial pollution. The toxicity of cyanide, sulfide and carbon monoxide has led to their unfortunate association with suicide attempts and capital punishment. Yet these gases have a positive side. NO is a key intercellular messenger and a similar role has been suggested for CO and H_2S. NO donors and NO gas are used in current therapies (Griffiths and Evans 2005; Kinsella 2006) and CO (Foresti et al. 2008) and H_2S (Szabo 2007) are in current clinical trials. To our knowledge no one has yet suggested a clinical role for HCN, but maybe this too is only a matter of time!

In this review we will summarise the interactions of these inhibitors with cytochrome oxidase, focusing primarily on mammalian systems. We will first discuss the molecular mechanisms involved, and then describe how this chemistry might play a role in cellular physiology and pathophysiology. There is a large literature in this area, which we will not have time to do justice to in a short

review. However, there are a range of books that provide good historical coverage (Coburn 1970; Medical and Biological Effects of Environmental Pollutants: Subcommittee on Hydrogen Sulfide 1979; Vennesland et al. 1981; Lancaster 1996) and a number of recent reviews (Kashiba et al. 2002; Wang 2002; Brown 2007; Cipollone and Visca 2007; Cooper and Giulivi 2007; Kaczorowski and Zuckerbraun 2007; Li and Moore 2007).

We will refer to the molecules exclusively in their neutral forms (HCN, CO, H_2S, NO) as these are likely to be the inhibitory species (Table 1). Due to the hydrophobic nature of the heme a_3/Cu_B oxygen reduction site (Rich et al. 1996), binuclear centre inhibitors prefer to bind as neutral species or as anions with an accompanying proton uptake. Therefore species with acidic or neutral pK_a (formate, sulfide, azide) bind more strongly as the pH is lowered within the physiological range (Stannard and Horecker 1948; Nicholls 1976; Nicholls and Kim 1982). However, pH changes in the physiological range have no effect on cyanide (Stannard and Horecker 1948) as with a high pK_a it is always present in the protonated state (HCN).

As noted above, there is an extensive literature reporting on the mechanism of inhibition by gaseous inhibitors. Before we look at the individual gases in detail there are some common pitfalls to the unwary in approaching this field. Four general points stand out. First the nature of the enzyme species that interacts with the inhibitor is frequently debated; the difficulty in preparing a homogenous oxidised enzyme preparation in a form that binds inhibitors readily is part of the problem here and readers are therefore pointed to the very useful review by Moody in this area (Moody 1996). Secondly the on rates and off rates of some these inhibitors can be quite slow making analysis by classical enzyme kinetics methods problematic. Thirdly as noted already the pH of the measurement can make a significant difference. Finally these gases can act as substrates as well as inhibitors for the enzyme, meaning that it cannot be assumed that the concentration added is the same as the concentration present at the time of inhibition. This is a particular problem for NO and H_2S and, if not corrected, the inhibitory constants can be wildly overestimated.

Figure 1 illustrates the site of action of these inhibitors and Table 1 summarises the inhibitory constants with respect to the oxygen kinetics. CO is a competitive inhibitor (only increasing K_m), whereas H_2S and HCN are non-competitive (only decreasing V_{max}). NO is more complex with two interactions, one being competitive and the other uncompetitive (decreasing both V_{max} and K_m, with no effect on V_{max}/K_m). Petersen (1977) produced a model of how these inhibition kinetics could be explained mechanistically. However, although ingenious there are some problems marrying these models to the known molecular sites of action, particularly for the non-competitive interaction of sulfide and cyanide. It is possible that these only appear non competitive as their very slow off rates mean that during the course of the assay a constant fraction of the enzyme population is effectively completely inactive. This would lead to a decreased V_{max}, with minimal effects on the oxygen K_m; this effect of irreversible inhibition mimicking non-competitive kinetics is also seen for other slow binding inhibitors of cytochrome oxidase such as psychosine (Cooper et al. 1993).

