

EDITORIAL

Changing views on carbon monoxide*

The paper by Andersson et al. published in this issue provides further evidence of the importance of carbon monoxide (CO) as an endogenously produced molecule [1]. A decade ago, carbon monoxide was known only as a lethal gas. However, since then it has been established as an important signalling molecule, produced in various cell types throughout the body. A better understanding of the role of CO as a signalling molecule could provide insight into the toxic mechanisms involved in CO poisoning. While hypoxia is generally held accountable for most of the effects experienced by victims of CO poisoning, some remain to be explained [2–4]. It has been suggested that CO functions as a neural messenger in the brain, and thus exposure to an external source of CO might interfere with signalling and cause neurological disturbance [5].

Carbon monoxide is produced *in vivo* by the catabolism of heme to yield CO and biliverdin, two molecules previously thought to be byproducts [6,7]. This process is activated by the enzyme heme oxygenase (HO), of which there are two isoforms: HO₁ and HO₂. HO₁ is found mainly in the spleen, where it degrades heme from senescent red blood cells. It can be induced by many agents, such as heme, inflammatory cytokines, prostaglandins, hormones and by oxidative stress and hypoxia [7,8]. HO₂ is the constitutive form of the enzyme and is present in neurones [7,8], endothelium and gastrointestinal tract [9] and testes [6]. Protein Kinase C, which is activated by neuronal depolarization, phosphorylates and activates HO₂ [6], thus providing a mechanism for the rapid formation of CO.

CO is known, for example in olfactory cells, to activate cytoplasmic guanylyl cyclase (cGC), leading to an increase in cyclic guanosine monophosphate (cGMP) [10]. However, there is also evidence indicating that CO plays a role in at least one cGMP-independent pathway, involving mitogen-activated protein kinases [11]. There is also controversy over the role of CO in cells in which nitric oxide (NO) concentrations regulate cGC activity. Despite the fact that CO *per se* activates cGC, inhibition of CO production did not lead to a fall in cGMP levels. Ingi et al. [12] argued that in such cells, CO inhibited the effect of NO on cGMP production. The mechanism for this is unclear and might include the inhibition of NO synthase by CO, thus reducing the production of NO, or interference with the interaction between NO and cGC.

Endogenously produced CO is thought to be involved in many processes, including smooth muscle relaxation mediated by the enteric nervous system. For example, this effect was observed in guinea-pigs *in vitro* [13]. Since heme oxygenase was found in the interstitial cells of Cajal, CO is thought to act as a messenger between these cells and gastrointestinal smooth muscle cells [9]. Further evidence stems from a study by the same group, who found that knockout mice lacking the

biosynthetic enzymes necessary to produce CO had depolarized neurons and decreased muscle relaxation due to reduced inhibitory junctional potentials [14]. Thus one function of CO is thought to be the inhibition of muscle contraction in the gut, where it acts as a signalling molecule at the neuromuscular junction. CO is also known to be involved in vasodilation [15,16] and studies have shown that CO causes bronchodilation in guinea-pigs [17,18] and has a role in modulating blood pressure under stress conditions *in vivo* [19].

There is recent evidence that CO is involved in signalling in the carotid body. Prabhakar et al. [20] found that HO₂ was localized to glomus cells of rat and cat carotid bodies. HO₂ inhibition caused an increase in carotid body activity, a response which was attenuated by the addition of exogenous CO. This study suggests that CO functions as an inhibitor of carotid body activity. However, the mechanism by which this occurs is still unclear. One possible explanation is that CO regulates the release of an excitatory neurotransmitter. CO could also affect vasodilation, which would lead to increased local oxygenation.

Besides its role as a signalling molecule in the peripheral nervous system, CO has more recently been implicated as a neurotransmitter in the brain. CO is found in large amounts in the brain, particularly in the hippocampus, cerebellum and olfactory cells [8]. Of the neuroendocrine tissues, the hypothalamus has the highest CO production rate in the brain [21]. These observations have prompted investigation into the possible role of CO as a mediator of long-term potentiation (LTP) and as a neuroendocrine modulator.

