The potential clinical impact of 20 years of nitric oxide research

RICHARD A. COHEN
Vascular Medicine Section, Whitaker Cardiovascular Institute, Evans Department of Medicine, Boston University Medical Center, Boston, Massachusetts 02118

ROBERT FURCHGOTT reported in 1980 that the physiological importance of the endothelium rested in its ability to release a diffusible vasodilator (10), later identified as nitric oxide. The deluge of research on nitric oxide over the 20 years that has followed his ingenious initial observation was recognized most recently with the 1998 Nobel Prize in Medicine or Physiology awarded to Furchgott, together with Louis Ignarro and Ferid Murad. The broad impact and importance of nitric oxide to cardiovascular physiology should now be obvious to all. Despite the fact that nitric oxide is the therapeutic constituent of nitroglycerin, making it one of the oldest effective therapeutic agents, the future is bright for new applications to clinical medicine that will result from further understanding the physiological and pathological roles of nitric oxide.

The year of 1998 also marked the introduction of a novel drug with the design based on nitric oxide research. The marketing of Viagra by Pfizer for erectile dysfunction had the added benefit of dramatically increasing the awareness by laymen of nitric oxide. I recently explained my field of research to a businessman during an airplane flight. I was amazed to discover that he not only knew that nitric oxide mediates penile erection (17) but also knew that the mechanism of action of Viagra involves enhancing the physiological action of nitric oxide by preventing the breakdown of cGMP (18). Explaining my research is now a lot easier and more fun! On a serious note, this opportunity for those of us involved in nitric oxide research to point out its relevance to health should not be missed. There are many classes of drugs with a rationale that may rely on enhancing the actions of nitric oxide, including antioxidants and phosphodiesterase inhibitors. In addition, new strategies to deliver nitric oxide itself in slow, rapid, or targeted release forms will provide many new therapeutic agents for disorders as diverse as pulmonary hypertension, arterial restenosis, liver failure, and schistosomiasis (16).

Early studies of vascular disease in animal models with hypercholesterolemia, diabetes, and hypertension have established that interference with nitric oxide function is a major consequence of cardiovascular risk factors and is potentially related to the progression of arteriosclerosis (5). Studies in humans have also demonstrated reduced coronary and forearm vasodilator responses to infusions of acetylcholine or methacholine, showing that this abnormality extends to the microvasculature of humans with these risk factors. Whereas the infusion of endothelium-dependent agonists in humans requires invasive studies, the recognition that the shear force developed by blood flow is a physiological stimulus for endothelium-dependent vasodilation has provided a rapid test of nitric oxide function in humans. This test relies on measuring the increase in brachial artery diameter that follows the hyperemia caused by forearm ischemic hyperemia produced by brief application of an arterial occlusion cuff. Because it requires only about 20 min, the test is feasible for large numbers of patients and promises to allow further studies of normal endothelial vascular function and pathology in humans (3). The technique has already provided an important link between basic studies in isolated animal blood vessels and clinical research in humans.

But what are the most exciting and promising current and future areas of basic nitric oxide research? What gaps remain in our knowledge that when filled will provide new understanding and therapies for human cardiovascular disease? In the March 1999 issue of AJ P: Heart and Circulatory Physiology several papers are published that provide an opportunity to comment on what the most promising areas are. Whereas the physiological significance of nitric oxide could only be hypothesized from the early studies of the effect of endothelium-dependent vasodilators on isolated blood vessels, it is now apparent that the influence of nitric oxide on the sympathetic nervous system is perhaps one of its more important physiological roles. This interaction was suggested first by showing that inhibitors of nitric oxide synthase increase regional vascular resistance and raise blood pressure (26). The studies in the March 1999 issue by Iida (12) focus on the levels of neural integration at which nitric oxide inhibits peripheral sympathetic vasoconstriction. The studies demonstrate the complexity of this interaction for different vascular beds, and they also suggest that further work is needed to elucidate the relationship in humans. One reason for the complexity is that nitric oxide influences sympathetic neurotransmission at several levels. Not only does the endothelium suppress vascular smooth muscle tone directly by releasing nitric oxide, but it also inhibits the release of norepinephrine at the sympathetic neuroeffector junction (7).
Furthermore, neurally released nitric oxide may modulate sympathetic vasoconstriction at pre- and postganglionic as well as postjunctional levels.

