**Review**

# Nitric Oxide in the Cardiovascular and Pulmonary Circulation – A Brief Review of Literatures and Historical Landmarks

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## Abstract

**Nitric oxide (NO) is an important gas molecule that plays a pivotal role in physiology and pathology in various systems. Our laboratory has been working on the hypertensive cardiovascular disorders and pulmonary edema for more than 30 years. In this brief review article, we have described the role of NO in hypertension, pulmonary disorders, sepsis, and to some extent, the endothelial factors on the arterial baroreceptors and cerebral blood flow. Our studies indicate that the vasodilatory effects of endogenous NO act primarily on the small resistance vessels. The large conduit vessels are less affected. In contrast to the earlier work suggesting that NO or endothelial function is impaired in hypertension, we have provided evidence to indicate that the NO release or function is enhanced in rats with hypertension. Chronic NO deprivation in rats with spontaneous hypertension facilitates the progression of hypertension to malignant phase with marked functional and structural changes in blood vessels of various organs. In most studies using isolated perfused lungs, our results show that NO exerts toxic effect on the lung injury following ischemia/reperfusion, air embolism, endotoxemia and hypoxia. Recent clinical investigations have revealed that the inducible NO synthase (iNOS) expression was increased in patients with enterovirus and other infections, suggesting a detrimental role of iNOS and NO in the acute lung injury. In this review article, we have also provided the experiences, results and stories in our laboratory during a relatively long period investigating the good and bad sides of NO on the cardiopulmonary functions. The purposes are two-fold: first, to share the experience and stories for scientific and educational purposes; and second, to encourage young investigators to continue work on many questions yet unanswered.**

**Key Words: nitric oxide, hypertension, ventricular hypertrophy, vascular changes, pulmonary edema, endothelial factors**

## Introduction

Nitric oxide (NO), a simple gas molecule found in smoke, smog, and other waste gases, is an environmental pollutant, destroyer of ozone, precursor of acid rain, and suspected carcinogen. Furchgott and

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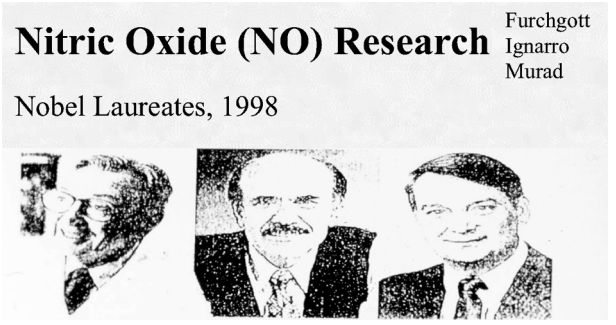


Fig. 1. Profs. Furchgott RF, Ignarro LJ, and Murad F received the honor of Nobel Prize in 1998 because their contribution to the research on nitric oxide (NO).

Fig. 2. Prof. Furchgott paid a short visit to Taiwan in 1984. We took the photograph with Prof. Yin, The Dean of National Defense Medical Center, and Prof. Lin.

Zawadzski discovered the endothelium-derived relaxing factor (EDRF) in 1980 (36), using endothelium denudation, sandwich, and cascade bioassay methods to confirm that substances released from the endothelial cell account for the vasorelaxation to acetylcholine (ACh). In fact, the discovery of endothelium derived vasoactive factors or substances such as protanoids (eicosanoids) including prostaglandins, thromboxanes and leukotrienes extend from 1930 to 1983. Many important studies have contributed to the biomedical sciences and created a number of Nobel laureates. EDRF was later identified as NO by Ignarro *et al*., Moncada and Higgs, Palmer *et al*. in 1987 and 1988 (55, 75, 87). Extensive studies on the physiological, patho-physiological and pharmacological aspects of NO have revealed that this gas molecule plays a key role in various organ systems (80). In 1992, an issue of *Science* elected NO as the molecule of the year (23). Because of the contribution to the investigations of NO (28-36, 46-56, 83-86, 90, 92), Furchgott, Ignarro and Murad earned the Nobel Prize in 1998 (Fig. 1). Although the research team of Moncada has been recognized to have great contribution to the research on NO function (24-27, 69, 71-81, 87), he has never received Nobel consideration. The reason remains unknown.