Carbon monoxide

Molecular mechanisms

CO inhibition is the simplest and best-characterised molecular mechanism (Chance et al. 1970). Cytochrome oxidase has four redox active metal centres. Cu_A is the initial electron acceptor from ferrocytochrome *c*. The electron is then

Table 1 Inhibition of cytochrome oxidase by gas inhibitors

Oxygen inhibition kinetics								
Inhibitor	pK	V_{max}	K_m	V_{max}/K_m	Mechanism	K_i	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})
HCN	9.3	⇓	–	⇓	NC	0.2 μM	5×10^{3}	5×10^{-4}
H_2S	7.0	⇓	–	⇓	NC	0.2 μM	1.5×10^{4}	6×10^{-4}
CO	N/A	–	⇑	⇓	C	0.3 μM	1.2×10^{5}	2×10^{-2}
NO (reduced enzyme)	N/A	–	⇑	⇓	C	0.2 nM	1×10^{8}	0.02
NO (oxidised enzyme)	N/A	⇓	⇓	–	UC	28 nM	1×10^{5}	N/A

Notes: The on and off rates are generally calculated from onsets and offsets of inhibition. Therefore in most cases k_{off}/k_{on} corresponds well to the inhibition constant (K_i), the small differences being likely due to the slightly different conditions used in different papers. In the case of HCN the enzyme species with the strongest binding to the inhibitor is a partially reduced enzyme intermediate (see text). The NO off rates from the oxidised enzyme are not indicated as NO is metabolised to nitrite, the resulting K_i being a complex kinetic constant arising from the NO on rate and the nitrite dissociation rate. Selections from a number of references were used to compile this table (Nicholls and Chance 1974; Nicholls 1975; Petersen 1977; Cooper et al. 2008).

C competitive, *NC* non-competitive, *UC* noncompetive, ⇓ decrease, ⇑ increase, – no effect, *N/A*, not applicable

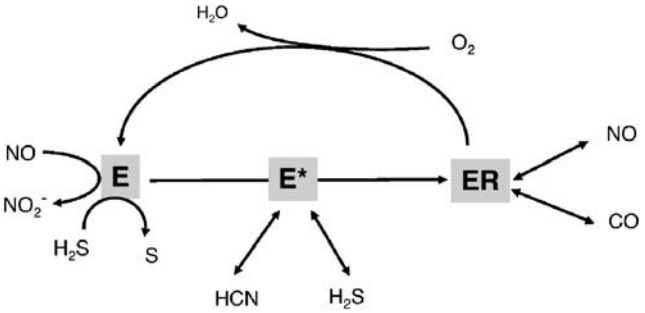

Fig. 1 Site of action of cytochrome oxidase gas inhibitors, *E* Oxidised enzyme, *E** turnover intermediate, *ER* reduced enzyme. Reversible inhibition is indicated by *double-sided arrows* and metabolism by *single-sided arrows*

passed to heme *a*, from where it travels to the binuclear heme a_3/Cu_B centre where the oxygen reduction reactions occur (Babcock and Wikström 1992). Oxygen does not bind to the enzyme until the binuclear centre is fully reduced (ferrous/cupric). The same situation applies for CO. CO therefore directly competes with oxygen binding and its inhibition is strictly competitive with respect to oxygen (Fig. 1, Table 1). Even in this case the inhibitor constant will be indirectly affected by the other substrate (ferrocytochrome *c*). The nature of electron transfer chains means that there will be a "cushioning" of inhibition by other parts of the chain (Chance 1965). For example the cytochrome *c* redox state will respond to the inhibition of the oxidase by becoming more reduced, hence increasing its rate of electron transfer to cytochrome oxidase. CO inhibition will therefore be less potent at lower mitochondrial electron transfer rates (Chance et al. 1970). Although CO is a substrate (Table 2) for cytochrome oxidase (Young and Caughey 1986a) the rate is so slow (k_{cat}=0.02 s^{-1}) that it does not interfere with kinetic measurements; indeed it is unlikely to have any physiological significance.