One clinical area impacted by research on the interaction of nitric oxide with the sympathetic nervous system is hypertension. It may be that a major mechanism by which antihypertensive agents, particularly angiotensin-converting enzyme inhibitors, lower blood pressure is by the enhancement of nitric oxide action (1). Also, the studies by lida (12) suggest that if the action of nitric oxide is impaired, for instance by diabetes, hyperlipidemia, or hypertension itself, then a drug that ordinarily inhibits sympathetic neural outflow might be less effective. Prospective clinical studies might examine this issue by determining whether the effectiveness of antihypertensive agents varies in different patients depending on an independent assessment of nitric oxide function. Furthermore, enhancement of nitric oxide function may enhance the therapeutic response to some antihypertensive agents.

Hormonal regulation of endothelial function is another area that will continue to yield results that are relevant for clinical medicine. Much of the reduced cardiovascular risk associated with female gender may well be due to the greater nitric oxide function associated with estrogen (15). The mechanism by which estrogen enhances nitric oxide is an active area of investigation that may result in new therapeutic agents. In the study by Knot et al. (19) published in the same March issue, the gender difference in nitric oxide function has been traced to a higher intracellular free calcium level in endothelial cells of coronary arteries of female rats. The higher calcium is one reason for greater nitric oxide synthase activity.

Despite years of research, one of the least understood areas of vascular function is its relationship to metabolism. It has only recently become evident how potent metabolic hormones are in regulating nitric oxide and in turn vascular function. Schröder et al. (27) show in their published study within the March issue that insulin, in the physiological range of 10–100 µU/ml (60–600 pmol/l), influences the tone of isolated skeletal muscle arterioles. Corroborating studies in cultured endothelial cells in which insulin was shown to stimulate the release of nitric oxide (37), a major local vasodilator effect of insulin appears to be endothelium dependent and prevented by inhibitors of nitric oxide synthase. A residual insulin-induced vasoconstriction remains unexplained but assures that physiological levels of insulin have multiple, yet to be explored, vascular effects. In addition to its endothelium-dependent effects, physiological concentrations of insulin may inhibit the action of vasoconstrictors in smooth muscle cells by enhancing the action of nitric oxide (14). Insulin-mediated, nitric oxide-dependent vasodilation has been shown in humans to be closely linked to the metabolic action of insulin to increase glucose uptake and is impaired in patients with insulin resistance (28). The implication that insulin resistance may at least in part represent impaired nitric oxide function could be further explored in patient studies by examining independently insulin-dependent and nitric oxide-dependent vasodilation. Insulin-resistant states might be treated indirectly by improving nitric oxide function.

A principal mechanism that is responsible for regulating the action of nitric oxide is its chemical reaction with and inactivation by superoxide anion. Whereas the physiological importance of nitric oxide is widely recognized, importance of the vascular superoxide anion is far less recognized or understood. Indeed, because the biological activity of nitric oxide depends on sufficiently low levels of superoxide anion, nitric oxide may be the “tail” wagged by the larger “dog” of superoxide anion. Consistent with previous reports on rabbit aorta and cow coronary artery (22, 23), Wambi-Kiese and Katusic in the March 1999 issue (32) show that nitric oxide-mediated, endothelium-dependent relaxation of cerebral arteries is impaired following inhibition of endogenous superoxide dismutase. The inhibition of this superoxide anion scavenging enzyme results in a rise in vascular superoxide anion that can react with and inhibit the action of nitric oxide (6). The potential sources of superoxide anion are multiple, including mitochondrial respiratory chain enzymes, xanthine oxidase, or NAD(P)H oxidase. Studied extensively only in neutrophils, the latter enzyme recently has been localized to normal vascular cells (9, 22, 33) and may play a prominent role in regulating nitric oxide function. Unfortunately, currently available inhibitors of this enzyme, the iodonium compounds, are known to be nonspecific. However, from the lack of effect of specific inhibitors of other known enzymes that generate superoxide anion, it would appear that NAD(P)H oxidase accounts for a major proportion of vascular superoxide anion. For instance, superoxide anion levels are high in the normal rat aortic adventitia where subunit proteins of NAD(P)H oxidase proteins are localized (33). Superoxide anion arising from this site inactivates nitric oxide, and superoxide anion levels are decreased by diphenylene iodonium, superoxide dismutase, or Tiron (4,5-dihydroxy-1,3-benzenedicarboxylicacid disodium salt), a cell-permeable superoxide scavenger (33). Unlike the neutrophil enzyme, the NAD(P)H oxidase found in vascular cells is constitutively active. The recognition that endogenous enzymes give rise to oxygen-derived free radicals necessitates making an important distinction between antioxidants used to oppose their effects; there are both antioxidant scavengers of free radicals and more specific antioxidant inhibitors of the enzymes that generate free radicals.

