Korean J Physiol Pharmacol
Vol 5: 87-92, February, 2001

Effect of Heme Oxygenase Induction by NO Donor on the Aortic Contractility

Chang-Kyun Kim, Uy Dong Sohn[1], and Seok-Yong Lee

Department of Pharmacy, College of Pharmacy, Sungkyunkwan University, Suwon 440-746; [1]College of Pharmacy Chung-Ang University, Seoul 156-756, Korea

Carbon monoxide (CO) binds to soluble guanylate cyclase to lead its activation and elicits smooth muscle relaxation. The vascular tissues have a high capacity to produce CO, since heme oxygenase-2 (HO-2) is constitutively expressed in endothelial and smooth muscle cells, and HO-1 can be greatly up-regulated by oxidative stress. Moreover, the substrate of HO, heme, is readily available for catalysis in vascular tissue. Although the activation of heme oxygenase pathway under various stress conditions may provide a defence mechanism in compromised tissues, the specific role of HO-1-derived CO in the control of aortic contractility still remains to be elucidated. The present study was done to determine the effect of HO-1 induction on the aortic contractility. Thus, the effects of incubation of aortic tissue with S-nitroso-N-acetylpenicillamine (SNAP) for 1 hr on the aortic contractile response to phenylephrine were studied. The preincubation with SNAP resulted in depression of the vasoconstrictor response to phenylephrine. This effect was restored by HO inhibitor or methylene blue but not by NOS inhibitor. The attenuation of vascular reactivity by preincubation with SNAP was also revealed in endothelium-free rings. $AlF4^-$-evoked contraction in control did not differ from that in SNP-treated group. These results suggest that increased production of CO was responsible for the reduction of the contractile response to phenylephrine in aortic ring preincubated with SNAP and this effect of SNAP was independent on endothelium.

Key Words: Heme oxygenase, Carbon monoxide, Vascular contractility, NO

INTRODUCTION

Carbon monoxide (CO) fulfills important physiological functions. Its lipophilicity and high diffusability allow this molecule to be involved in cell to cell communication as second messenger gases (Maines, 1997). CO is formed by the heme oxygenase (HO) isoenzymes from the substrate ferrous protoporphyrin (heme, FePP) (Maines, 1997) leading ultimately to the equimolar production of bilirubin, a compound with antioxidant properties (Stocker et al, 1987; Dennery et al, 1995). CO binds to soluble guanylate cyclase (sGC) to lead its activation, but has lower potency and efficacy than nitric oxide (NO) (Stone & Marletta, 1994).

Corresponding to: Seok-Yong Lee, College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea. (Tel) 82-31-290-7718, (Fax) 82-31-290-7738, (E-mail) sylee@skku.ac.kr

There are three known isoforms of HO. HO-1 is inducible (Shibahara et al, 1985), where HO-2 and HO-3 are constitutively expressed (Trakshel et al, 1986; McCoubrey et al, 1997). HO-3 is highly homologous to HO-2 but has not been characterized fully. HO-2 is the predominant form expressed in the central nervous system, where CO is thought to act as a neurotransmitter (Verma et al, 1993). HO-1, an inducible enzyme, is highly expressed in erythropoietic tissues, where its function is heme degradation. The HO-1 gene is exquisitely sensitive to a large variety of stimuli and agents that cause oxidative stress, such as heat shock (Raju & Maines, 1994), ischemia-reperfusion (Raju & Maines, 1996), hypoxia (Morita et al, 1995) and endotoxins (Rizzardini et al, 1994). The vascular tissues have a high capacity to produce CO, since HO-2 is constitutively expressed in endothelial and smooth muscle cells (Werkstrom et

al, 1997) and HO-1 can be greatly up-regulated by oxidative stress in the blood vessels (Ewing et al, 1994). Moreover, the substrate of heme oxygenase, heme, is readily available for catalysis in both vascular and myocardial tissues (Maines, 1997). Although the activation of heme oxygenase pathway under various stress conditions may provide a defence mechanism in compromised tissues because of the potent antioxidant and vasoactive properties (Abraham et al, 1996), the specific role of HO-1-derived CO in the control of aortic contractility still remains to be elucidated. The present study was done to elucidate the effect of HO-1 induction on the aortic contractility.