Cellular physiology

CO is produced by heme oxygenase in mammalian tissues, and is thought to be a physiological signal regulating synaptic transmission and vascular function (Koehler and Traystman 2002; Durante et al. 2006). Pharmacological levels of CO have been found to be protective when added to cells and organisms, inhibiting: apoptosis, inflammation, platelet aggregation, and proliferation of vascular smooth muscle. Higher levels of CO are toxic, and CO is a major cause of accidental and nonaccidental poisoning. The mechanism of CO-induced death is thought to be high affinity binding of CO to hemoglobin and myoglobin, resulting in tissue hypoxia that inhibits cytochrome oxidase synergistically with CO. The mechanisms by which CO exerts its physiological and pharmacological effects are obscure. Some effects are mediated by activation of soluble guanylate cyclase, but very high levels of CO are normally required to activate the cyclase. Recently evidence was presented that CO exerts its anti-inflammatory effect on cells by inhibiting cytochrome oxidase, resulting in ROS production by mitochondria (Zuckerbraun et al. 2007).

In cells 20 μM CO inhibited cellular respiration by 40% at 20 μM O_2, the number varying with O_2 concentration as expected for a competitive inhibitor (D'Amico et al. 2006). Is this degree of inhibition consistent with the data on the purified enzyme (Table 1)? CO inhibits cytochrome oxidase in competition with oxygen with a K_i of 0.3 μM. Assuming a cytochrome oxidase oxygen K_m of 1 μM, a CO concentration of 20 μM equates to a modified oxygen K_m of 68 μM. At 20 μM O_2 cell respiration should therefore be inhibited by approximately 80%. The slightly weaker inhibition observed in whole cell respiration likely arises from cytochrome oxidase activity being only partially rate limiting for cellular respiration prior to the addition of CO, resulting in a lowered oxygen K_m, which has the effect of making a competitive inhibitor less effective.

Heme oxyenase-1 catalyses cellular CO production. Transfection of cells with heme oxygenase-1 resulted in

Table 2 Metabolism of gas inhibitors by cytochrome oxidase

Nitric oxide, overall reaction: $NO+OH^- \Rightarrow NO_2^- + e^- + H^+$
(a) $NO+Cu_B^{2+\ -} \Rightarrow NOCu_B^{2+}$
(b) $NOCu_B^{2+}+OH^- \Rightarrow NO_2^- + Cu_B^+ + H^+$
Carbon monoxide, overall reaction: $CO+1/2O_2 \Rightarrow CO_2$
(a) $Cu_B^{2+}Fe^{3+}+CO+H_2O \Rightarrow Cu_B^+Fe^{2+}+CO_2+2H^+$
(b) $Cu_B^+Fe^{2+}+1/2O_2+2H^+ \Rightarrow Cu_B^{2+}Fe^{3+}+H_2O$
Hydrogen sulfide, overall reaction: $H_2S+1/2O_2 \Rightarrow S^0+H_2O$
(a) $Cu_B^{2+}Fe^{3+}+H_2S \Rightarrow Cu_B^{2+}–SH^-\ Fe^{3+}+H^+$
(b) $Cu_B^{2+}Fe^{3+}–SH^- \Rightarrow Cu_B^+SH^{\cdot}\ Fe^{3+}$
(c) $Cu_B^+SH^{\cdot}\ Fe^{3+\ -} \Rightarrow Cu_B^+\ Fe^{2+}+S^0+H^+$
(d) $Cu_B^+\ Fe^{2+}+1/2O_2+2H^+ \Rightarrow Cu_B^{2+}Fe^{3+}+H_2O$

