Tolerance to the Organic Nitrates
New Ideas, New Mechanisms, Continued Mystery

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Nitroglycerin (NTG) and other organic nitrates continue to be important drugs that are often used in cardiovascular medicine. All organic nitrates undergo denitritification, releasing nitric oxide (NO) through a biotransformation process that remains poorly understood to date. NO activates the enzyme soluble guanylyl cyclase in smooth muscle cells, causing increased levels of the second messenger cGMP. Finally, cGMP activates a cGMP-dependent protein kinase. This enzyme mediates vasorelaxation through inhibitory phosphorylation of different proteins involved in the regulation of intracellular Ca^{2+} levels.

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All of the organic nitrates have potent short-term vascular, hemodynamic, and clinical effects. A critical limitation in their clinical application is the development of tolerance, whereby their initial effects are rapidly lost during sustained administration. Although there seems to be little doubt that NO, or some NO adjunct, mediates the pharmacodynamic effect of nitrates, the mechanism(s) underlying the development of tolerance remain an important and vexing problem for cardiovascular pharmacology.

Nitrate Tolerance: The State of the Art

The phenomenon of nitrate tolerance has been recognized for more than a century and has been intensely investigated for 3 decades. Despite these efforts, a unifying hypothesis concerning the mechanism of tolerance continues to be elusive. Initial investigations focused on abnormalities in biotransformation and denitrification of organic nitrates. Almost 30 years ago, Needleman and Johnson\(^1\) proposed that tolerance might result from impaired nitrate metabolism caused by the reduced bioavailability of sulfhydryl groups. Although sulfhydryl depletion is no longer thought to play a causal role in tolerance,\(^2\) abnormalities in nitrate biotransformation remain the subject of active investigation.\(^3\) Importantly, investigations in this area are severely limited by our poor understanding of the biotransformation pathway and by significant analytical problems in quantifying NO and/or NO adjunct production rates.

Approximately 10 years ago, research in the field of nitrate tolerance shifted toward the description of systemic, counter-regulatory responses to organic nitrate therapy. Human studies demonstrated that nitrate therapy led to stimulation of the sympathetic nervous system, activation of the renin-angiotensin axis, and evidence of plasma volume expansion.\(^4\) Such changes were thought to represent a homeostatic response aimed at restoring hemodynamic equilibrium, thus reversing the effects of nitrates. On the basis of these observations, subsequent efforts were made to prevent or alleviate these systemic, counter-regulatory responses using pharmacological interventions. Despite some contradictory results, in the majority of human studies, these interventions (including angiotensin-converting enzyme inhibitors, angiotensin II antagonists, and diuretics) were not successful.\(^4\)

Other lines of investigation suggested that nitrate tolerance was caused by abnormalities in the transduction of NO signaling, resulting in decreased vascular and hemodynamic responses to nitrates, a phenomenon that has been termed end-organ tolerance. A number of mechanisms for this hyporesponsiveness have been examined, including the decreased activity of guanylate cyclase, increased activity of phosphodiesterases leading to increased rates of cGMP catabolism, and, finally, a reduction in the bioavailability of NO mediated by increased vascular production of free radical species, particularly superoxide anion.\(^4,5\)

The Oxidative Stress Hypothesis

The observation that therapy with NTG increases vascular free radical production has had a major impact in the field of nitrate pharmacology.\(^5\) The development of the free radical hypothesis of nitrate tolerance introduced the important concept that the endothelium plays a critical role in mediating nitrate effects during sustained therapy. The demonstration that therapy with NTG, and possibly other NO donors, increases superoxide production, thus limiting the bioavailability of both endogenous and exogenous NO, provided an internally consistent explanation for many basic and clinical observations in the field of nitrate tolerance. Studies in animal models and humans confirmed that NTG treatment enhances superoxide anion production, a finding that has been associated with the presence of endothelial dysfunction and heightened vascular responsiveness to vasoconstrictors.\(^7,8\) The mechanisms of these phenomena have been actively investigated, and it is now clear that augmented superoxide anion bioavailability is caused by abnormalities in the function of both membrane-bound reduced nicotinamide adenine dinu
cleotide phosphate [NAD(P)H] and xantine oxidases and endothelial NO synthase. To date, the exact trigger(s) of these phenomena remain unknown, although enhanced angiotensin II and endothelin-1 production, protein kinase C activity, and changes in the metabolism of L-arginine and tetrahydrobiopterin have all been implicated. Despite this uncertainty, the demonstration that NTG therapy increases superoxide anion bioavailability has provided a plausible explanation for the phenomenon of nitrate tolerance. Although the exact unifying hypothesis remains undeveloped, these observations concerning the importance of endothelium-derived free radicals suggest that a complete understanding of nitrate tolerance is within our reach.