METHODS

Chemicals

S-nitroso-N-acetyl penicillamine (SNAP), zinc protoporphrine IX (ZnPP-IX), phenylephrine, L-NMMA and methylene blue were purchased from Sigma (USA). All concentrations are expressed as the final molar concentration (M) in the organ chamber. Stock solutions were prepared each day, and kept on ice during the course of the experiment. Unless stated otherwise, chemicals were dissolved in distilled water. Stock solution of ZnPP-IX was prepared in subdued light by dissolving the solid in 30% v/v 0.1 N NaOH. When fully dissolved, the pH was titrated using HCl for final pH of 7.6~7.8. AlF_4^- was made by simultaneous addition of 5 mM NaF and 10 μM $AlCl_3$.

Preparation of aortic ring

Male Sprague-Dawley rats (200~250 g) were sacrificed by decapitation and exsanguination. The descending aorta was isolated, cleaned of adherent tissue and prepared as rings 3~5 mm long. Rings were suspended in an organ chamber between two clips, one of which was connected to a force transducer (Grass, USA) to measure the isometric force. The organ chamber was filled with 5 ml of Krebs-Henseleit buffer (KHB) consisting of (mM) NaCl 120, KCl 4.6, $CaCl_2$ 2.4, KH_2PO_4 1.2, $MgSO_4$ 1.2, Glucose 9.9, $NaHCO_3$ 25, K^+EDTA 0.03 at 37°C. The chamber was bubbled continuously with 95% O2~5% CO2. The aortic rings were stretched over 90 min to passive tensions of 1.5 g, which were determined to be optimal tension for contractile responses in this preparation.

Experimental protocol

Aortic rings were contracted with 125 mM KCl to determine the maximal vasoconstrictor response. The organ chamber was then washed out 4 times over 60 min with KHB and the aortic rings were allowed to relax to baseline tension before continuing the protocol. The aortic rings were incubated with 500 μM SNAP for 1 hr and then washed out every 15 min over 3 hrs with KHB. A concentration-response curve to phenylephrine was performed by increasing the concentration of phenylephrine. To determined whether induction of HO-1 modulated vasoconstrictor responses to phenylephrine, aortic rings were pretreated with ZnPP-IX (100 μM), L-NMMA (100 μM) or methylene blue (10 μM) for 30 min before beginning the phenylephrine concentration response. Room lights were extinguished during any experiments utilizing ZnPP-IX on account of the light sensitivity of porphyrins.

Statistics

Results are expressed as a percentage of 125 mM KCl-induced contraction. Each data are expressed as the mean $\pm$ S.E.M. of experiments and were analyzed using Student's *t*-test. Differences were considered significant when $p < 0.05$.

RESULT

Phenylephrine (3 nM~3000 nM) produced a concentration-dependent increase in contractility in untreated rings. Preincubation with SNAP for 1 hr significantly reduced the contractile response to phenylephrine (Fig. 1). To determine whether the vasodepressor effect of SNAP treatment on the phenylephrine concentration response was due to induction of HO, aortic rings were pretreated with the HO inhibitor ZnPP-IX (100 μM) 30 min before obtaining concentration responses to phenylephrine. ZnPP-IX alone had no effect on the contractile response to phenylephrine (data not shown), however, ZnPP-IX abolished the vasodepressor effect of SNAP, suggesting that induction of HO was responsible for the attenuation of contractile response in SNAP-treated aorta (Fig. 2).

