REPORT OF A SCIENTIFIC MEETING

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Association of University Anesthesiologists Satellite Symposium: The Role of the Endothelium in Vascular Control. Asheville, North Carolina, May 2–4, 1993.

The satellite symposium "The Role of the Endothelium in Vascular Control" was held on May 2–4 in Asheville, North Carolina. This was the second in a new series of timely, "Gordon Conference-style" scientific sessions linked to the annual meeting of the Association of University Anesthesiologists. Registrants from more than 30 institutions gathered to hear ten interdisciplinary presentations organized in three sessions: overviews of nitric oxide, endothelin, and prostaglandins; the molecular and cellular actions of nitric oxide; and the role of nitric oxide in therapeutics. Scientific posters were on display throughout the meeting and were discussed in a separate session.

Overviews. The opening session, moderated by W. David Watkins, M.D., Ph.D. (University of Pittsburgh) featured three basic scientists renowned for their work with vascular mediators. Louis J. Ignarro, Ph.D. (University of California, Los Angeles) recounted the recent history of nitric oxide (NO), in which he has played a leading role, from its early recognition as a "vascular spasmolytic" to its identification as endothelium-derived relaxing factor. He summarized the physiologic actions of NO in low concentrations, primarily inhibition of vascular smooth muscle tone and platelet function, the effect of which is mediated by intracellular cyclic guanosine monophosphate. An extremely small and diffusible molecule, NO is synthesized from L-arginine by NO synthase, which exists in both constitutive and inducible isoforms. Constitutive NO synthase is activated by the calcium–calmodulin complex and seems to be inhibited by NO itself in a negative feedback system. In addition to its endothelial action, NO is a neurotransmitter of nonadrenergic, noncholinergic neurons, which, when stimulated, relax smooth muscle in erectile tissue as well as in intestine and lung. Thus, NO-mediated vasodilatation may arise from either endothelial or nonadrenergic, noncholinergic neuronal stimulation. Many important physiologic and pathophysiologic roles of NO *in vivo,* however, remain to be defined.

Masashi Yanigasawa, M.D., Ph.D. (University of Texas, Southwestern) described endothelin (ET)-1, -2, and -3, three isopeptides recognized for their extremely potent vasoconstrictor activity. ET-1, the best known member of the family of ETs, is up-regulated by various chemical (cytokines, agonists of phosphoinositide turnover) and mechanical (shear, stress) factors and down-regulated by atrial natriuretic peptide and NO. ETs are catalyzed from inactive intermediates termed "big endothelins" by a membrane-bound metalloprotease, endothelin-converting enzyme. Two receptor subtypes have been identified: ET_A is expressed in vascular smooth muscle cells, is selective for ET-1, and mediates ET-induced vasoconstriction, whereas ET_B is expressed in endothelial cells, is nonselective for the three ETs, and mediates ET-induced NO release, resulting in vasodilatation. Clinically, endothelin production correlates with the ratio of pulmonary to systemic vascular resistance and is elevated in the pulmonary vasculature of patients with primary pulmonary hypertension.

In constant-flow models designed to minimize the flow–resistance interaction, Philip Kadowitz, Ph.D. (Tulane University) has analyzed prostaglandin responses in the pulmonary circulation. He described the broad range of prostanoid activity, from the intense vasoconstriction of thromboxane A_2 to the vasodilatation seen with acetylcholine and prostaglandins E_1 and E_2. Prostacyclin exhibits effects like those of NO, eliciting vasodilation, bronchodilation, and potentially inhibition of platelet aggregation. Recent studies with thromboxane receptor blocking agents (*e.g.,* SQ 30741) have shown them to block thromboxane A_2–mediated responses selectively and in a competitive and reversible manner. With improved pharmacologic profiles, the newer thromboxane antagonists may prove to be useful therapeutic agents in the treatment of acute lung injury.

Molecular and Cellular Actions of Nitric Oxide. The second session, which narrowed the focus to NO, was moderated by Leonard L. Firestone, M.D. (University of Pittsburgh). Jack Lancaster, Ph.D. (University of Pittsburgh) opened the session with a general overview of the chemistry of NO, emphasizing its rare properties among biologic messenger and effector molecules, such as its paramagnetism, diffusibility, and the chemical processes that drive its interactions. The primary cellular targets of NO are iron (both heme and nonheme), responsible for most messenger and cytotoxic effector actions; oxygen species, involved in immune activation and ischemic injury; and thiols, whose role is less well understood. Recent studies with electron paramagnetic resonance spectroscopy have revealed the presence of NO in virtually all tissues after *Escherichia coli* endotoxin injection. In cultured hepatocytes, NO appears to sensitize the cell to oxidative injury. Diffusion simulations have allowed the prediction that NO acts as a predominantly paracrine rather than autocrine molecule.

Michael A. Marletta, Ph.D. (University of Michigan) detailed the enzymology underlying conversion of L-arginine to citrulline and NO by NO synthase (NOS). The constitutive class of NOS forms NO in vascular endothelial cells and in neurons, whereas inducible NOS is a different gene product that produces NO in stimulated macrophages. A current goal of investigators is to design agents that selectively inhibit these NOS isoforms. NOS seems to function as a "self-sufficient" P-450, possessing both a reductase (FAD and FMN, the bound flavins) and a heme domain (heme and 6R-tetrahydro-L-biopterin, or H_4B). NADPH is required as a cofactor, and it appears that reducing equivalents are shuttled from NADPH *via* the flavins to the heme, where arginine conversion takes place. The reaction can be inhibited by alkyl-substituted L-arginine analogs, such as N^G-methyl-L-arginine, also consistent with P-450 type catalysis.