There is evidence, though controversial, that CO is involved in the induction of LTP, a process that leads to an increase in synaptic efficiency in the hippocampus, believed to be important for learning and memory. It is thought that CO might act as a retrograde messenger, by delivering information from the post-synaptic to the pre-synaptic neuron, leading to an increase in transmitter release. HO₂ inhibitors blocked the induction of LTP in the CA₁ region of the hippocampal slices [22–24], and attenuated LTP in the dentate gyrus [25]. However, a study by Poss et al. [26] showed that LTP was not affected in knockout mice lacking the gene for HO₂. As the results are inconsistent and the mechanism by which CO is involved in LTP is still unknown, it is unclear whether CO has a role in LTP.

CO also appears to have a neuromodulatory effect in the hypothalamus. A study in female rats has shown that CO plays a role in the release of gonadotropin-releasing hormone (GnRH) [27]. GnRH release was stimulated in hypothalamus preparations incubated with HO and haematin, a heme molecule cleaved by HO to yield CO. As both a heme oxygenase inhibitor and a CO-scavenger molecule blocked this effect, GnRH release was thought to be mediated by CO.

CO is also thought to act as an inhibitory neurotransmitter involved in oxytocin release from rat hypothalamus [28]. The study showed that when rat hypothalamus was incubated with

*The views expressed here are those of the authors and should not be taken as those of the UK Department of Health.

hemin, a substrate of heme oxygenase, KCl- or serotonin-stimulated oxytocin release was inhibited. The evidence suggested that CO was responsible for the modulating action.

Another role for CO in the hypothalamus is in mediating the release of corticotropin-releasing hormone (CRH) [29,30], however, there is controversy over what the effect might be [28]. In support of other studies showing an inhibitory role for CO in the neuroendocrine axis, hemin dose-dependently inhibited KCl- and IL-1 β -stimulated CRH release *in vitro* [29]. While more studies are needed to clarify the role of CO, it seems possible that CO regulates the release of hormones from the hypothalamus.

CO might also be involved in inflammatory processes. A study by Otterbein et al. [11] reported that CO could be responsible for anti-inflammatory effects associated with HO₁. CO inhibited the expression of pro-inflammatory cytokines (TNF- α , IL-1 β , MIP-1 β), while increasing the expression of the anti-inflammatory cytokine IL-10, both *in vivo* and *in vitro*. CO mediated these effects not through cGMP but through mitogen-activated protein kinases.

A study by Andersson et al. [31] demonstrated that CO was produced endogenously in the nose and paranasal sinuses in humans, and is probably involved in upper and lower airway regulation and inflammation. HO₁ and high levels of HO₂ were measured in the respiratory epithelium and in connection with smooth muscle of arteries and veins in the submucosa. In their most recent paper (published in this issue) [1], they report increased levels of exhaled CO in patients with upper respiratory tract infection or allergic rhinitis. This further supports the idea that CO is involved in upper airway inflammation, and might function as a mediator. Their results agree with the findings of Otterbein et al. [11], who reported anti-inflammatory effects of CO.

CO has also been found in macrophages. Arias-D'iaz et al. [32] investigated the capacity of human pulmonary macrophages to produce CO. In the presence of LPS (lipopolysaccharide), TNF- α production was accompanied by an increase in CO concentration and cell cGMP content. CO is thought to be involved in the production of cGMP in pulmonary macrophages, and could be involved in lung defence against endotoxin shock [15].

An interesting and somewhat ironic point about carbon monoxide is that it seems to be associated both with causing oxidative stress and with producing protective effects during stress conditions. Many studies have shown that hypoxia caused by accidental inhalation of CO is associated with increased oxidative stress compared with that caused by lack of oxygen alone [5,33]. Under hypoxic conditions, xanthine dehydrogenase is converted to xanthine oxidase, which produces superoxide radicals and hydrogen peroxide, followed by lipid peroxidation [34]. Another pathway thought to cause oxidative stress involves the binding of CO to reduced cytochrome *c* oxidase in the mitochondria. This binding interferes with the electron transport chain and leads to the production of partially reduced oxidative species [35]. On the other hand, it has been shown that the expression of HO₁ is increased under stress conditions, including oxidative stress and inflammation [15]. During hypoxic stress, CO is increased sevenfold in vascular smooth muscle cells, and is thought to produce vasodilation in response to hypoxia. It is surprising that CO is thought to have a protective effect during hypoxia, when it is often CO itself that is responsible for hypoxia.

