Editorial

Acute CO poisoning is known to produce both immediate and delayed cellular injury to various regions of the brain. The classical teaching is that CO causes tissue hypoxia (1). It binds very tightly to heme centers of respiratory proteins such as hemoglobin and cytochrome c oxidase, outcompeting O_2 (1, 2). Accordingly, CO decreases both the O_2 carrying capacity of the blood and O_2 utilization by the mitochondria. Toxic exposure to CO also produces an oxidative stress which is secondary to inhibiting mitochondrial respiration: reactive oxygen species (ROS) are released that cause oxidative damage to proteins, lipids, and nucleic acids. In this issue of *The Journal* Ischiropoulos and co-workers (3) expand on this theme. They describe a possible connection between CO toxicity and increased nitric oxide (NO) production in the brain. Ischiropoulos et al. find that CO exposure leads to perivascular deposits of nitrotyrosine, a marker of peroxynitrite ($OONO^-$) production. Peroxynitrite is the oxidant formed on NO/superoxide (O_2^-) reaction which they implicate in brain damage. These findings are potentially very important. However, more work is needed to determine the significance of the findings and to establish the causality of NO.

The original insight of Beckman and co-workers was to appreciate that the reactivity of O_2^- may be enhanced through its rapid reaction with NO (4). They proposed that this increased reactivity—attributed to formation of peroxynitrite—would be manifest as toxicity in biological systems. There is now a wealth of experimental evidence supporting their prediction. Nonetheless, an equally substantive body of data has established that NO can ameliorate O_2^--mediated cytotoxicity. Accordingly, the same inherent reactivity of peroxynitrite can have salutary consequences. Such diametrically opposed effects can be understood by appreciating that the target(s) (reaction substrate) of $OONO^-$ when it is cytotoxic is different from that when it is protective. Peroxynitrite's target is dictated by the redox state of the cell, particularly by the rates of NO and O_2^- production and by the concentration of intracellular thiol.

This point is illustrated by an exercise in understanding the pathophysiological implications of NO/O_2^- reactions with mitochondrial aconitase. Both superoxide and peroxynitrite inactivate aconitase, but the O_2^- is $\sim$ 100 times faster (5). It follows, that NO production will slow the rate of O_2^- inactivation by forming $OONO^-$. Moreover, mitochondria are very rich in glutathione, which reacts faster with peroxynitrite than with superoxide. Thus, NO generation also diverts O_2^- away from aconitase toward protective thiols. NO will confer resistance to O_2^- under these conditions. On the other hand, the scenario is quite different when glutathione is depleted by oxidant stress. Any peroxynitrite formed would be free to react with aconitase. The damage sustained by the enzyme may be even worse than that incurred from O_2^- alone, as $OONO^-$ is the more powerful oxidant. Aconitase is one of many proteins and lipids that may be oxidized by peroxynitrite when the protection conferred by glutathione is overwhelmed (6).

The measurement of peroxynitrite poses a significant problem as it is short-lived. Evidence for its production in vivo rests largely on detection of nitrotyrosine, a stable product of its reaction with proteins. The specificity of this assay in tissues in unknown and its interpretation is complicated by the low-yield of nitrotyrosine under physiological conditions; most of the peroxynitrite is consumed in other reactions. Limitations notwithstanding, nitrotyrosine appears to be a useful marker of NO-related oxidative stress—much as malondialdehyde is a marker of O_2-related stress—to which peroxynitrite undoubtedly contributes. Indeed, extensive protein nitration occurs in a variety of inflammatory diseases including atherosclerosis. ARDS, and arthritis. The immunoradiochemical assay developed by Ischiropoulos and co-workers to quantify nitrotyrosine (3) is an important step forward. The demonstration of an increase in oxidative stress, however, should not be confused with causality.

How does CO increase levels of nitrotyrosine? By binding to heme irons in proteins, CO may competitively displace NO which is normally bound by virtue of its higher affinity. By disrupting mitochondrial electron transport, it will also promote escape of O_2^-. The simultaneous liberation of NO and O_2^- would generate $OONO^-$. Such displacement of NO (or release of O_2^-) from blood-borne hemoglobin might explain the perivascular predominance of nitrotyrosine. Additionally, $OONO^-$ formation could arise from activation of *N*-methyl-D-aspartate receptors when damaged brain cells release glutamate. CO, NO, and O_2 may further cycle to exacerbate radical damage (7).

Is $OONO^-$ casual in brain poisoning by CO? In weighing the role of NO/O_2^-, one should not forget that O_2 is inevitably displaced from hemes before NO and that aerobic metabolism is inhibited before O_2^- is released from mitochondria. Accordingly, nitrotyrosine is more likely to be a marker of the lost capacity of hemoglobin to carry O_2 and of the mitochondria to function, than of $OONO^-$ causality in CO toxicity. Of course there is always the possibility that NO production is increased by cells to protect themselves against O_2^- or to reverse CO hypoxia by dilating blood vessels. In the final analysis, Ischiropolous et al. increase our understanding of the biochemical events associated with CO poisoning, but questions regarding the role of NO/O_2^- remain.

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