Only the reactions at the heme a_3/Cu_B binuclear centre are indicated. Of the three gases NO is best characterised (Cooper et al. 1997; Giuffre et al. 2000; Torres et al. 2000; Cooper 2002). The CO reaction we have chosen to illustrate is an anaerobic water–gas reaction followed by oxygen-catalysed oxidation of the reduced heme a_3/Cu_B binuclear centre (Nicholls 1979; Timkovich and Thrasher 1988); we disfavour the more complex scheme (Young and Caughey 1986a) requiring the simultaneous binding of three gas molecules (two CO and one O_2) to the enzyme. The H_2S reaction is the least well characterised and the illustration above is at least not inconsistent with the experimental data (Wever et al. 1975; Nicholls and Kim 1981; Nicholls and Kim 1982; Hill et al. 1984). Note *in vivo* there will be added complexity as all these metabolic reactions will be occurring in tandem with other inhibited species not discussed here e.g. ferrous heme a_3-NO, ferrous heme a_3-CO and ferric heme a_3-SH^-. The chemistry above assumes a rapid transfer of electrons from cytochrome *c* to the binuclear centre. In the absence of this oxygenated intermediates build up, following CO (Nicholls 1979) or H_2S addition (Nicholls and Kim 1982), particularly the 607 nm ferryl intermediate.

an inhibition of cellular respiration at cytochrome oxidase by CO, but this was only marked (75% inhibition) at low (1%) oxygen (D'Amico et al. 2006). Induction of endogenous heme oxygenase-1 with inflammatory mediators has also been found to cause inhibition of cellular respiration, attributed to CO inhibition of cytochrome oxidase, but the inhibition was small (D'Amico et al. 2006). In general, inhibition of cytochrome oxidase by endogenously produced CO is thought to be insignificant, partly because CO binds so tightly to hemoglobin and myoglobin that free levels of CO are likely to be very low.

Nitric oxide

Molecular mechanisms

NO inhibition is more complex than CO (Mason et al. 2006). There is a major component that behaves like CO, in that it is competitive with oxygen (Brown and Cooper 1994); this presumably also requires the presence of two electrons in the binuclear centre, though there is some evidence that the singly reduced centre can also bind NO (Giuffre et al. 2002). However, there is an additional interaction at the oxidised enzyme (Cooper et al. 1997); this is presumed to be via binding to Cu_B^{2+} as similar rates ($\sim 10^5$ M^{-1} s^{-1}) are seen in the fully oxidised enzyme and forms where heme a_3 is superoxidised to ferryl, Fe(IV)=O (Giuffre et al. 2000). The enzyme becomes reduced and the NO oxidised to nitrite (Torres et al. 2000), Table 2. Kinetically this results in uncompetitive inhibition with respect to the oxygen kinetics. Another means of distinguishing the uncompetitive from the competitive mode of inhibition is that the latter is fully reversible by light, because light dissociates NO (and CO) from ferrous heme a_3. Although some authors favoured only the competitive reaction (Antunes et al. 2004) and some the uncompetitive (Pearce et al. 2003), recent kinetic models have successfully incorporated both modes of inhibition (Cooper et al. 2008). NO therefore can be seen as a strong inhibitor AND a good substrate for cytochrome oxidase (Cooper 2002). Depending on the balance of these effects the activity of cytochrome oxidase towards NO can therefore result in the inhibition of cell respiration or the removal of NO from the cell.

Cellular physiology

Nitric oxide (NO) is produced from L-arginine in mammalian cells by three distinct NO synthases (nNOS, iNOS and eNOS), and regulates multiple physiological functions including vascular tone, platelet aggregation, smooth muscle relaxation, and synaptic function, as well as cytotoxicity of the innate immune system (Alderton et al. 2001). The main physiological target of NO is the heme group of soluble guanylate cyclase, which it reversibly activates at about 1–2 nM NO (Bellamy et al. 2002).

