Nitric Oxide and Carbon Monoxide, Collaborative and Competitive Regulators of Hypertension

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Blood pressure is one of the vital parameters of the body that is normally maintained in homeostasis by a complex multifactorial mechanism mediating constriction or dilation of vessels. Hypertension ensues when the responses to vasorelaxant signals become inefficient or vascular tissues are injured by inflammatory insults, leading to a decrease in arterial compliance and patency. This pathologic condition is best exemplified in atherosclerosis, one of the most common diseases afflicting humans worldwide. It is now generally recognized that nitric oxide (NO) and carbon monoxide (CO), two gasotransmitters synthesized by inducible NO synthase (iNOS) and heme-oxygenase-1 (HO-1) respectively, play important roles in the compensatory regulation of the blood pressure during the development of hypertension. Nonetheless, much remains elusive regarding how these two stress systems interact with each other. Knowledge about their crosstalk will prove essential in the better understanding of the mechanisms underlying the disease process as well as in the design of potential therapeutic strategies. In this review, we provide an overview of the functions of NO and CO related to cardiovascular health. By dissecting the current findings in the literature, we discuss possible theories about the dynamics and interplay of their actions. *(Chang Gung Med J 2009;32:12-21)*

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Key words: nitric oxide (NO), carbon monoxide (CO), hypertension

Hypertension is characterized by increased vascular contractility, a concomitant increase in oxidative stress, enhanced vascular inflammation and vascular remodeling.[1] There is ample evidence showing that hypertension is associated with cardiovascular diseases, which affect more than one-forth of the adult population worldwide.[2] Its pandemic impact suggests the importance of a better understanding of the mechanisms underlying this pathological condition. Much of our current knowledge on hypertension have been generated from studies with spontaneously hypertensive rats (SHR), our closest genetic animal model of essential hypertension.[3,4] It has been generally accepted that hypertension is related to endothelial dysfunction in the peripheral, coronary and renal circulations. For instance, endothelium-dependent relaxation of isolated aortic rings from SHR are impaired when compared to

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Received: Sep. 29, 2007; Accepted: May 9, 2008
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those from normotensive Wistar-Kyoto (WKY) rats.[5] Under normal conditions, the endothelium protects the structural integrity of the vascular wall as well as promotes vasoconstriction or vasodilation in response to various stimuli. Furthermore, it is involved in inflammatory, thromolytic and coagulant processes.[6] Two important mediators of the many endothelial functions are nitric oxide (NO) and carbon monoxide (CO).[7] Indeed, it has become increasingly clear that impaired regulation of the systems synthesizing these two gasotransmittors upon induction by pathologic stress, namely inducible nitric oxide synthase (iNOS) and heme oxygenase-1 (HO-1), constitutes one of the pathogenic mechanisms of hypertension. With growing interest and advancing research endeavors, many excellent reviews on iNOS/NO[8,9] and HO-1/CO[10,11] have become available in recent years. Yet, because these two fields have evolved independently of each other, studies generally have investigated the role of one pathway or the other in the control of biological activities, with little emphasis on the possible interactions between these two closely related systems.[12] Hence, the present review will focus on the interdependence of NO and CO function during the development and progression of hypertension.

