Physiol. Res. 51: 335-339, 2002

MINIREVIEW

The Role of Carbon Dioxide in Free Radical Reactions of the Organism

A. VESELÁ, J. WILHELM

Department of Medical Chemistry and Biochemistry, Second Faculty of Medicine, Charles University, Center for Experimental Cardiovascular Research, Prague, Czech Republic

Received August 6, 2001
Accepted November 22, 2001

Summary

Carbon dioxide interacts both with reactive nitrogen species and reactive oxygen species. In the presence of superoxide, NO reacts to form peroxynitrite that reacts with CO_2 to give nitrosoperoxycarbonate. This compound rearranges to nitrocarbonate which is prone to further reactions. In an aqueous environment, the most probable reaction is hydrolysis producing carbonate and nitrate. Thus the net effect of CO_2 is scavenging of peroxynitrite and prevention of nitration and oxidative damage. However, in a nonpolar environment of membranes, nitrocarbonate undergoes other reactions leading to nitration of proteins and oxidative damage. When NO reacts with oxygen in the absence of superoxide, a nitrating species N_2O_3 is formed. CO_2 interacts with N_2O_3 to produce a nitrosyl compound that, under physiological pH, is hydrolyzed to nitrous and carbonic acid. In this way, CO_2 also prevents nitration reactions. CO_2 protects superoxide dismutase against oxidative damage induced by hydrogen peroxide. However, in this reaction carbonate radicals are formed which can propagate the oxidative damage. It was found that hypercapnia *in vivo* protects against the damaging effects of ischemia or hypoxia. Several mechanisms have been suggested to explain the protective role of CO_2 *in vivo*. The most significant appears to be stabilization of the iron-transferrin complex which prevents the involvement of iron ions in the initiation of free radical reactions.

Key words

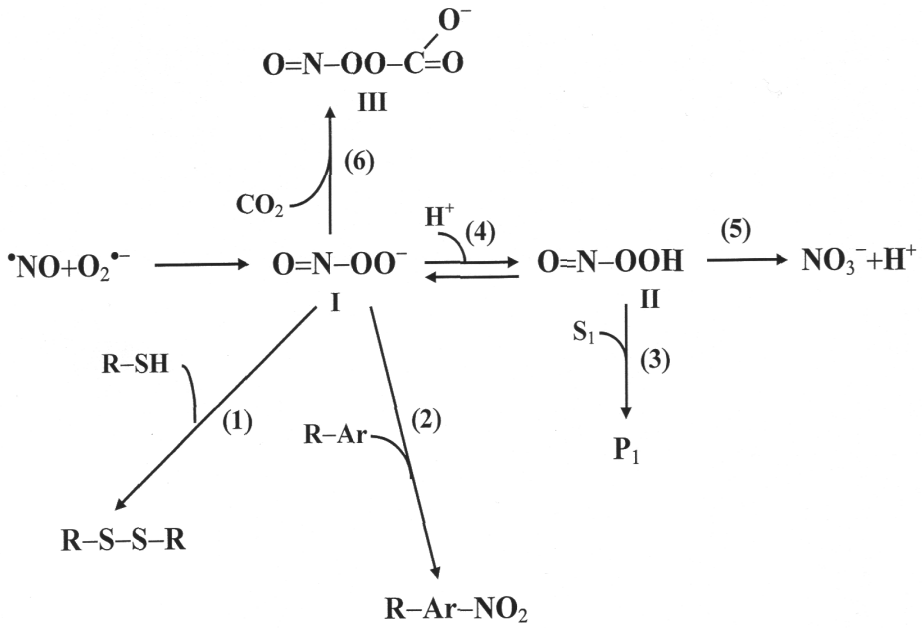
CO_2 • peroxynitrite • Free radicals • Oxidative damage • Hypercapnia

Carbon dioxide is an important component of biological acido-basic reactions which produces various physiological effects. A relatively new role for CO_2 was recently reported. It was found that CO_2 interacts with various free radical species and, depending on many factors, it can either propagate or inhibit free radical chain reactions.