The affinity of reduced cytochrome oxidase for NO in the absence of oxygen is about 0.2 nM (Table 1). Half inhibition of cellular respiration occurs at 60 nM NO at 30 μM O_2, and at 250 nM at 150 μM O_2 (Brown and Cooper 1994). Thus at physiological oxygen levels, soluble guanylate cyclase is about 50-fold more sensitive to NO than cytochrome oxidase. Although changes in cellular conditions can decrease this gap in sensitivity (Cooper and Giulivi 2007), it is still difficult to envisage a situation, other than heterogeneity, where significant inhibition of cytochrome oxidase is not coincident with almost full activation of guanylate cyclase. In vivo levels of NO are unclear, but likely to be somewhere in the range 0.1–100 nM (Bellamy et al. 2002). However, cells in culture expressing eNOS, nNOS or particularly iNOS clearly show NO- and oxygen-dependent inhibition of cytochrome oxidase and cellular respiration (Clementi et al. 1999; Brown 2007; Brown and Borutaite 2007; Cooper and Giulivi 2007; Leavesley et al. 2008). Therefore, it is still an open question whether NO significantly inhibits cellular respiration at cytochrome oxidase *in vivo* (Brown 2007; Brown and Borutaite 2007; Cooper and Giulivi 2007).

NO or its derivatives can inhibit other mitochondrial respiratory chain components: NO reversibly inhibits complex III at about 1 μM; *S*-nitrosating reagents such as *S*-nitrosthiols or N_2O_3 can inactivate complex I, and peroxynitrite can irreversibly inhibit most mitochondrial proteins by oxidation (Brown 2007; Cooper and Giulivi 2007).

Hydrogen cyanide

Molecular mechanisms

HCN binds tightly to the binuclear centre of oxidised cytochrome oxidase (Antonini et al. 1971). There is a weak interaction with the reduced enzyme, but the low affinity (200 μM) renders this unlikely to be relevant to enzyme inhibition (Antonini et al. 1971; Nicholls and Chance 1974). Although in the final inhibited state HCN is bound to oxidised heme a_3, in the isolated enzyme the rate of formation of this species cannot account for the observed rate of onset of inhibition (Mitchell et al. 1992; Berka et al. 1993). It is clear that there is a form of the enzyme populated in turnover that rapidly binds HCN. The current view is that the HCN-sensitive turnover intermediate requires only one electron in the enzyme, though the initial binding itself is to the oxidised form of Cu_B (Mitchell et al.

1992; Wilson et al. 1994). There is no evidence that HCN can be metabolised by cytochrome oxidase.

Cellular physiology

HCN can be generated by brain cells (Gunasekar et al. 2000; Gunasekar et al. 2004) and neutrophils (Stelmaszynska 1986), and has been suggested to act as a neuromodulator, but the evidence is very limited (Cipollone and Visca 2007). Opiates have been found to increase HCN production by neurons, PC12 cells and rat brain in vivo (Borowitz et al. 1997). Rates of HCN production were measured as about 10 nmol/min/g brain, and the cyanide levels in the brain were reported as 2–10 μM. HCN levels in human blood become toxic between 20 and 50 μM. Different methods have estimated endogenous HCN levels to be from 0.3 (Ishii et al. 1998) to 2 μM (Lundquist and Sorbo 1989). However, much of this may be bound to methemoglobin.

Cyanide will inhibit any ferric heme proteins with a free ligand position, including catalase. Isolated cytochrome oxidase (Table 1) is reversibly inhibited by HCN with a K_i of between 0.1 and 10 μM, dependent on the redox state of the enzyme, but independent of oxygen concentration (Nicholls and Chance 1974; Petersen 1977; Pearce et al. 2003; Leavesley et al. 2008). Cellular respiration is half inhibited by about 10–50 μM added cyanide (Villani et al. 1998; Leavesley et al. 2008). Again the increase from the in vitro value may be explained by the extent of control of respiration by cytochrome oxidase activity. Comparison of the estimated endogenous HCN levels with the levels required to inhibit cellular respiration suggest that such inhibition by endogenous HCN is unlikely. However, as we currently know so little about endogenous HCN production, this conclusion may be premature.