Overview of NO in hypertension

Generated from a two-step oxidation of L-arginine to L-citrulline,[13] NO is known to be an important autocrine and paracrine signaling molecule in the regulation of various cell functions, including modulation of vasomotor tone[14] and cell adhesion to the endothelium,[15] as well as inhibition of platelet aggregation[16] and vascular smooth muscle cell proliferation.[17] It is synthesized by three distinct isoforms of the nitric oxide synthases (NOS), which differ both in their structure and function.[18] Endothelial NOS (eNOS or NOS III) and neuronal NOS (nNOS or NOS I) are Ca^{2+} -dependent and constitutively expressed. In contrast, under normal physiological conditions, the expression of inducible NOS (iNOS or NOS II) is minimal or absent.[19] However, this latter can be induced, independent of the Ca^{2+} concentrations, to very high levels by cytokines or other proinflammatory agents during infection in most types of vascular cells, including endothelial cells,[20] cardiac myocytes,[21] hepatocytes[22] and macrophages.[23] NO is pleiotropic in nature: on one hand, constitutive production of NO is critical for its cytoprotective action on the cardiovascular system, as exemplified in the case of defective eNOS.[24] On the other hand, excessive or inappropriate NO output by iNOS upon pathologic induction can be as deleterious as insufficient NO because of its cytotoxic effects.[25] This harmful aspect of the enzyme is at least partly contributed by a simultaneous production of superoxide anions by iNOS that can scavenge NO to form peroxynitrite. This uncoupled reaction is favored in the presence of low concentrations of substrate L-arginine or tetrahydrobiopterin (BH_4), a key cofactor of NOS deficient in SHR yet required for the enzyme to dimerize and to produce NO instead of superoxide.[26] Peroxynitrite is a potently damaging oxidant that could create a considerable amount of oxidative stress and injury to the vascular bed. Furthermore, it mediates reactions such as protein nitration, DNA single-strand breakage and guanidine nitration that are both cytotoxic and mutagenic.[27] Therefore, it is believed that overproduction of superoxide in SHR may lead to the development of hypertension through decreased availability of NO and chronic damage to the cardiovascular system.[28] Indeed, superoxide anion levels in SHR increase in an age-dependent manner in concordance with the development of elevated blood pressure.[29] Evidence for the damaging role played by superoxide is conversely provided by the protective effect of superoxide dismutase delivered to a rat model of angiotensin II-induced hypertension.[30] Consistent with these findings, our laboratory has demonstrated that exogenous BH_4 significantly improved acetylcholine-induced relaxation, suppressed iNOS expression and reduced NO, peroxynitrite and superoxide formation, altogether attenuating the progression of hypertension.[31] Therefore, we proposed that the relative deficiency of BH_4 may be responsible, at least in part, for the pathology in SHR.[31] While it can be concluded that iNOS uncoupling contributes to increased oxidative stress, iNOS might also decrease intracellular BH_4 and L-arginine availability to eNOS, thereby leading to an impairment in eNOS-derived NO production.[32] In this regard, we postulated that the decline of eNOS activity and/or expression may contribute to the development of hypertension, whereas the increase of iNOS expression is a consequence of the pathological state associated with the vascular insult,[33] as observed in SHR models.[34]

The relative contributions of iNOS uncoupling versus iNOS-dependent eNOS uncoupling merit further investigation. All in all, when the pro-oxidant nature of NO takes over its vasodilator function, iNOS may be considered as a detrimental player in the disease pathogenesis. Indeed, it has been shown that iNOS deficiency protects the heart from ventricular hypertrophy and congestive heart failure resulting from systolic overload.[35] Similarly, studies have shown that iNOS knockout mildly reduced infarct-induced mortality, improved ventricular function, and lowered myocardial nitrotyrosine and plasma nitrate content, as well as decreased programmed cell death during both the acute and chronic phases of myocardial infarction.[36-38]