Interactions of CO_2 with reactive nitrogen species derived from NO are of great importance. Among them, peroxynitrite originating from the reaction between NO and superoxide plays a central role (Beckman and Koppenol 1996). NO is produced intracellularly by NO synthase. It diffuses outside the cell where it can encounter superoxides which are produced mainly by NADPH oxidase localized in the cell membrane of

Fax+4202 24920590
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

various types of cells. Besides being located in phagocytes, which are the major producers of superoxide, NADPH oxidase has also been found in endothelial cells or smooth muscle cells (Maly and Schurer-Maly 1995). The reaction between superoxide and NO is diffusion-limited and proceeds with a higher rate than dismutation of superoxide catalyzed by superoxide dismutase (Beckman and Koppenol 1996). The principal reactions of peroxynitrite are summarized in **Scheme 1**.



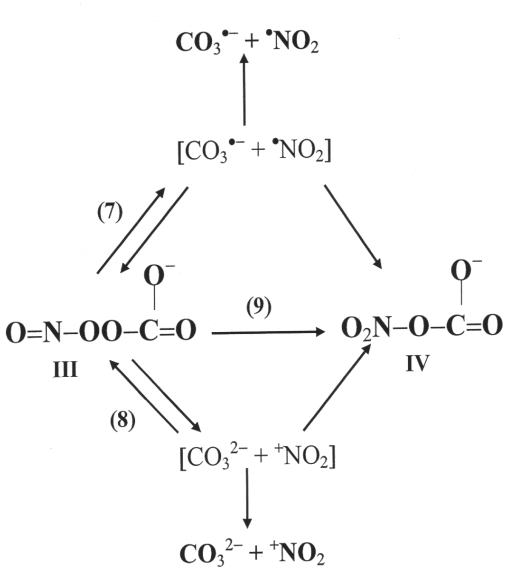
Peroxynitrite (**I**) is a strong oxidant and can directly oxidize sulfhydryl groups to disulfides – reaction (1). This reaction leads to inhibition of various enzymes. Peroxynitrite also mediates nitration of aromatic compounds, especially in the presence of transition metal ions – reaction (2). In the presence of proteins, nitration of tyrosine in position 3 ensues. This 3-nitrotyrosine is used as an indicator of reactive nitrogen species *in vivo* (Khan *et al.* 1998). Under physiological pH, peroxynitrite rapidly protonates to peroxynitrous acid (**II**). This acid is unstable and can interact with various biomolecules (hemoglobin, cytochrome c, methionine, ascorbate, tryptophan) by one-electron exchange reactions to produce corresponding oxidation products (Denicola *et al.* 1996) – reaction (3). The decomposition of peroxynitrous acid leads to nitrate – reaction (5). It has been found that peroxynitrite is unstable in a bicarbonate buffer. Here it reacts with CO_2 to produce nitrosocarbonate anion (**III**) (Uppu *et al.* 1996, Lemercier *et al.* 1997) – reaction (6). This reaction is kinetically of the second order with a rate constant of about 10^4 (Denicola *et al.* 1996). It is thus one of the most rapid reactions of peroxynitrite. The rate constant for the reaction with CO_2 is higher than the rate constant for the reaction of peroxynitrite with R-SH. However, the intracellular concentration of sulfhydryl compounds is higher than that of CO_2 and therefore the main intracellular substrate for peroxynitrite is R-SH. In the extracellular space, the concentration of R-SH is about ten-fold lower than in the cell and the concentration of CO_2 is higher; therefore nitrosocarbonate is formed preferentially.

Further reactions of nitrosocarbonate are illustrated in **Scheme 2**. The product of these reactions is nitrocarbonate (**IV**) which might be formed by three pathways.

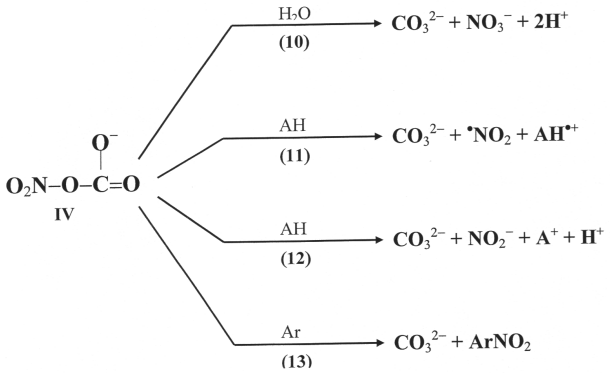
The first possibility is represented by a homolytic scission, reaction (7), producing radicals $^{\bullet}NO_2$ and $CO_3^{\bullet-}$. These radicals can recombine to recreate nitrocarbonate or can diffuse out and initiate free radical reactions. $CO_3^{\bullet-}$ is relatively less reactive than $^{\bullet}OH$, the most reactive oxygen radical; however, it is more stable and can diffuse from the site of origin and thus propagate the oxidative damage. $^{\bullet}NO_2$ can initiate free radical reactions by hydrogen abstraction from, for instance, fatty

acids, tyrosine, or tocoferol. $^{\bullet}NO_2$ radicals also dimerize producing an equimolar mixture of NO_3^- and NO_2^-.