Hydrogen sulfide

Molecular mechanisms

The binding and inhibition kinetics of H_2S are, if anything, even more complex than those of NO (Wever et al. 1975; Hill et al. 1984). As is the case with NO, H_2S is both a substrate and an inhibitor for cytochrome oxidase (Nicholls and Kim 1981; Nicholls and Kim 1982), Table 2. Inhibition is non-competitive with respect to cytochrome *c* and oxygen (Petersen 1977). Sulfide binds to oxidised states of the binuclear centre; the final inhibited state has two bound sulfide molecules, one bound to reduced Cu_B and the other bound to oxidised heme a_3 (Hill et al. 1984). However, in order to get to this state as many as three H_2S molecules can potentially interact with the enzyme. As with NO, this interaction includes oxidation of gas (presumably to elemental sulfur) and reduction of the enzyme (Table 2). This reaction is coupled to the consumption of molecular oxygen and the formation of higher oxidation state (ferryl) enzyme intermediates (Petersen 1977). It is rapid enough to be of potential physiological importance.

Cellular physiology

Hydrogen sulfide (H_2S) has been proposed to be an endogenous signalling molecule in mammals, synthesized from L-cysteine by either cystathionine beta-synthase (CBS) or cystathionine gamma-lyase (CSE), and to regulate vascular tone, myocardial contractility, neurotransmission, insulin secretion and a variety of pathologies (Kashiba et al. 2002; Kimura 2002; Wang 2002; Wang 2003; Bhatia 2005; Lowicka and Beltowski 2007).

Biological effect of H_2S have been attributed to activation of ATP-sensitive potassium channels (K_{ATP}). In principle, this activation might be mediated by inhibition of cytochrome oxidase by H_2S, decreasing cellular ATP and thereby activating the K_{ATP} channels, which are inhibited by ATP. The measured sensitivity (EC_{50}) of both relaxation and K_{ATP} currents of mesenteric arteries was about 25–116 μM (Cheng et al. 2004; Tang et al. 2005) which is compatible with the H_2S-induced K_{ATP} channel activation being mediated by inhibition of cytochrome oxidase.

Isolated cytochrome oxidase is reversibly inhibited (Table 1) by H_2S with a K_i of 0.2 μM, which is independent of oxygen concentration (Petersen 1977). Respiration of isolated mitochondria is half inhibited at 10 μM H_2S (Yong and Searcy 2001), and that of intact cells at about 30 μM (Leschelle et al. 2005). However, this is complicated by the fact that H_2S is also an excellent substrate for the mitochondrial respiratory chain, so that at low concentrations it stimulates oxygen consumption and increases membrane potential, whereas at high concentrations (>20 μM) it inhibits oxygen consumption at cytochrome oxidase (Yong and Searcy 2001; Goubern et al. 2007).

Estimated endogenous sulfide levels are often erroneous because of extraction and binding problems (Ubuka 2002). Recent measurements of free H_2S by electrode found it essentially undetectable (<100 nM total sulfide) in blood plasma in a wide range of animals and conditions (Whitfield et al. 2008). However, (Doeller et al. 2005) another recent electrode method has shown that tissue homogenates, vascular cells and intact aorta produce 1–10 μM free H_2S in the presence of cysteine and low oxygen. Thus, the *in vivo* levels of H_2S are still unclear, but high nanomolar to low μM H_2S seems a reasonable guess at present, at least in non-pathological conditions. There is no evidence as yet that endogenous H_2S significantly inhibits respiration *in vivo*.

Inhaled H_2S has been shown to induce a "suspended animation-like state" in mice, that is mice were given

sufficient H_2S to inhibit whole body oxygen consumption (via inhibition of cytochrome oxidase) by around 90% for 6 hours, and when resuscitated where found to be undamaged and behaviourally normal (Blackstone et al. 2005; Volpato et al. 2008). The mystery here is that if a similar level of respiratory inhibition were induced by hypoxia, it would rapidly cause organism death and massive cell death in brain, heart and other tissues, whereas the H_2S-induced state actually protects against lethal hypoxia (Blackstone and Roth 2007). This suggests that H_2S has protective effects, independent of inhibition of cytochrome oxidase.