Nitroglycerin and nitroprusside are two nitro-vasodilators. Before and at the time of discoveries of EDRF and NO, our laboratory studied the effect of nitrovasodilators and other vasodilatory agents on the blood flow, blood volume distribution, vascular resistance, exchange and capacitance functions and systemic hydraulic vascular load in animals and patients (3, 7-9, 11, 15, 20, 21, 68, 97). While we did not realize that the vascular action of nitro-vasodilators was mediated through the release of NO, we continued our work on the involvement of NO in hypertension and lung injury. In this review article, we summarize the experimental results obtained in our laboratory and compare them to those from other laboratories. We also present some historical records with respect to the stories that involved in the NO studies in Taiwan.

*Some Historical Landmarks*

Prof. C. I. Lin was the classmate of one of the authors, H. I. Chen, in the National Defense Medical Center (NDMC). Assigned to New York University at Brooklyn for his Ph.D. degree in electrophysiology of the heart, Professor Lin studied under Professor Vassalle. During the laboratory rotation, he had an opportunity to learn the technique of vessel segment preparation in Prof. Furchgott's laboratory, but he did not pay much attention to the significance of EDRF.

After Taiwan began to enjoy economic growth in the 1980s, the National Science Council (NSC) developed a program to invite international scholars for short or long visits to Taiwan. When H. I. Chen was appointed the Director of the Medical Research and Clinical Research Center, National Defense Medical Center and Triservice General Hospital (TGH), Professor Lin served as Chairman in Pharmacology. They had the opportunity to invite Professor Furchgott to come to Taiwan (Fig. 2). His lectures on the discovery of EDRF were stimulating. He is a scientist with humble and gentle personality. He declared his finding of EDRF as accidental; yet, the clever designs that demonstrated how the relaxing substance was released from the endothelial cells in response to ACh by techniques such as endothelial denudation, sandwich preparation, and cascade bioassay clearly revealed the study's significance (Fig. 3). Later on, Prof. Murad visited Taiwan. He and his wife, Carol, took a short trip to Hualien and gave a special lecture in the Tzu Chi University (Fig. 4). Some months later, Prof. Ignarro also paid a short visit to Taiwan, and gave a

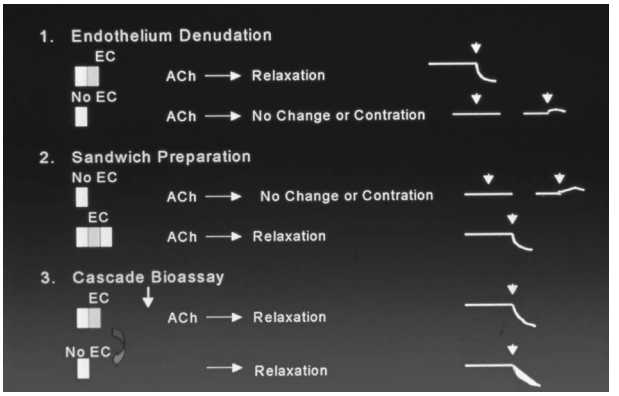


Fig. 3. Prof. Furchgott gave a lecture on the discovery of endothelium-derived relaxing factor (EDRF) by techniques with endothelium denudation, sandwich preparation and cascade bioassay (redrawn by Chen H. I.).

Fig. 5. After a humorous lecture in National Cheng Kung University in Tainan, Prof. Ignarro gave some words before the dinner party.

Fig. 4. Prof. Murad gave a lecture at Tzu Chi University on the finding of cyclic guanosine monophosphate (cGMP) and its biological functions.

humorous lecture in National Cheng Kung University in Tainan (Fig. 5). The visiting and lectures by these scientists have stimulated the research on NO in Taiwan.