Much of the role of CO in the body remains a mystery. This single molecule is thought to be involved in smooth muscle relaxation, memory formation, hormone release in the hypothalamus and inflammation. It is clear that it often functions as a signalling molecule, and sometimes has an inhibitory role. But the full picture is far from clear. Studies providing new information about CO are appearing frequently, and it is becoming increasingly apparent that CO is a very versatile molecule. As many of the pathways remain unclear, it is difficult to understand how CO can be involved in so many different processes.

References

- Andersson JA, Uddman R, Cardell LO. Increased carbon monoxide levels in the nasal airways of subjects with a history of seasonal allergic rhinitis and in patients with upper respiratory tract infection. *Clin Exp Allergy* 2002; 32:224–7.
- Feldman RG. *Occupational and Environmental Neurotoxicology*. Philadelphia: Lippencott-Raven Publishers 1999; 378–99.
- Institute for Environment and Health. IEH Assessment on Indoor Air Quality in the Home (2): Carbon Monoxide. Norwich: Page Bros 1998.
- Thom SR, Taber RL, Mendiguren II, Clark JM, Hardy KR, Fisher AB. Delayed neuropsychologic sequelae after carbon monoxide poisoning: Prevention by treatment with hyperbaric oxygen. *Ann Emergency Med* 1995; 25:474–80.
- Piantadosi CA, Zhang J, Demchenko IT. Production of hydroxyl radical in the hippocampus after CO hypoxia or hypoxic hypoxia in the rat. *Free Radic Biol Med* 1997; 22:725–32.
- Snyder SH, Jaffrey SR, Zakhary R. Nitric oxide and carbon monoxide: parallel roles as neural messengers. *Brain Res Rev* 1998; 26:167–75.
- Dawson TM, Snyder SH. Gases as biological messengers: nitric oxide and carbon monoxide in the brain. *J Neurosci* 1994; 14:5147–59.
- Verma A, Hirsh DJ, Glatt CE, Ronnett GV, Snyder SH. Carbon monoxide: a putative neural messenger. *Science* 1993; 259:381–3.
- Farrugia G, Szurszewski JH. Heme oxygenase, carbon monoxide, and interstitial cells of Cajal. *Microsc Res Tech* 1999; 47:321–4.
- Ingi T, Ronnett GV. Direct demonstration of a physiological role for carbon monoxide in olfactory receptor neurons. *J Neurosci* 1995; 15:8214–22.
- Otterbein LE, Bach FH, Alam J et al. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat Med* 2000; 6:422–8.
- Ingi T, Cheng J, Ronnett GV. Carbon monoxide: an endogenous modulator of the nitric oxide-cyclic GMP signaling system. *Neuron* 1996; 16:835–42.
- Utz J, Ullrich V. Carbon monoxide relaxes ileal smooth muscle through activation of guanylate cyclase. *Biochem Pharmacol* 1991; 41:1195–201.
- Xue L, Farrugia G, Miller SM, Ferris CD, Snyder SH, Szurszewski JH. Carbon monoxide and nitric oxide as coneurotransmitters in the enteric nervous system: evidence from genomic deletion of biosynthetic enzymes. *Proc Natl Acad Sci USA* 2000; 97:1851–5.
- Marilena G. New physiological importance of two classic residual products. carbon monoxide and bilirubin. *Biochem Mol Med* 1997; 61:136–42.
- Zhang F, Kaide JI, Rodriguez-Mulero F, Abraham NG, Nasjletti A. Vasoregulatory function of the heme-heme oxygenase-carbon monoxide system. *Am J Hypertens* 2001; 14:62S–67S.