L-NMMA (100 μM) significantly increased the con-

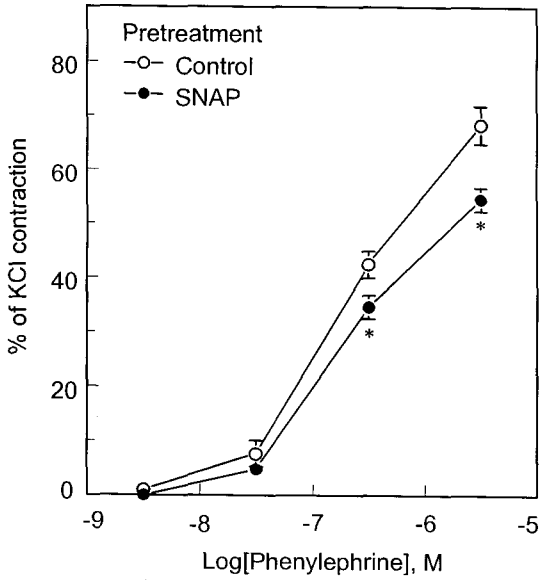

Fig. 1. Effects of preincubation with 500 μM SNAP for 1 hour on the aortic contractile response to phenylephrine. All data are expressed as a percentage of the initial 125 mM KCl-induced contraction. Each value represents the mean±S.E.M. of 9~10 experiments. *P<0.05

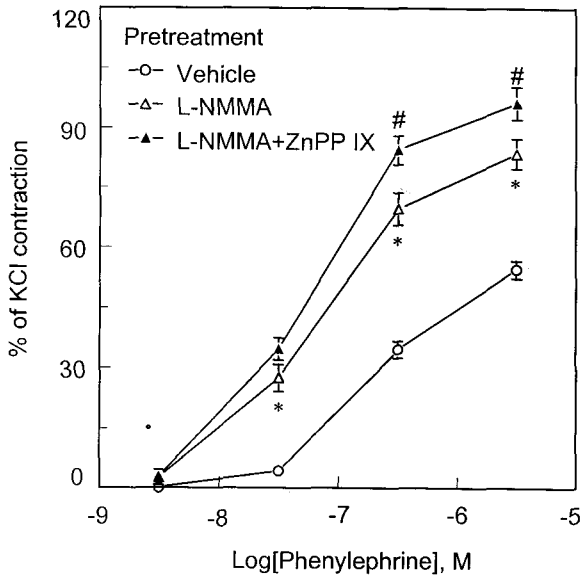

Fig. 3. Effects of preincubation with 500 μM SNAP for 1 hour on the aortic contractile response to phenylephrine in the presence of L-NMMA (100 μM) or ZnPP-IX (100 μM). All data are expressed as a percentage of the initial 125 mM KCl-induced contraction. Each value represents the mean±S.E.M. of 9~10 experiments. *P<0.05 compared with vehicle group, #P<0.05 compared with L-NMMA alone group.

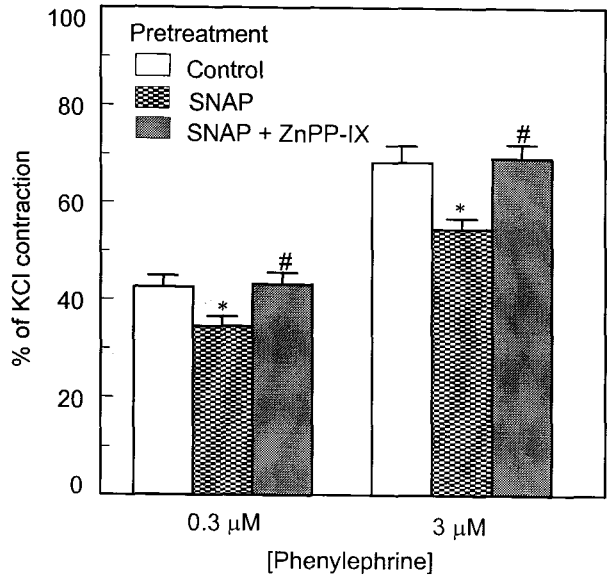

Fig. 2. Effects of preincubation with 500 μM SNAP for 1 hour on the aortic contractile response to phenylephrine in the presence of ZnPP-IX (100 μM). All data are expressed as a percentage of the initial 125 mM KCl-induced contraction. Each value represents the mean±S.E.M. of 9~10 experiments. *P<0.05 compared with control group, #P<0.05 compared with SNAP alone group.