*NO in Arterial Hemodynamics and Hypertension*

In 1997, our laboratory studied the acute effects of NO blockade with $N^{\omega}$-intro-L-arginine monomethyl ester (L-NAME) on the arterial hemodynamics of steady and pulsatile components (13, 14, 45). Acute blockade of the endogenous NO increased the arterial pressure and total peripheral resistance with a decrease in heart rate, but only slightly influenced the pulsatile hemodynamics such as characteristic impedance and pulse wave reflection. Aminoguanidine, a relatively specific inducible NO synthase (iNOS) inhibitor essentially did not exert significant effect on the steady and pulsatile hemodynamic parameters, suggesting that blockade of constitutive NOS (cNOS), but not iNOS was involved in these changes. The complete assessment of arterial hemodynamics requires methods using Fourier transformation or frequency analysis. It happened that several students (K. C. Chang and C. T. Hu) from the Department of Engineering, National Taiwan University joined the laboratory work and introduced the mathematic methodology for the complete hemodynamic studies (7, 8). We used L-NAME to elucidate the role of endogenous NO in arterial hemodynamic changes in normotensive and spontaneously hypertensive rats (SHR). Our research showed that L-NAME altered the steady hemodynamics, but not the pulsatile parameters in both normotensive Wistar Kyoto rats (WKY) and SHR (45). In contrast to the earlier work indicating that EDRF or NO was impaired in hypertension, we found higher elevations of arterial pressure and total peripheral resistance following L-NAME in SHR than in WKY, which suggests that NO release or function is enhanced, rather than impaired in rats with hypertension (13, 45). Since 1992, chronic NO blockade to elevate the arterial pressure has become a new model of hypertension (1, 89). One medical student, H. R. Chang, conducted his animal experiments during the winter and summer vacation. He found that early deprivation of NO in SHR at an age of 5 wks (prehypertensive stage) facilitated the hypertension to malignant phase within 4 wks. The magnitude of increase in arterial pressure was much greater in SHR than that in WKY and inhibition of iNOS with aminoguanidine did not affect the changes of arterial pressure in SHR and WKY. Accordingly, the enhanced NO release or function through the cNOS, not the iNOS in SHR is a compensatory mechanism to keep the blood pressure at a lower level in SHR. Chang also observed when the blood pressure level of SHR reached the malignant phase, the rat developed signs of stroke (5). He and other coworkers continuously extended the experiment using an isolated and perfused mesenteric vascular

bed in rats between 12 and 15 wks of age with established hypertension and normotensive WKY (4). ACh and NO donors (sodium nitroprusside or S-nitroso-N-acetyl penicillamine) produced dose-dependent vasorelaxation in WKY and SHR, while the magnitude of endothelium-dependent or -independent vasodilation was greater in SHR than that in WKY. In addition, SHR demonstrated higher vasoconstrictory responses to norepinephrine or phenylephrine than WKY. The results further support that endothelium-dependent or -independent vasoconstriction and vasodilation is enhanced in SHR compared with normotensive WKY.

Recently, Hsieh *et al*. (37, 38) employed chronic NO deprivation with L-NAME in 5-week-old WKY and SHR. He and coworkers used various histopathological and immunohistochemical techniques to observe the structural changes in cerebral arteries. The studies revealed that early NO deprivation accelerated the hypertension. Chronic treatment with L-NAME decreased the body weight, but increased heart weight in SHR. Immunohistochemical stain with antibody against macrophage/monocyte (ED1) demonstrated severe perivascular inflammation, and Periodic acid-Schiff (PAS) staining revealed arteriolar hyalinosis and increased arteriolar injury score. The perivascular inflammatory changes and hyalinosis were observed at only 2 wks following L-NAME, intensifying with continuous administration. The lumen diameter and medial cross-sectional area in SHR were smaller than those in WKY, and further reduced in SHR and WKY following NO deprivation. An analysis of the vascular remodeling indicates eutrophic vascular smooth muscle cells (VSMCs) with hypotrophic medial changes in untreated SHR, but not in untreated WKY. Hypertrophic VSMCs and eutrophic medial changes developed in SHR following L-NAME treatment. Hypertrophic VSMCs and hypotrophic medial changes occurred in all L-NAME treated rats, but not in untreated WKY. The vascular remodeling is significant in reducing the intravascular tension. In the kidney, NO deprivation resulted in severe glomerular sclerosis, arteriolar hyalinosis and impairment of renal function, revealing severe proteinuria, eye fundus, and brain lesions in SHR. However, TUNEL assay did not disclose significant apoptotic changes following L-NAME administration for 4 wks. The use of non-specific and specific NOS inhibitors suggests that the blockade of the cNOS (possibly eNOS) is the major culprit to cause these structural and functional changes (12).

