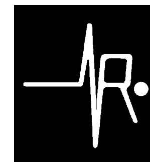
Available online at www.sciencedirect.com
SCIENCE DIRECT
Free Radical Biology & Medicine 40 (2006) 1332 – 1339
www.elsevier.com/locate/freeradbiomed



Original Contribution

Carbon monoxide, oxidative stress, and mitochondrial permeability pore transition

Claude A. Piantadosi *, Martha S. Carraway, Hagir B. Suliman

Departments of Medicine and Anesthesiology, Duke University Medical Center, P.O. Box 3315, Durham, NC 27710, USA

Received 10 July 2005; revised 1 November 2005; accepted 29 November 2005
Available online 21 December 2005

Abstract

The cellular effects of carbon monoxide (CO) are produced primarily by CO binding to iron or other transition metals, which may also promote prooxidant activities of the more reactive gases, oxygen and nitric oxide. We tested the hypothesis that prooxidant effects of CO deregulate the calcium-dependent mitochondrial pore transition (MPT), which disrupts membrane potential and releases apoptogenic proteins. Rats were exposed to either CO (50 ppm) or hypobaric hypoxia (HH) for 1, 3, or 7 days, and liver mitochondria harvested to study protein expression and sensitivity to MPT by calcium and oxidants. Both exposures induced hypoxia-sensitive protein expression: hypoxia-inducible factor 1α (HIF-1α), heme oxygenase-1 (HO-1), and manganese SOD (SOD2), but SOD2 induction was greater by CO than by HH, especially at 7 days. Relative to HH, CO also caused significant early mitochondrial oxidative and nitrosative stress shown by decreases in GSH/GSSG and increases in protein 3-nitrotyrosine (3-NT) and protein mixed disulfide formation. This altered MPT sensitivity to calcium through an effect on the "S-site," causing loss of pore protection by adenine nucleotides. By 7 days, despite continued CO, nitrosative stress decreased and adenine nucleotide protection was restored to preexposure levels. This is the first evidence of functional mitochondrial pore stress caused by CO independently of its hypoxic effect, as well as a compensatory response exemplifying a mitochondrial phenotype shift. The implications are that cellular CO can activate or deactivate mitochondria for initiation of apoptosis in vivo.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Mitochondria; Permeability pore; Hypoxia; Apoptosis; ROS; Nitric oxide; Free radical

Introduction

The biochemistry of carbon monoxide is important in both toxicity and physiology: Carbon monoxide (CO) is a potentially dangerous metabolic poison, especially to the brain and heart [1], but it is also produced endogenously. CO is generated during heme degradation by the heme oxygenase (HO) enzymes [2], and in particular, the inducible isoform, HO-1, is adaptive to oxidative stress [3,4] and inflammation [5]. Endogenous CO has been proposed to play a physiological role in cell signaling for more than a decade [6].

The effects of CO on mitochondrial oxidation-reduction (redox) state are well recognized because CO binds the reduced iron (Fe^{2+}) in the a_3 heme of the terminal electron acceptor of the respiratory chain, cytochrome *c* oxidase [7–9]. CO increases mitochondrial reactive oxygen species (ROS) production in vitro and in vivo [10,11] and mitochondria metabolize CO by oxygenation to CO_2 [9]. CO also binds to reduced transition metals in other metalloenzymes including guanylate cyclase and cytochrome P_{450} [7]. Therefore, CO-heme ligand formation tends to interfere with redox reactions involving molecular oxygen (O_2) as well as nitric oxide ($NO^•$).

Because of its effects on mitochondrial redox state, we considered that CO might alter the sensitivity of mitochondria to calcium-dependent large amplitude swelling [12], or mitochondrial pore transition (MPT), also called mitochondrial outer

Abbreviations: CO, carbon monoxide; HO, heme oxygenase; ROS, reactive oxygen species: $NO^•$, nitric oxide; MPT, mitochondrial pore transition; ANT, adenine nucleotide translocase; COHb, carboxyhemoglobin; HH, hypobaric hypoxia; HIF-1α, hypoxia-inducible factor lα; SSA, sulfosalicylic acid; RCR, respiratory control ratio; tBu, tertiary butyl hydroperoxide; SOD2, manganese superoxide dismutase; DTNB, 5,5′-dithiobis(nitrobenzoic acid); 3-NT, 3-nitrotyrosine; HPLC, high-performance liquid chromatography; ECD, electrochemical detection.

* Corresponding author. Fax: +1 919 684 6002.
E-mail address: piant001@mc.duke.edu (C.A. Piantadosi).

0891-5849/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.freeradbiomed.2005.11.020

membrane permeabilization (MOMP [13], for review). The MPT is modulated by so-called pore proteins, including cyclophilin D, the voltage-dependent anion channel (VDAC or porin), the adenine nucleotide translocases (ANT), and outer membrane anti- and proapoptotic proteins, such as those of the proto-oncogene Bax-Bcl_2 family, which interact with pore-forming proteins to facilitate (e.g., Bax) or stabilize (e.g., Bcl_{xl}) the pore [14,15].