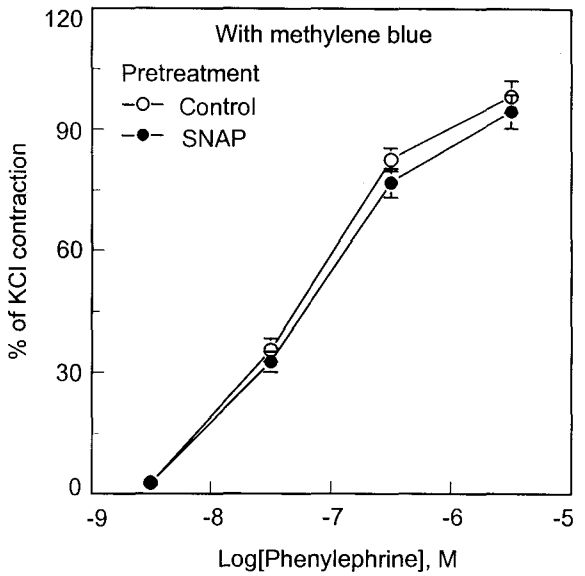

Fig. 4. Effects of preincubation with 500 μM SNAP for 1 hour on the aortic contractile response to phenylephrine with presence of methylene blue. All data are expressed as a percentage of the initial 125 mM KCl-induced contraction. Each value represents the mean±S.E.M. of 9 experiments.

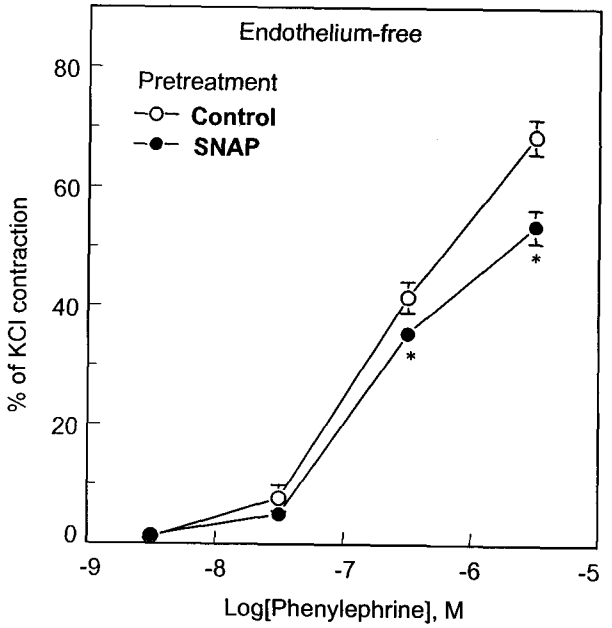

Fig. 5. Effects of preincubation with 500 μM SNAP for 1 hour on the contractile response of endothelium-free aortic ring to phenylephrine. All data are expressed as a percentage of the initial 125 mM KCl-induced contraction. Each value represents the mean±S.E.M. of 10 experiments. *P<0.05