Studies to examine the effects of NO on the ventricular hypertrophy (VH) continue to-date. Chang *et al*. (6) has established reductions of VH and fibrosis in SHR through chronic L-arginine administration, while Hu *et al*. (44) found that chronic NO deprivation causes severe VH with decreases in left ventricular cGMP and nitrate/nitrite and increase in collagen content. The extent of VH in terms of left ventricular weight to body weight ratio, total number, numerical density and size of cardiomyocytes were correlated well with steady hemodynamic parameters, such as aortic pressure and total peripheral resistance, as well as to pulsatile hemodynamics like arterial impedance, compliance and pulse wave reflection.


Fig. 6. A memorial photography of Prof. Robert K. S. Lim, the Founder of Chinese Physiological Society and the Father of Physiological Sciences across the strait.

*NO in Pulmonary Circulation and Lung Injury*

After graduating from the Medical School of NDMC, H. I. Chen joined the research team of Professors Robert K. S. Lim, J. T. Lu, and C. Y. Chai on studies in analgesia and antipyresis. Professor Lim (Fig. 6), who founded the Chinese Physiological Society in Beijing as well as the Chinese Journal of Physiology, returned to Taipei with his instruments, journals, and books, and established numerous laboratories in the Kohlberg Memorial Research Laboratory located in Taipei Veterans General Hospital (VGH). Most physiologists across the strait acknowledge him as the father of Physiological Sciences in China. Professor Lu served as Dean and Director of NDMC and VGH, while Professor Chai taught Physiology at NDMC, in addition to working part-time at the Kohlberg Medical Research Center.

Many physiologists and anatomists from the United States and other Medical Colleges in Taiwan came to learn techniques and research procedures from Professor Lim. They soon discovered that he

had suffered from esophageal cancer for many years, but he refused surgical and medical treatment except for sounding to ameliorate difficulty in swallowing. After six months, the group got together to discuss possible interpretations of several experimental results and Professor Lim left Taiwan the following day. He deceased a short time later, after finishing a monograph and some paper preparations; yet, his legacy lives on. During his brief stay in Taiwan, Professor Lim's spirit for scientific research opened the door for physiological and medical investigations, leaving a lasting contribution to biomedical sciences in Taiwan that continues today.

After completing graduate training in the United States, H. I. Chen was supported by the school and the hospital to set up a well-equipped laboratory, where he helped many young investigators to work on the hemodynamic mechanisms of pulmonary edema caused by various challenges and disorders, such as phorbol myristate acetate (PMA), platelets, air embolism, hypoxia, ischemia-reperfusion, endotoxemia (lipopolysaccharide, LPS) administration, infections etc. (16, 19, 22, 41, 42, 57-61, 63, 66, 93, 95). Most experimental data obtained in whole animal and isolated perfused lungs have suggested that endogenous and/or exogenous NO are detrimental to the acute lung injury (ALI). Nitric oxide synthase (NOS) inhibitors such as L-NAME, aminoguanidine, and dexamethasone effectively reduce or prevent ALI. In one study (94), we used the NO sensor for direct and real-time recording of NO release from the isolated lung, and may well be the first to discover that NO release intensifies under hypoxic ventilation to counterbalance hypoxia-induced pulmonary vasoconstriction. Furthermore, L-NAME reduces the NO release, thereby increasing hypoxic pulmonary vasoconstriction.

*NO in Sepsis and Clinical Diseases*

Septicemia or endotoxin shock is one of the major causes of death in the United States and other countries (2, 82, 88, 98). It has been known that activation of inducible NOS (iNOS) to produce large amounts of NO accounts for the systemic hypotension, hyperreactiveness to vasoconstrictors and finally multiple organ failure (70, 82, 88, 91, 98, 99). We found that administration of endotoxin (lipopolysaccharide, LPS) induced severe ALI with increases in iNOS, tumor necrosis factor $\alpha$ and interleukin-1$\beta$. The findings suggested that proinflammatory cytokines were also involved in the sepsis-induced ALI (96).