The second possibility is represented by a heterolytic scission, reaction (8), producing carbonate and NO_2^+. In the hydrophobic environment of a membrane, NO_2^+ can nitrate protein tyrosine by the mechanism of electrophilic substitution. In the presence of water outside the membranes, NO_2^+ quickly reacts to produce NO_3^- and cannot effectively nitrate proteins.



The third pathway leading to nitrocarbonate is the direct rearrangement – reaction (9) (Uppu *et al.* 1996).



Nitrocarbonate is subjected to other reactions as summarized in **Scheme 3**. In an aqueous environment, the most probable reaction is hydrolysis, reaction (10), producing carbonate and nitrate. In a nonpolar environment of the membrane, other reactions are possible. They can concern either one-electron oxidation of substrates, reaction (11), producing radical species, or two-electron oxidation of substrates, reaction (12), producing ionic species. In the nonpolar environment, nitration of aromatic residues is also possible – reaction (13).

To sum up, in an aqueous environment CO_2 effectively scavenges peroxynitrite transforming it to nitrate thus preventing the oxidative effects of peroxynitrite. In the nonpolar environment of membranes, CO_2 supports free radical reactions and can accentuate the ensuing oxidative damage.

Other interactions of CO_2 with reactive nitrogen species are observed in the absence of superoxide, when peroxynitrite is not produced. In this case NO is oxidized by molecular oxygen:

$$2NO + O_2 \longrightarrow 2NO_2 \qquad (14)$$

Nitric dioxide reacts with NO to N_2O_3:

$$NO + NO_2 \rightleftharpoons N_2O_3 \qquad (15)$$

N_2O_3 can initiate nitrosation of primary and secondary amines and can oxidize sulfhydryl groups of proteins or glutathione (Caulfield *et al.* 1996). Some anions, like phosphates or bicarbonates, react with N_2O_3 to form nitrosyl compounds:

$$N_2O_3 + HCO_3^- \longrightarrow NOHCO_3 + NO_2^- \qquad (16)$$

These compounds can nitrosylate amines:

$$NOHCO_3 + R\text{-}NH_2 \longrightarrow R\text{-}NH\text{-}NO + H_2CO_3 \qquad (17)$$

or hydrolyze to HNO_2:

$$NOHCO_3 + H_2O \longrightarrow HNO_2 + H_2CO_3 \qquad (18)$$

Under physiological pH, hydrolysis proceeds faster. In this way, anions act as scavengers of N_2O_3 preventing the nitration reactions. In the extracellular space, the concentration of bicarbonate is higher than that of phosphate and therefore bicarbonate is the principal scavenger of N_2O_3 *in vivo* (Caulfield *et al.* 1996).

CO_2 may also interfere with free radicals in the reactions of superoxide dismutase (SOD). This enzyme catalyzes the dismutation of superoxide to oxygen and hydrogen peroxide. If hydrogen peroxide is not decomposed by other enzymes, it can damage SOD by a peroxidase-type mechanism:

$$SOD\text{-}Cu^{2+} + H_2O_2 \rightleftharpoons SOD\text{-}Cu^{1+} + O_2^{\bullet-} + 2H^+ \qquad (19)$$

$$SOD\text{-}Cu^{1+} + H_2O_2 \longrightarrow SOD\text{-}Cu^{2+}\text{-}OH^{\bullet} + OH^{-} \quad (20)$$

The intermediate of reaction (20) SOD-Cu^{2+}-$OH^{\cdot}$ is similarly reactive as a hydroxyl radical and inactivates the enzyme by interaction with a histidine residue:

$$SOD\text{-}Cu^{2+}\text{-}OH^{\bullet} \xrightarrow{\textbf{His}} Cu^{2+} + inactiveSOD \quad (21)$$

In the presence of bicarbonate, the active enzyme intermediate reacts with it and generates the unmodified enzyme and carbonate radical:

$$SOD\text{-}Cu^{2+}\text{-}OH^{\bullet} + HCO_3^{-} \longrightarrow SOD\text{-}Cu^{2+} + H_2O + CO_3^{\bullet-} \quad (22)$$