Overview of CO in hypertension

The major cellular source of CO is heme oxygenase (HO), a ubiquitously expressed protein that catalyzes the oxidative degradation of heme to biliverdin, CO and iron, with biliverdin subsequently converted to bilirubin by biliverdin reductase.[39] HO exists in 2 major isoforms, HO-1 and HO-2, which are products of different genes. McCoubrey *et al*. also cloned a putative third HO isozyme sharing a ~90% amino acid sequence homology to HO-2.[40] While HO-2 and HO-3 are constitutively expressed and produce most of the endogenous CO under normal conditions, the expression level of HO-1, a.k.a. the stress protein HSP32, often falls below the detectable level on reverse transcriptase-polymerase chain reaction (RT-PCR) or Western blot.[41,42] However, like iNOS, HO-1 is highly inducible by a vast array of stimuli, including oxidative stress, heat shock, ultraviolet radiation, ischemia-reperfusion (I/R), heavy metals, lipopolysaccharide, cytokines, and NO and its substrate heme.[43] It is also found highly expressed in the endothelium and foam cells of atherosclerotic lesions in both humans and animals.[44] Biliverdin and bilirubin, two products of HO-1, have been long recognized as potent antioxidants.[45] They can efficiently scavenge peroxy radicals and inhibit lipid peroxidation.[46,47] Hence, HO-1 has emerged as an important mediator of antioxidant and tissue-protective actions.[48] Consistent with this premise, it has been shown that hearts from HO-1 knockout mice have greater susceptibility to I/R injury.[49] Conversely, cardiac-specific overexpression of HO-1 leads to attenuated myocardial injury after I/R in transgenic mice.[50] Observations from these animal studies were convincingly supported by the first human case of HO-1 deficiency, which displayed early atherosclerotic changes in the vasculature as reflected by the presence of fatty streaks and fibrous plaque.[51,52] Notably, an upregulated HO-1 system not only increases the production of biliverdin and bilirubin, but also normalizes the endogenous CO concentration. CO was originally considered a toxic metabolic waste product. The cytoprotective function of CO was unveiled in 1984 when McGrath and Smith demonstrated the relaxation of rat coronary artery in response to exogenous CO.[53] Subsequently, different research groups have provided evidence of the ability of CO to relax vascular tone in the heart similar to that of NO. This discovery is of significance because the HO-1/CO system is believed to constitute a novel cardiac defense mechanism[54,55] protecting cells and tissues when they are exposed to different stress stimuli. For instance, CO perfusion and pretreatment with hemin to promote HO activity were found to suppress in a concentration-dependent manner the phenylephrine-induced vasoconstriction in rat tail artery; upon withdrawal of CO, the vascular contractility was recovered.[56] The vasorelaxant effect of endogenous CO was concomitantly revealed in one study when HO activity was inhibited with zinc protoporphyrin-IX, which increased the perfusion pressure in isolated rat liver.[57] In another study, inhibition of HO decreased the diameter in resistance vessels.[58]

Although the precise physiological role of HO-1 in hypertension remains to be further elucidated, the suggested vasoprotective actions of HO-1 are likely conferred by its anti-inflammatory and antioxidant properties that protect the cardiovascular tissues against both primary and secondary damage inflicted on cells, as well as by its vasodilator abilities that can prevent the progress of abnormal vascular contractility and vascular remodeling. Hence, abnormal functions of HO-1 have been linked to the pathogenesis and maintenance of hypertension.[59] This concept helps to explain the reduced expression of HO-1 detected in the aorta and pulmonary, mesenteric and tail arteries of young SHRs in comparison with that in normotensive WKY rats of all ages. On the other hand, overexpression of HO-1 or administration of CO reversed the blood pressure development in young SHRs and other animal models of hyperten-

sion.[60-63]

Crosstalk between iNOS and HO-1 in hypertension

The physiological effects of NO and CO should be considered in an integrated environment. The potential interactions between them are of special interest in this regard because they have similar cardiovascular functions sharing control of vascular contractility and both are generated in the vascular wall.[64] Most importantly, for both the iNOS/NO and HO-1/CO systems, soluble guanylate cyclase (sGC) is the common transduction mediator that dictates the downstream signaling cascade in many cell types.[65-68] The activation of sGC results in a transient increase in cyclic guanosine 3, 5-monophosphate (cGMP). One of the downstream targets of cGMP is the cGMP-dependent protein kinase which, by phosphorylating regulators for calcium metabolism and transport, promotes a decrease in intracellular calcium levels.[69-71] A drop in the intracellular calcium concentration generally leads to relaxation in vascular smooth muscle cells.[72] Hence, dysfunction of this sGC/cGMP pathway has been reported to lead to hypertension.[73] For example, Kloss *et al*. have demonstrated that both the function and expression of sGC were significantly decreased in the aortas of prehypertensive and old SHR when compared with age-matched WKY rats.[74]