The MPT is triggered primarily by the action of calcium on cyclophilin D, a cyclosporin A-inhibitable isomerase involved in mitochondrial initiation of apoptosis, e.g., through cytochrome *c* release, required for apoptosome assembly and caspase-3 activation [13]. The MPT release of apoptogenic proteins is modulated by oxidative and nitrosative modification of pore protein thiols [16–19]. Thus, we tested the hypothesis that CO at levels encountered endogenously regulates the sensitivity to MPT in vivo by pore thiol oxidation. We further proposed that such mitochondrial protein modifications would not be caused by cellular responses to hypoxia, e.g., from carboxyhemoglobin (COHb) formation, but by mitochondrial oxidative and/or nitrosative stress.

Oxidant regulation of the MPT involves at least two types of sites at pore-related proteins including the adenine nucleotide translocator [16–18]. One site contains critical protein thiols ("S-site") while the other responds to the redox state of the pyridine nucleotides ("P-site") [17]. The concept that thiols at the S-site regulate MPT is based on facilitation of pore opening by many oxidants and diazine carbonyls [16,18,19]. Although the ANT is not required for MPT [20], adenine nucleotide binding, especially of ADP, is highly protective [18] except after certain types of oxidative stress [20,21]. Further progress in understanding MPT regulation by oxidants has been hindered by lack of precise information on the molecular organization of the "pore" and the exact nature of the "S-site." Moreover, the effects of CO and CO-related chemical changes on MPT control are unknown, especially with respect to nitric oxide. Both NO and CO bind cytochrome *c* oxidase, and NO can modify susceptibility to MPT both by altering mitochondrial ROS production and by nitrosylation of pore-associated proteins. Finally, we chose to evaluate these effects with respect to ATP, which is needed for apoptosis [22].

Methods

Materials

All materials were obtained from Sigma Chemical Co. (St. Louis, MO) unless indicated otherwise.

Animal studies

Adult male Sprague-Dawley rats were exposed in room air (21% O_2) to CO (50 ppm) for 1, 3, or 7 days ($n = 8$ per group) on a protocol approved by the Institutional Animal Care and Use Committee. CO levels were monitored continuously with a gas detector (Toxilog, Biosystems Inc., Rockfall, CT) and regulated to within 2.5 ppm. A control group was exposed to hypobaric hypoxia (HH; ~15,000 feet, 430 Torr) in an altitude chamber for 1, 3, or 7 days ($n = 8$ per group). The chamber altitude was returned to sea level daily for 5 min to clean the cages and replenish the animal's food and water. HH exposures were monitored continuously using a calibrated altimeter and regulated to within +500 feet. A group of air-control rats ($n = 8$) was selected at random from the same batches that were exposed to CO or HH. After exposure, the animals were given pentobarbital (100 mg/kg ip), and under deep anesthesia, 0.5 ml heparinized arterial blood was drawn followed by exsanguination. The blood was used immediately to measure hemoglobin and carboxyhemoglobin on a CO oximeter (Instrumentation Laboratories, Model 480) calibrated for the rodent.

Tissue harvesting

The abdomen was opened and the circulation perfused with cold 60 ml 0.9% NaCl by puncturing the left ventricle with an 18 gauge needle and sectioning the inferior vena cava. Approximately 100 mg of liver was excised and placed in a 2-ml polypropylene centrifuge tube and distilled water added by weight to produce 10% w/w tissue suspensions. This tissue was diced and sonicated on ice for 1 min (Model 450; Branson Ultrasonics; Danbury, CT). The sonicates were centrifuged at 2000 *g* for 30 s on a microcentrifuge (Model DW-41N-115 Qualitron, Inc, Witz Scientific; Holland, OH) and introduced by injection into sealed gas chromatograph vials (2 ml borosilicate glass; Alltech Associates, Inc.; State College, PA) containing 2 μl of 60% sulfosalicylic acid (SSA) and 20 μl of dd-H_2O and purged with CO-free nitrogen. The vials were agitated on the needle to mix the sample and the SSA and returned to ice while CO was released. The rest of the liver was excised rapidly and a part snap-frozen in liquid nitrogen and stored at −80°C for protein and Western blot analyses. About 2 g fresh liver was placed in cold buffer (0.32 M sucrose, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.4), minced with scissors, and hand-homogenized, and fresh mitochondria isolated [23].

Tissue CO analysis

CO in the headspace gas of the sealed gas chromatograph vials was analyzed using a reduction gas analysis GC (RGA5 Process Gas Analyzer; Trace Analytical; Menlo Park, CA) [24,25]. CO-free carrier gas was obtained by filtering nitrogen through a stabilized zeolite Getter (PS11-MC1 Ambient Temperature Gas Purifier; SAES Getters USA Inc.; Colorado Springs, CO). The GC was calibrated with certified gas containing 4.89 ppm CO in nitrogen (Scott Specialty Gases; Plumsteadville; PA) by using 50 to 550 μl of calibration gas in 2-ml vials purged with CO-free nitrogen to produce a standard curve [26].

Mitochondrial isolations

Mitochondrial preparations were paired and one CO and one HH-exposed animal were studied together. Homogenates were centrifuged at 1330 *g* at 4°C, the pellets were resuspended in fresh isolation buffer and centrifuged for 3 min at 1330 *g*, and

Download English Version:

https://daneshyari.com/en/article/1911580

Download Persian Version:

https://daneshyari.com/article/1911580

Daneshyari.com