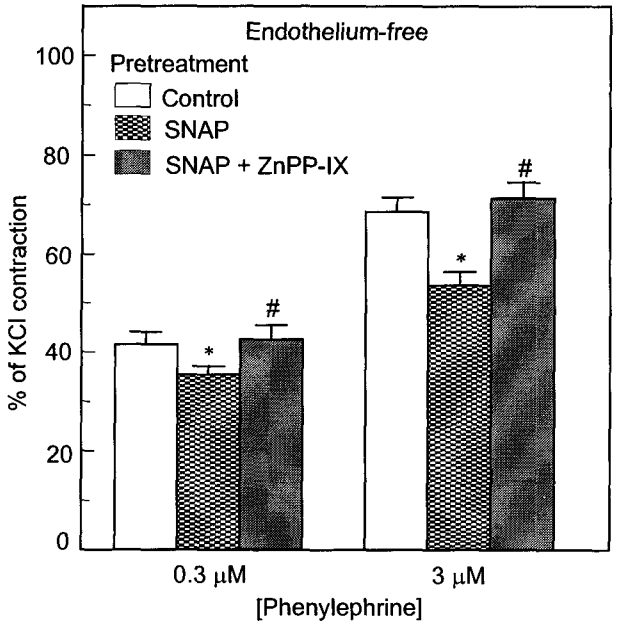

Fig. 6. Effects of preincubation with 500 μM SNAP for 1 hour on the contractile response of endothelium-free aortic ring to phenylephrine in the presence of ZnPP-IX (100 μM). All data are expressed as a percentage of the initial 125 mM KCl-induced contraction. Each value represents the mean±S.E.M. of 8~10 experiments. *P<0.05 compared with control group, #P<0.05 compared with SNAP alone group.

tractile response to phenylephrine in SNAP-pretreated aortic rings, but it didn't substantially restore the ZnPP-IX-sensitive attenuation of contractile response to phenylephrine in SNAP-pretreated aortic rings, suggesting that attenuated contractile response in SNAP-pretreated aorta is not related to NO production level (Fig. 3).

Methylene blue, an inhibitor of soluble guanylate cyclase, significantly increased the contractile response to phenylephrine in untreated rings, and significantly restored the vascular responsiveness in SNAP-treated aortic rings (Fig. 4).

HO can be induced in the endothelial cells and vascular smooth muscle. Thus, the effect of denudation of endothelium was studied. In endothelium-free aortic rings, SNAP treatment also significantly reduced the vascular reactivity to vasoconstrictor and this effect was attenuated by ZnPP-IX (Fig. 5, 6).

To determine whether the changes in intracellular signaling mechanism are involved in attenuation of contractile response in SNAP-treated aorta, effects of G protein activator AlF_4^- was studied. AlF_4^--evoked contraction in control did not differ from that in SNAP-treated group (Fig. 7).

DISCUSSION

Oxidative stress results in the induction of a number of enzymes, some of which may contribute to the development of septic shock, including NO synthase, cyclooxygenase, and HO-1 (Yamanaka et al, 1993; Camhi et al, 1995; Choi & Alam, 1996; Scott et al, 1996).

It has been suggested that HO-1 is highly induced in response to LPS-elicited oxidative stress and increased levels of HO-1 may be protective in septic shock (Keyse et al, 1990; Applegate et al, 1991; Otterbein et al, 1995). However, how HO might produce this beneficial effects and whether modulation of vascular tone is involved is not known. Because systemically administered LPS induces iNOS in several different tissues including vascular tissues and the large amount of NO can induce HO-1 in vascular tissue, it can be suggested that LPS may increase in HO-1 level through the induction of iNOS. Thus, to determine whether HO induced by oxidative stress