The research team used the isolated perfused lung model to reveal that the major site of NO production through the whole blood is in the lung (63). NO production, mediated by the iNOS system, is toxic to the endothelium in the pulmonary microvasculature in isolated perfused lungs (57). Red blood cells (RBC) or hemoglobin and static inflation can also attenuate the ALI following hypoxia or ischemia/reperfusion (58, 59). Recently, we advanced our studies to clinical patients (43, 60). In cases with Japanese B encephalitis, viral destruction of the depressor area in the medulla causes central sympathetic activation, whereas rupture of intracranial mycotic aneurysms results in increased intracranial pressure. The hemodynamic mechanism of ALI or acute respiratory distress syndrome (ARDS) in these two disorders may operate through the similar sequences as proposed by our previous reports (17, 18). We also reveled that the ALI in cases of lymphangitis with breast carcinoma and fat embolism, where blockade of lymphatics, capillaries, and venules in breast lymphangitis causes the development of ALI. In cases of ALI associated with fat embolism, we found that ALI cannot be solely attributed to fat embolic blockade of lymphatic drainage. Several mediators, such as cGMP, 5-hydroxytryptamine, NO and cytokines may play a contributing role.

During the summers from 2001-2003, we encountered a total of 48 children suffering from hand, foot, and mouth disease (60). Chest radiography on admission revealed clear lung; however, 21 out of 48 cases developed severe dyspnea, hyperglycemia, leukocytosis, and decreased blood oxygen tension. Arterial pressure (AP) and heart rate (HR) fluctuation ensued. Spectral analysis of the AP and HR variabilities showed elevations in sympathetic activity, AP and HR at the onset of respiratory stress. Thereafter, parasympathetic activity increased with declines in AP and HR. These children died within 4 hours after the onset of ARDS. Before death, chest radiography revealed severe lung infiltration. Similar to Japanese B encephalitis, destruction of the medullary depressor area caused initial sympathetic activation. Reverse-transcriptase chain reaction (RT-PCR) found marked iNOS expression in the lung parenchyma, suggesting iNOS may also be involved in the pathogenesis of ARDS in patients with enterovirus 71 infection. At the present time, we are preparing manuscripts in patients with rabies, leptospirosis, hypercalcemia, and scrub typhus. The Department of Pathology had several autopsy cases in which various causes led to death from ARDS. Cooperation with Dr. Y. H. Hsu, Chairman, Department of Pathology, has enabled our laboratory to extend knowledge in the clinical investigation of the pathogenesis of ARDS in a variety of disorders.

Our laboratory has recently developed an unrestrained and conscious rat model (65), which we have employed to investigate the physiological and chemical indicators for early and late stages of sepsis in conscious rats. The data suggest that the changes

in AP, HR, white blood cells, nitrate/nitrite, methyl guanidine (an index of hydroxyl radical production), and chemical substances such as blood urea nitrogen, creatinine, and lactic dehydrogenase may serve as indicators for the early stages of sepsis. Increases in creatinine phosphokinase, glutamic oxaloacetic transaminase and amylase occur during late stages of sepsis (64). Hsu *et al*. (39, 40) found that an antioxidant N-acetylcysteine and an anesthetics (propofol) could ameliorate the LPS-induced organ damage in conscious rats. In a recent work, we have revealed that inhibition of iNOS potentiates multiple organ dysfunction in conscious rats with endotoxin shock (62). The findings are somewhat controversial, because previous studies suggest that iNOS inhibition is beneficial to lung injury in anesthetized rats and isolated lungs. The discrepancy requires further studies. Continuous work conducted in the conscious rat model is now ongoing in the laboratory, and we have just published and submitted papers to show that insulin and pentobarbital attenuate the ALI induced by endotoxin in conscious rats (19, 61).

*Others Investigation with Endothelial Factors*

In 1989, H. I. Chen took a sabbatical leave in the Cardiovascular Center, University of Iowa to work with Prof. F. M. Abboud on the baroreflex control on the circulation. We found that the prostacyclin released from the endothelium cells could activate the barore-ceptor activity (10). Prostacyclin also reduced the acute resetting of baroreceptors (67) and prevented the marked increase in cerebral blood flow in response to acute hypertension (100).

*Final Remarks*

To the best of our knowledge, many papers related to NO on organ functions were published by many excellent investigators in various institutions. We regret that these studies were not fully included in this brief review. We expect that investigators in this field provide a more comprehensive review of the research work on NO in this country. The purpose of this review is to motivate young investigators to work on questions that remain to be answered.

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