So, while bicarbonate protects SOD, the carbonate radical can initiate free radical reactions and oxidative damage. The examples of targets of carbonate radical are given below. It quickly oxidizes tyrosine:

$$CO_3^{\bullet-} + Tyr\text{-}OH \longrightarrow Tyr\text{-}O^{\bullet} + HCO_3^{-} \quad (23)$$

and also sulfhydryl groups. Reaction of carbonate radical with nitrite produces radical species:

$$CO_3^{\bullet-} + NO_2^{-} \longrightarrow HCO_3^{-} + {}^{\bullet}NO_2 \quad (24)$$

$^{\bullet}NO_2$ can initiate oxidative damage (see above). Carbonate radicals can also dimerize. In this reaction, chemiluminescence is generated which can be used for the detection of CO_2 participation in oxidative damage (Goss *et al.* 1999).

The overview of CO_2 involvement in free radical reactions as described above can be used to explain the protective role of hypercapnia against free radical damage induced by hypoxia *in vivo* (Barth *et al.* 1998, Vannucci *et al.* 1995, 1997). The scavenging of peroxynitrite plays an important role. With regard to the role of iron ions in the initiation and propagation of free radical reactions, the most probable explanation of protective effects of CO_2 seems to consist in stabilization of the iron-transferrin complex. Oxidative stress dysregulates iron metabolism and increases the levels of catalytic metal ions. Transferrin is the major iron binding protein in human blood plasma. It is a single polypeptide with two domains, each of which is capable of binding one ferric ion in association with the synergistic binding of an anion. *In vivo* this anion is usually bicarbonate (Edeker *et al.* 1995). The capacity to bind iron enables transferrin to protect cells from oxidative damage. The fact that iron binding by transferrin requires bicarbonate anions offers an explanation of the different effects of lactic acid and hypercapnic acidosis on lipid peroxidation (Rehncrona *et al.* 1989). The association of iron with transferrin is dependent not only on pH but also on the bicarbonate concentration and thereby on pCO_2. The binding constant (K) of iron transferrin (T) can be defined by the equations:

$$Fe^{3+} + HCO_3^{-} + H_3\text{–}T \rightleftharpoons Fe\text{–}T\text{–}HCO_3^{-} + 3H^{+} \quad (25)$$

$$K = \frac{[Fe^{3+}]\,[HCO_3^{-}]\,[H_3\text{-}T]}{[Fe\text{-}T\text{-}HCO_3^{-}]\,[H^{+}]^3} \quad (26)$$

$$[Fe\text{-}T\text{-}HCO_3^{-}] = \frac{K\,[HCO_3^{-}]\,[Fe^{3+}]\,[H_3\text{-}T]}{[H^{+}]^3} \quad (27)$$

It follows from these equations that the increase in $[HCO_3^{-}]$ concomittant to the increase in $[H^{+}]$ in hypercapnic acidosis would tend to stabilize the transferrin iron complex. On the other hand, hydrogen ions donated by lactic acid would decrease the stability and tend either to dissociate iron from transferrin or to decrease its ability to bind iron delocalized from other sources (Rehncrona *et al.* 1989).

Numerous other mechanisms potentially exist whereby CO_2 protects the tissues from hypoxic-ischemic damage. An increase in blood pCO_2 shifts the oxygen-hemoglobin dissociation curve to the right (Bohr effect), the result of which is a decrease in the affinity of hemoglobin for oxygen. Therefore, at the capillary level, CO_2 would tend to raise pO_2, increase the gradient for any given oxyhemoglobin saturation, and facilitate transfer of O_2 to the tissue for oxidative processes. CO_2 might also preserve cardiac function during systemic hypoxia. The inhibition of systemic lactate production by CO_2 inhalation during hypoxia would serve to maintain optimal cardiovascular function. Finally, CO_2 has several effects on metabolism. Hypercapnia leads to a generalized suppression of oxidative matabolism and tissue lactate production is also reduced (Vannucci *et al.* 1995).

Chronic hypercapnia is commonly found in patients with severe hypoxic lung disease and is associated with greater elevation of pulmonary arterial pressure than that due to hypoxia alone. It has been shown that chronic hypercapnia inhibits hypoxic pulmonary vascular remodeling and right ventricular

hypertrophy, and protects against hypoxia-induced impairment of endothelial function (Ooi *et al.* 2000).