While it is possible that both iNOS and HO-1 independently contribute to the modulation of vascular functions via the respective NO- and CO-mediated sGC activation, the presence of another vasoactive signaling factor likely influences the vascular effects of each system. In fact, the modes of action of these two systems and their regulations strongly support their interdependence that is subject to change at different developmental stages of hypertension. For example, Kajimura *et al*. have shown that CO becomes a stimulatory modulator of sGC when the tissue level of NO is low.[75] It was documented that the effect of CO on cGMP production might be ascribed, at least in part, to the displacement and release of NO from its intracellular storage pool(s).[76,77] Interestingly, we have also provided evidence from our time-course study that the expression of HO-1 appears to occur earlier than that of iNOS.[78] Thus, while the up-regulation of HO-1 and iNOS could serve, as distinct entities, to oppose the elevation of blood pressure during the development of hypertension in SHR,[78] HO-1 may act by potentiating the activity of the iNOS/NO system, which is a much more efficient activator of sGC.[79] Intriguingly, the same author also reported that CO could be a negative modulator inhibiting sGC activity when the tissue NO level is high.[75,80] Maines has proposed a few other possible forms of negative regulation of NO production by HO-1, reflecting the hemoprotein nature of NOS.[81] These include limited availability of heme for NOS production,[81,82] an accelerated turnover rate of NOS presented as an HO-1 substrate of the P450 type,[81,83] and direct binding of CO to the heme moiety of NOS leading to its inactivation.[81,84] The mechanistic nature and the advantages of such differential regulations mediated by HO-1 are unknown. By controlling the NOS production, Maines postulated that the HO-1 system would modulate the negative feedback regulation that the synthase activity product exerts on its own production.[81,85] As described above, NO could be both hemodynamically beneficial and cytotoxic, depending on the rate of NO production and the chemical fate of the NO produced.[86] Because iNOS is a significant source of oxidative stress, its negative roles as a generator of maladaptative responses have been suggested. In contrast, unlike the highly reactive NO, which by itself is a free radical, CO is chemically stable. Therefore, it is also tempting to postulate that the inactivation of iNOS could represent a natural compensatory mechanism of the HO-1 system working in concert with iNOS in response to hypertension. If this was true, it would seem that the endpoints of this feedback loop would be decreased NO transformation to reduce oxidative stress and increased CO production to perform NO-equivalent signaling functions. Some interesting data suggestively support this premise. Huang *et al*. have found in a hippocampus model that expression of iNOS in 23-week-old SHR was about fourfold lower than that in age-matched control rats and 4-week SHR rats while HO-1 levels remained elevated.[87] Our laboratory has also provided data suggesting that the HO-1/CO system takes over and acts as a major modulator for the maintenance and restoration of blood pressure when the iNOS/NO system is suppressed during the development of hypertension.[78] In the same line of thought, Sammut *et al*. demonstrated that CO is a major contributor to the regulation of vascular tone

in aortas expressing high levels of HO-1.[88] In certain circumstances, however, these very same complementary actions that promote a reduction in blood pressure could be counterbalanced by the ability of CO to inhibit both the synthesis and vascular response to NO. Thus, the relative importance and role that CO plays in modulating the vascular tone with respect to NO will likely vary depending on the underlying physiological state and the amount of CO being generated.[89]