- 17 Cardell LO, Lou YP, Takeyama K, Ueki IF, Lausier J, Nadel JA. Carbon monoxide, a cyclic GMP-related messenger, involved in hypoxic bronchodilation in vivo. *Pulm Pharmacol Ther* 1998; 11:309–15.
- 18 Kinhult J, Uddman R, Cardell LO. The induction of carbon monoxide-mediated airway relaxation by PACAP 38 in isolated guinea pig airways. *Lung* 2001; 179:1–8.
- 19 Motterlini R, Gonzales A, Foresti R, Clark JE, Green CJ, Winslow RM. Heme oxygenase-1-derived carbon monoxide contributes to the suppression of acute hypertensive responses in vivo. *Circ Res* 1998; 83:568–77.
- 20 Prabhakar NR, Dinerman JL, Agani FH, Snyder SH. Carbon monoxide: a role in carotid body chemoreception. *Proc Natl Acad Sci USA* 1995; 92:1994–7.
- 21 Brann DW, Bhat GK, Lamar CA, Mahesh VB. Gaseous transmitters and neuroendocrine regulation. *Neuroendocrinology* 1997; 65:385–95.
- 22 Stevens CF, Wang Y. Reversal of long-term potentiation by inhibitors of haem oxygenase. *Nature* 1993; 364:147–9.
- 23 Zhuo M, Small SA, Kandel ER, Hawkins RD. Nitric oxide and carbon monoxide produce activity-dependent long-term synaptic enhancement in hippocampus. *Science* 1993; 260:1946–50.
- 24 Zhuo M, Laitinen JT, Li XC, Hawkins RD. On the respective roles of nitric oxide and carbon monoxide in long-term potentiation in the hippocampus. *Learn Mem* 1999; 6:63–76.
- 25 Ikegaya Y, Saito H, Matsuki N. Involvement of carbon monoxide in long-term potentiation in the dentate gyrus of anesthetized rats. *Jpn J Pharmacol* 1994; 64:225–7.
- 26 Poss KD, Thomas MJ, Ebrilidze AK, O'Dell TJ, Tonegawa S. Hippocampal long-term potentiation is normal in heme oxygenase-2 mutant mice. *Neuron* 1995; 15:867–73.
- 27 Lamar CA, Mahesh VB, Brann DW. Regulation of gonadotrophin-releasing hormone (GnRH) secretion by heme molecules: a regulatory role for carbon monoxide? *Endocrinology* 1996; 137:790–3.
- 28 Kostoglou-Athanassiou I, Forsling ML, Navarra P, Grossman AB. Oxytocin release is inhibited by the generation of carbon monoxide from the rat hypothalamus – further evidence for carbon monoxide as a neuromodulator. *Brain Res Mol Brain Res* 1996; 42:301–6.
- 29 Pozzoli G, Mancuso C, Mirtella A et al. Carbon monoxide as a novel neuroendocrine modulator: inhibition of stimulated corticotropin-releasing hormone release from acute rat hypothalamic explants. *Endocrinology* 1994; 135:2314–7.
- 30 Grossman A, Costa A, Forsling ML et al. Gaseous neurotransmitters in the hypothalamus. The roles of nitric oxide and carbon monoxide in neuroendocrinology. *Horm Metab Res* 1997; 29:477–82.
- 31 Andersson JA, Uddman R, Cardell LO. Carbon monoxide is endogenously produced in the human nose and paranasal sinuses. *J Allergy Clin Immunol* 2000; 105:269–73.
- 32 Arias-D'iaz J, Vara E, Garcia C, Villa N, Balibrea JL. Evidence for a cyclic guanosine monophosphate-dependent, carbon monoxide-mediated, signaling system in the regulation of TNF- α production by human pulmonary macrophages. *Arch Surg* 1995; 130:1287–93.
- 33 Thom SR, Ischiropoulos H. Mechanism of oxidative stress from low levels of carbon monoxide. *Health Effects Institute Research Report* Number 80, 1997.
- 34 Thom SR. Dehydrogenase conversion to oxidase and lipid peroxidation in brain after carbon monoxide poisoning. *J Appl Physiol* 1992; 73:1584–9.
- 35 Zhang J, Piantadosi CA. Mitochondrial oxidative stress after carbon monoxide hypoxia in the rat brain. *J Clin Invest* 1992; 90:1193–9.

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