Reactions with cytochrome *c*

All four gaseous ligands can potentially interact with the enzyme substrate, cytochrome *c*. CO can only bind to reduced cytochrome *c* when the distal methionine ligand is displaced e.g. at alkaline pH. Although HCN can react with oxidised and reduced cytochrome *c*, the binding is of too low an affinity to suggest any physiological importance (Nicholls and Mochan 1967). H_2S can directly reduce cytochrome *c* (Nicholls and Kim 1982) and NO can interact with oxidised and reduced cytochrome *c* (Sharpe and Cooper 1998). These reactions, although faster than those of HCN and CO at physiological pH, are still slower and of much lower affinity than effects at the oxidase itself. However, there is one caveat to these observations. The interaction of cardiolipin dramatically enhances the ligand reactivity of cytochrome c, making gas interactions far more likely (Kagan et al. 2005; Vlasova et al. 2006).

Interactions between the gasses, and between gases and hypoxia

The inhibition of cytochrome oxidase by combinations of gas molecules has not been extensively studied. Ligands that bind to the same redox state of the enzyme will replace each other, so an excess of CO will replace NO and an excess of H_2S will replace HCN (Nicholls et al. 1972). In principal, partial inhibition of cytochrome oxidase in mitochondria or cells by one gas will make the oxidase more rate limiting for respiration, and therefore more sensitive to the other gases. But how this relates to the steady state inhibition constant has not been well characterised. In general one would just be replacing one inhibitory reaction by another, although there are conflicting studies that suggest that NO might be able to relieve cyanide inhibition (Pearce et al. 2003; Leavesley et al. 2008). As H_2S is rapidly consumed by acting as a substrate for the respiratory chain, inhibition of cytochrome oxidase by any other gas (or indeed H_2S or hypoxia) will inhibit H_2S consumption, and thus raise H_2S levels available to cause oxidase inhibition.

Hypoxia is also an inhibitor of cytochrome oxidase, and a major cause of human pathology. NO and CO inhibit cytochrome oxidase largely in competition with oxygen, and they therefore increases the apparent K_m for oxygen of cytochrome oxidase and cellular respiration. This is important because in the absence of inhibitors this K_m is about 0.5 μM O_2, which is substantially below the physiological range of oxygen concentrations found in the body; so the rate of oxygen consumption by cytochrome oxidase (which accounts for roughly 90% of all oxygen consumption in the body) is normally not limited and therefore independent of oxygen levels and oxygen supply. However, the presence of 60 nM NO, raises this K_m to 30 μM O_2 (Brown and Cooper 1994), which is the median level of oxygen found in the brain and higher than that found in the heart; at this level of NO the oxygen consumption rate of cellular respiration is proportional to oxygen level and oxygen supply. Note however the complications that: (a) the NO synthases and heme oxygenase (that makes CO) are oxygen dependent (because oxygen is a substrate), and therefore gas synthesis declines with oxygen level, (b) NO is a potent vasodilator thus increasing oxygen supply via activating soluble guanylate cyclase at NO concentrations 50-fold lower than required to inhibit cytochrome oxidase, and (c) inhibition of cytochrome oxidase will increase tissue oxygen levels, partially relieving inhibition by NO or CO.

Conclusions

Gases with the ability to inhibit cytochrome oxidase are becoming increasingly relevant to cellular physiology. Although much is known about the molecular mechanism question marks remain especially as to the details of H_2S inhibition. All these gases are likely to exert toxic effects via cytochrome oxidase when given in overdose. Inhibition in cell culture is readily observed. But the extent of any physiological effects in vivo are still a matter of active debate.

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