Alex L. Loeb, Ph.D. (University of Pennsylvania) characterized the microcirculatory response to NO in various vascular beds. The overall response is determined by a balance of factors, including neuronal and endothelial influences and local tissue-specific properties, which likely account for the marked regional heterogeneity observed. Inhibition of NOS causes large increases in vascular resistance in most tissues of awake or anesthetized rats. In several regional circulations, this response is increased during isoflurane anesthesia. Recent data indicate that although hypercapnia-induced increase in cerebral blood flow is attenuated by NOS inhibitors, the hypoxic cerebral blood flow response apparently is not NO-mediated. In the renal circulation, blood flow is decreased and resistance increased by NOS inhibition; these renal effects are prevented by systemic administration of an angiotensin II antagonist, while the systemic blood pressure response is largely preserved. The NO responsiveness of microvessels is altered

in many disease states, probably by changes in endothelial cell function that may alter the expression of NOS enzymes or reduce NO synthesis.

John F. French, Ph.D. (Marion Merrell Dow, Cincinnati) addressed the potential role of NOS inhibitors in septic shock, which carries a mortality rate of 40–60% and is the most common cause of death in intensive care units in the United States. A key focus in the search for an effective treatment is NOS inhibitors, and results at the cellular level with several arginine compounds were reported. NH_2-homoarginine, the most potent NOS inhibitor, attenuates the increase in cyclic guanosine monophosphate levels induced by interleukin 1, and all compounds studied caused a concentration-dependent decrease in inhibition of nitrite production. These effects have been seen in both vascular smooth muscle and cardiac myocytes. CP-arginine, which is most potent in the central nervous system, seems to be selective for constitutive NOS. Constitutive NOS appears to play a major role in septic shock.

Poster Discussion. The poster discussion, led by Roger Johns, M.D. (University of Virginia), centered on four general areas of interest. One group of posters was concerned with the variable effects of different anesthetic agents on NO release and the potential confounding by anesthetic-induced vasodilation. Other posters addressed technical considerations in investigations of NO, in particular, the most feasible methods to detect the presence of NO in biologic systems. The topic of greatest controversy was whether or not NO has a negative feedback influence on NOS under physiologic conditions. Both pro and con positions led to the same conclusion, that the current indirect evidence for both sides of the argument must be confirmed with direct measurements. Finally, the physiologic and pathophysiologic influences of NO in whole organisms were considered. Remarks emphasized the complexity of the homeostatic mechanisms that operate in intact systems and the inherent difficulty in ascribing actions to a single mediator.

Role of Nitric Oxide in Therapeutics. Greg Koski, M.D. (Harvard University) moderated the final session, beginning with Allan M. Lefer, Ph.D. (Jefferson Medical College), who described the influence of NO in ischemia–reperfusion injury. Myocardial postischemic reperfusion is characterized by endothelial dysfunction, followed by neutrophil accumulation and, ultimately, myocardial necrosis. These effects are associated with loss of basal NO production and were mitigated by replacement of lost NO through infusion of authentic NO, sydnonimine- or cysteine-containing NO donors, or, less potently, L-arginine. A similar response was seen in the postischemic splanchnic circulation. Such therapeutic use of NO was not found to have cardiotoxic or negative inotropic properties.

The therapeutic use of NO and NOS inhibitors in animal and human septic shock was reviewed by Robert F. Lodato, M.D., Ph.D. (University of Texas, Houston). Both exogenous endotoxin and endogenous cytokines induce NO release, potentially mediating the extreme vasorelaxation and hypotension characteristic of septic shock. In a canine model, hypotension produced by endotoxin, tumor necrosis factor, or interleukin 1 was reversed by infusion of N^G-methyl-L-arginine and then restored by administration of excess L-arginine. NOS inhibition may also restore vascular sensitivity to adrenergic vasoconstrictor agents in refractory septic shock. However, potential cardiocirculatory toxic effects are under investigation. Very preliminary experience with clinical use of low-dose N^G-methyl-L-arginine has been encouraging. Selective inhibition of inducible NOS and the addition of NO donors to NOS inhibition may hold promise.

The closing presentation was delivered by Warren M. Zapol, M.D. (Harvard University), who summarized recent studies using inhaled NO gas in animals and humans. Disorders involving pulmonary hypertension and ventilation–perfusion mismatching are treated with intravenous vasodilators with only partial success, because of systemic hypotension and exacerbated shunting. Inhalation of 10–80-ppm NO gas has selectively decreased pulmonary arterial pressures in newborn animals, and clinical trials are under way in infants with persistent pulmonary hypertension. Chronic NO inhalation produced good results in children with congenital heart disease, and inhalation for as many as 56 days resulted in no apparent adverse effects. Inhaled NO also appears to act as a bronchodilator in animal models. Safety considerations are paramount in the clinical use of inhaled NO, and an effort is under way to develop universal safety standards.

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