Contrary to the protective effects of hypercapnia, in vitro studies indicate a dual role of CO_2. In an aqueous environment, it scavenges reactive nitrogen species thus preventing nitration and oxidative damage. On the other hand, in the nonpolar environment of membranes, CO_2 can propagate free radical reactions by formation of carbonate radicals and in this way accentuate the oxidative damage. Carbonate radicals are also produced during the interactions of CO_2 with reactive oxygen species in a reaction mechanistically similar to that of SOD. This suggests that CO_2 might also support free radical-mediated damage in an aqueous medium.

Acknowledgements

This work was supported by a grant MSM 111300002.

References

BARTH A, BAUER R, GEDRANGE T, WALTER B, KLINGER W, ZWIENGER U: Influence of hypoxia and hypoxia/hypercapnia upon brain and blood peroxidative and glutathione status in normal weight and growth-restricted newborn piglets. *Exp Toxicol Pathol* **50**: 402-410, 1998.

BECKMAN JS, KOPPENOL WH: Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly. *Am J Physiol* **271**: C1424-C1437, 1996.

CAULFIELD JL, SINGH SP, WISHNOK JS, DEEN WM, TANNENBAUM SR: Bicarbonate inhibits N-nitrosation in oxygenated nitric oxide solutions. *J Biol Chem* **271:** 25859-25863, 1996.

DENICOLA A, FREEMAN BA, TRUJILLO M, RADI R: Peroxynitrite reaction with carbon dioxide/bicarbonate: kinetics and influence on peroxynitrite-mediated oxidations. *Arch Biochem Biophys* **333:** 49-58, 1996.

EDEKER BL, RASMUSSEN GT, BRITIGAN BE: Bicarbonate and phosphate ions protect transferrin from myeloperoxidase-mediated damage. *Leukocyte Biol* **58:** 59-64, 1995.

GOSS SPA, SINGH RJ, KALYANARAMAN B: Bicarbonate enhances the peroxidase activity of Cu,Zn-superoxide dismutase. *J Biol Chem* **274:** 28233-28239, 1999.

KHAN J, BRENNAN DM, BRADLEY N, GAO B, BRUCKDORFER R, JACOBS M: 3-nitrotyrosine in the proteins of human plasma determined by an ELISA method. *Biochem J* **330**: 795-801, 1998.

LEMERCIER J, PADMAJA S, CUETO R, SQUADRITO GL, UPPU RM, PRYOR WA: Carbon dioxide modulation of hydroxylation and nitration of phenol by peroxynitrite. *Arch Biochem Biophys* **345:** 160-170, 1997.

MALY FE, SCHURER-MALY CC: How and why cells make superoxide: the "phagocytic" NADPH oxidase. *News Physiol Sci* **10**: 233-238, 1995.

OOI H, CADOGAN E, SWEENEY M, HOWELL K, O'REGAN RG, MCLOUGHLIN P: Chronic hypercapnia inhibits hypoxic pulmonary vascular remodeling. *Am J Physiol* **278:** H331-H338, 2000.

REHNCRONA S, HAUGE HN, SIESJO BK: Enhancement of iron-catalyzed free radical formation by acidosis in brain homogenates: Difference in effect by lactic acid and CO_2. *J Cereb Blood Flow Metabol* **9:** 65-70, 1989.

UPPU RM, SQUADRITO GL, PRYOR WA: Acceleration of peroxynitrite oxidations by carbon dioxide. *Arch Biochem Biophys* **327:** 335-343, 1996.

VANNUCCI RC, TOWFIGHI J, HEITJAN DF, BRUCKLACHER RM: Carbon dioxide protects the perinatal brain from hypoxic-ischemic damage: an experimental study in the immature rat. *Pediatrics* **95:** 868-874, 1995.

VANNUCCI RC, BRUCKLACHER RM, VANNUCCI SJ: Effect of carbon dioxide on cerebral metabolism during hypoxia-ischemia in the immature rat. *Pediat Res* **42:** 24-29, 1997.

Reprint requests

Doc. Dr. J. Wilhelm, Department of Medical Chemistry and Biochemistry, Second Faculty of Medicine, Charles University, Plzeňská 221, 150 00 Prague 5, Czech Republic. E-mail: jiri.wilhelm@lfmotol.cuni.cz