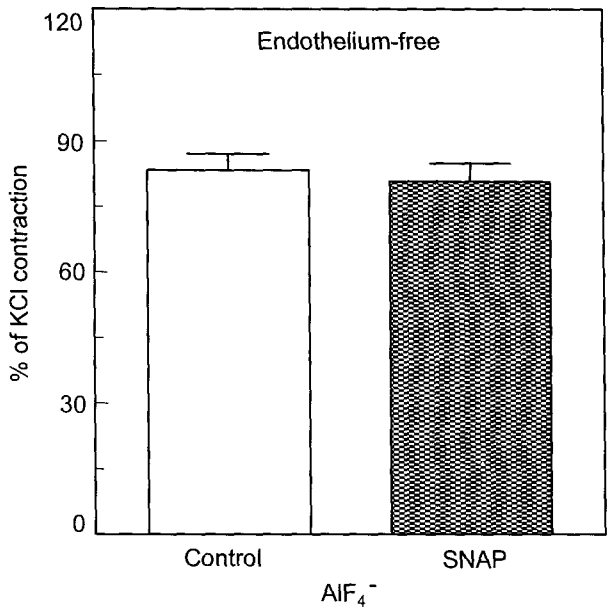

Fig. 7. Effects of G protein activator AlF_4^- on the endothelium-free aortic ring. Aortic rings of SNAP group were preincubated with 500 μM SNAP for 1 hour. All data are expressed as a percentage of the initial 125 mM KCl-induced contraction. Each value represents the mean ±S.E.M. of 7 experiments.

might influence vascular tone, we studied the effects of incubation of aortic tissue with NO donor for 1 hr in vitro on the aortic contractile response to phenylephrine.

Our results demonstrate that preincubation of aortic tissue with SNAP attenuated the contractile response to phenylephrine. HO inhibitor ZnPP-IX reversed the vasodepressor effect of SNAP on the concentration response of phenylephrine. These results suggested that SNAP induced HO and increased HO resulted in increased production of CO, which was responsible for the vasodepressor effect of SNAP since alternative products of HO-1, including biliverdin and free iron, did not account for the vasodepression (Johnson et al, 1996). CO elicits vasodilation by stimulating guanylate cyclase and perhaps by activating calcium-activated potassium channels (Furchgott & Jothianandan, 1991; Zakhary et al, 1996; Wang et al, 1997).

HO-2, a constitutive form, is detected in endothelial cells and smooth muscle cells of rat aorta. It was not clear whether endothelium is related to the vasodepressor effect of induced HO. In this study, the denudation of endothelium did not affect the vasodepressor effect of SNAP. This result means HO was induced mainly in smooth muscle of aorta.

In summary, preincubation with SNAP (NO donor) resulted in depression of the vasoconstrictor response to phenylephrine. This effect was reversed by the HO inhibitor, suggesting that increased production of CO was responsible for the vasodepression. These effects were independent of the presence of endothelium.

REFERENCES

Abraham NG, Drummond GS, Lutton JD, Kappas A. The biological significance and physiological role of heme oxygenase. *Cell Physiol Biochem* 6: 129−168, 1996

Applegate LA, Luscher P, Tyrrell RM. Induction of heme oxygenase: A general response to oxidant stress in cultured mammalian cells. *Cancer Res* 51: 974−978, 1991

Camhi SL, Lee P, Choi AM. The oxidative stress response. *New Horiz* 3: 170−182, 1995

Choi AM, Alam J. Heme oxygenase-1: Function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. *Am J Respir Cell Mol Biol* 15: 9−19, 1996

Dennery PA, McDonagh AF, Spitz DR, Rodgers PA, Stevenson DK. Hyperbilirubinemia results in reduced oxidative injury in neonatal Gunn rats exposed to hypoxia. *Free Radic Biol Med* 19: 395−404, 1995

Ewing JF, Raju VS, Maines MD. Induction of heart heme oxygenase-1 (HSP32) by hyperthermia: possible role in stress-mediated elevation of cyclic 3':5'-guanosine monophosphate. *J Pharmacol Exp Ther* 271: 408−414, 1994

Furchgott RF, Jothianandan D. Endothelium-dependent and -independent vasodilation involving cyclic GMP: Relaxation induced by nitric oxide, carbon monoxide and light. *Blood Vessels* 28: 52−61, 1991

Johnson RA, Lavesa M, Deseyn K, Scholer MJ, Nasjletti A. Heme oxygenase substrates acutely lower blood pressure in hypertensive rats. *Am J Physiol* 271: H132−H1138, 1996