Conversely, diverse NO releasing agents were found to possess the ability to modulate HO-1 protein expression and activity. For example, NO has been reported to avidly induce HO-1 expression and CO production in different cell types.[90-95] It was suggested that mitogen-activated protein kinases (MAPK) ERK and p38 pathways are underlying mechanisms by which NO regulates HO-1 gene expression.[96-98] That NO is the initial element in the cascade of events leading to HO-1 up-regulation was ascertained by the use of hydroxocobalamin, an NO scavenger that considerably decreased HO-1 activation by NO donors.[91,99] Consistent with this finding, it has been shown that the dilation of pial arterioles in piglets by CO could be blocked by *N*-nitro-L-arginine, an inhibitor of NO production; sodium nitroprusside, an NO donor, reversed this tempered vasodilation.[100] It was thus speculated that NO is in fact a permissive factor for the vascular effect of CO.[100,101] Of note, peroxynitrite itself produces a concentration-dependent increase in HO-1 protein expression.[102] The regulation of HO-1 gene expression by NO may represent an elegant example of preconditioning wherein exposure of tissues to oxidative stress results in an upregulation of endogenous defensive proteins that confer resistance to the subsequent insults. Our recent studies on alpha-lipoic acid, a natural antioxidant reported to protect against oxidative injury in various disease processes, gave an example of how HO-1 expression could be induced through the pro-oxidative production of reactive species followed by subsequent activation of the p44/42 MAPK pathway in vascular smooth muscle cells.[103] Alternatively, given the highly reactive and short-lived nature of NO in comparison with the structural stabilities in CO, it was also postulated that, in addition to regulating biological processes through its rapid pharmacological action, NO exerts delayed and long-lasting effects via induction of the HO-1/CO/bilirubin pathway.[104]

Conclusions

Although further investigation is required to clarify the precise action of these two gaseous molecules, we believe that NO and CO function interdependently, each dynamically influencing the other to regulate the signal transduction related to vasodilation processes. In certain physiological or pathophysiological situations, it is possible that iNOS and HO-1 co-operate to maintain cellular homeostasis upon exposure to oxidative stress. Under other conditions, however, one enzymatic pathway may counter-regulate, compensate or prevail over the other. The interplay and crosstalk between CO and NO, being synergistic or antagonistic, provides an integrated mechanism for the fine-tuning of their vasodilator functions during the development of hypertension.[105] Despite the lack of a consensus regarding how the two stress systems influence each other, it seems reasonable to generalize, based on the current findings on NO and CO, that iNOS is the destructive player contributing to oxidative stress while HO-1 is the defensive player mounting against it. Given this, methods that inhibit iNOS[106] or upregulate HO-1[107] may become invaluable antihypertensive measures. Of course, the metabolism of NO and CO is more complex than it appears and both signaling molecules could, at times, evoke an opposing set of actions in the regulation of blood pressure depending on specific temporal and spatial contexts. With many of these mysteries being unsolved and still a matter of debate, only a clear understanding of the mutual relationship between the two systems and their intimately linked regulation would allow the development of targeted therapeutic strategies to prevent or treat vascular dysfunction.

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一氧化氮、一氧化碳──兩者在血壓調控之角色

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血壓爲人體生命重要指標之一，它透過複雜機轉來調控血管收縮與舒張，以維持血壓之恆定。當血管對血管舒張劑反應訊號不良或血管組織受發炎損害時，高血壓便接踵而來，以動脈粥狀樣化所導致高血壓爲例，這種疾病爲困擾人類一種慢性疾病。新近研究顯示一氧化氮 (NO) 與一氧化碳 (CO)，分別爲 iNOS 與 HO-1 酶合成，爲高血壓發展過程扮演重要血壓恆定調控角色。然而有關這兩種系統之間在人體遭受壓力改變下如何互相作用則仍不清楚，有待進一步探討。然而對此兩系統間交互作用之相關新知將提供有關高血壓疾病發展過程重要機轉之瞭解以便將來作爲設計新治療藥物之重要參考策略。本篇回顧性文章，將提供有關 NO 與 CO 功能及其對心血管生理與病理相互關係，並參考最新文獻發現作爲詳細研究之剖析。因此，我們將以本實驗室過去研究成果及最新文獻發現兩系統間之動力平衡與彼此相互作用之可能理論基礎作詳細討論以供讀者參考。(長庚醫誌 2009;32:12-21)

關鍵詞：一氧化氮，一氧化碳，高血壓

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受文日期：民國96年9月28日；接受刊載：民國97年5月9日
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