The relevance of NAD(P)H oxidase to vascular pathology is also becoming evident. The activity of the enzyme has been shown to increase in angiotensin II-induced hypertension (8) and nitrate tolerance (24), and both are ameliorated by superoxide anion scavengers or antioxidants. The superoxide anion generated from this enzyme may not only reduce nitric oxide-induced relaxation but also may contribute to formation of other reactive oxygen derivatives, including hydrogen peroxide, which has been implicated in vascular hypertrophy (36). Bringing the importance of NAD(P)H oxidase to a
clinical level, a polymorphism in p22phox, a membrane-bound subunit of the enzyme, has recently been reported to be associated with altered cardiovascular disease risk (13). Whereas these studies of NAD(P)H oxidase are in an early stage, it is by now clear that inactivation of nitric oxide by increased levels of superoxide anion occurs in a wide variety of vascular pathologies, including diabetes mellitus (4) and atherosclerosis (20).

The chemical reaction between nitric oxide and superoxide anion is extremely rapid, with a reaction constant of \(6.7 \times 10^9 \text{ mol}^{-1} \text{L} \cdot \text{s}^{-1}\) (11). The peroxynitrite that is formed is a highly reactive intermediate, and its potential clinical importance seems to be growing steadily with continued research. Because peroxynitrite reacts with tyrosine groups of proteins, its formation may be detected by immunohistochemical staining of nitrotyrosine in tissues (35). Increases in the release of nitric oxide (2, 21) and superoxide anion in vascular disease are bound to form peroxynitrite. Underlying its potential clinical importance, nitrotyrosine staining has been found in human atherosclerotic plaques, chronic hepatitis, ulcerative colitis, multiple sclerosis, Parkinson's disease, and Alzheimer's disease (35). The paper by Thom et al. (30) in the March issue brings further complexity and significance to this area. The studies of these investigators suggest that environmental levels of carbon monoxide increase both nitric oxide levels and the formation of peroxynitrite. Furthermore, they find that the oxidant stress induced by carbon monoxide is associated with an inflammatory response that is prevented by an inhibitor of nitric oxide synthase. Because nitric oxide itself inhibits endothelial cell leukocyte adhesive mechanisms, this observation suggests that formation of peroxynitrite may stimulate inflammation.

The importance of oxygen-derived free radicals in the breakdown and regulation of nitric oxide activity or in the formation of peroxynitrite may also provide a long-sought after therapeutic rationale for the use of antioxidants in treating vascular disease. An exasperated clinician-investigator currently throws up his hands when confronted with patients who ask if they should add "designer" antioxidants to their self-prescribed vitamin C and E intake. However, current and future clinical research may provide support for antioxidant therapy. The first scientific report of the clinical benefit of vitamin E in patients with coronary disease has been published (29), and antioxidants potentially represent an especially rich interface between basic cardiovascular research and clinical medicine. An amazing feature of abnormal endothelial vascular function, even in patients for whom the abnormality may be longstanding, is its reversibility. The impaired endothelium-dependent vasodilation observed in patients with prolonged diabetes may be rapidly reversed by vitamin C (31). Furthermore, the chronic effects of a risk factor, such as diabetes mellitus, can be effectively reproduced in isolated arteries (4) or the human forearm (34) by brief exposure to elevated glucose. Endothelial cell dysfunction in humans might represent the cumulative effect of multiple daily insults to the vasculature by uncontrolled cardiovascular risk factors. The rise in plasma triglycerides following a single high-fat meal has been reported to reduce flow-mediated, endothelium-dependent dilation of the brachial artery. The impaired vasodilatation is prevented by orally administered vitamin E and C (25). Along with risk factor modification and exercise, which also improve vascular function, antioxidants may finally be shown to have a scientifically proven clinical role. The apparent sensitivity of the vasculature to very short-term exposure to cardiovascular risk factors may represent a harsh reality for us all. However, current and future research may provide the means to prevent the harmful effects of these risk factors on the vasculature and potentially their long-term clinical consequences that include heart attack and stroke.

Address for reprints requests: R. A. Cohen, Vascular Biology Unit, Boston Medical Center R408, 80 E. Concord St., Boston, MA 02118 (Email: racohen@ned-med1.bu.edu).

REFERENCES