Keyse SM, Applegate LA, Tromvoukis Y, Tyrrell RM. Oxidant stress leads to transcriptional activation of the human heme oxygenase gene in cultured skin fibroblasts. *Mol Cell Biol* 10: 4967−4969, 1990

Maines MD. The heme oxygenase system: A regulator of second messenger gases. *Annu Rev Pharmacol Toxicol* 37: 517−554, 1997

McCoubrey WM, Huang TJ, Maines MD. Isolation and characterization of a cDNA from the rat brain that encodes heme protein heme oxygenase-3. *Eur J Biochem* 247: 725−732, 1997

Morita T, Perrella MA, Lee ME, Kourembanas S. Smooth

muscle cell-derived carbon monoxide is a regulator of vascular cGMP. *Proc Natl Acad Sci USA* 92: 1475–1479, 1995

Otterbein L, Sylvester SL, Choi AM. Hemoglobin provides protection against lethal endotoxemia in rats: The role of heme oxygeanse-1. *Am J Respir Cell Mol Biol* 13: 595–601, 1995

Raju VS, Maines MD. Coordinated expression and mechanism of induction of HSP32 (heme oxygenase-1) mRNA by hyperthermia in rat organs. *Biochem Biophys Acta* 1217: 273–280, 1994

Raju VS, Maines MD. Renal ischemia/reperfusion upregulates heme oxygenase-1 (HSP32) expression and increases cGMP in rat heart. *J Pharmacol Exp Ther* 277: 1814–1822, 1996

Rizzardini M, Carelli M, Cabello, Porras MR, Cantoni L. Mechanism of endotoxin-induced heme oxygenase mRNA accumulation in mouse liver: synergism by glutathione depletion and protection by N-acetylcysteine. *Biochem J* 304: 477–483, 1994

Scott JA, Machoun M, McCormack DG. Inducible nitric oxide synthase and vascular reactivity in rat thoracic aorta: Effect of aminoguanidine. *J Appl Physiol* 80: 271–277, 1996

Shibahara S, Muller R, Taguchi H, Yoshida T. Cloning and expression of cDNA for rat heme oxygenase. *Proc Natl Acad Sci USA* 82: 7865–7869, 1985

Stocker R, Glazer AN, Ames BN. Antioxidant activity of albumin-bound bilirubin. *Proc Natl Acad Sci USA* 84: 5918–5922, 1987

Stone JR, Marletta MA. Soluble guanylate cyclase from bovine lung: Activation with nitric oxide and carbon monoxide and spectral characterization of the ferrous and ferric states. *Biochemistry* 33: 5636–5640, 1994

Trakshel GM, Kutty RK, Maines MD. Purification and characterization of the major constitutive form of testicular heme oxygenase: The non-inducible isoform. *J Biol Chem* 261: 11131–11137, 1986

Verma A, Hirsch DJ, Glatt CE, Rommett GV, Snyder SH. Carbon monoxide: a putative neural messenger. *Science* 259: 381–384, 1993

Wang R, Wu L, Wang Z. The direct effect of carbon monoxide on Kca channels in vascular smooth muscle cells. *Pflügers Arch* 34: 285–291, 1997

Werkstrom V, Ny L, Persson K, Andersson KE. Carbon monoxide-induced relaxation and distribution of haem oxygenase isozymes in the pig urethra and lower oesophagogastric junction. *Br J Pharmacol* 120: 312–318, 1997

Yamanaka S, Iwao H, Yukimura T, Kim S, Miura K. Effect of the platelet-activating factor antagonist, TCV-309, and the cyclo-oxygenase inhibitor, ibuprofen, on the haemodynamic changes in canine experimental endotoxic shock. *Br J Pharmacol* 110: 1501–1507, 1993

Zakhary R, Gaine SP, Dinerman JL, Ruat M, Flavahan NA, Snyder SH. Heme oxygenase 2: endothelial and neuronal localization and role in endothelium-dependent relaxation. *Proc Natl Acad Sci USA* 93: 795–798, 1996