**New Evidence Concerning the Role of Phosphodiesterases**

In the present issue of *Circulation*, Kim et al emphasize the potential importance of vascular phosphodiesterase IA1 (PDEIA1) upregulation in the development of tolerance. They demonstrate in a rat model that sustained in vivo administration of NTG, in moderate doses, increases the expression and activity of Ca2+-dependent PDEIA1. PDEIA1, in smooth muscle cells, catalyzes cGMP, removing its inhibition on cellular Ca2+ influx. Increased PDE activity reduces vascular sensitivity to all cGMP-dependent vasodilators, including NTG. The authors further demonstrate that PDE upregulation is specific for the isoform IA1 and that NTG is not associated with significant changes in PDE5 activity. Accordingly, using the highly selective PDE1 inhibitor vinpocetine, they were able to reverse tolerance in an ex vivo assay. They concluded that their results support an important role of PDE1 upregulation in both the development of tolerance and the heightened vascular responsiveness to vasoconstrictors that follow NTG therapy. Indeed, angiotensin II and norepinephrine increase smooth muscle cell Ca2+ influx, one of the cofactors for the calmodulin-linked PDE IA1. The increased expression and activity of the Ca2+-dependent PDEIA1 is compatible with an exaggerated decrease in cGMP concentrations in response to vasoconstrictors, causing an augmented cellular uptake of Ca2+. Thus, increased activity of PDEIA1 might ultimately allow higher Ca2+ influx in response to vasoconstrictors, heightening vascular sensitivity to such agents.

The idea that PDE upregulation is involved in nitrate tolerance is not new. Different investigations have suggested that PDE inhibition can reverse nitrate tolerance. Despite this interest, no human in vivo study has demonstrated a clear effect of PDE inhibition on nitrate tolerance. In addition, the existence of distinct isoforms of PDE serve to add complexity to the interpretation of the results. In contrast to the results of Kim et al, previous reports have documented that nitrate tolerance can be reversed with the PDE5 inhibitor zaprinast. As pointed out by Kim et al, vinpocetine is less selective for PDE5, suggesting that these previous reports may indicate a significant inhibitory effect of zaprinast on PDE1. Although the enzymatic kinetics of the 2 compounds support this claim, the fact that the unspecific PDE inhibitor dipyridamole had no effect on nitrate tolerance remains difficult to explain.

An important ambiguity in the context of these studies is the impact of PDE inhibitors on the short-term response to NTG. As seen in Figure 5, vinpocetine seems to increase vascular responses to NTG (albeit not significantly so), even before continuous NTG treatment. This finding has also been documented with other PDE inhibitors. If these agents increase NTG responses independently of tolerance, conclusions concerning their capacity to reverse tolerance should be made with caution. The importance of a nonspecific augmentation of nitrate effects by PDE inhibition is emphasized by the interaction found between sildenafil and nitrates. Silde- nafil, a highly selective PDE5 inhibitor, displays a potent interaction with organic nitrates, augmenting the short-term response to NTG and the effect of isosorbide-5-mononitrate during sustained therapy. These observations emphasize that the results of any study in which nitrate tolerance is reversed by a specific intervention should be interpreted with caution. This concept is best articulated by the experience with N-acetylcysteine supplementation. When given to patients made tolerant to NTG, N-acetylcysteine restored the hemodynamic effect of the nitrate, a finding that was thought to provide evidence for reversal of tolerance mediated by thiol supplementation. It is now recognized that this agent can augment the vascular and hemodynamic effects of NTG, even when given acutely, and that the interaction between NTG and N-acetylcysteine is not dependent on the mechanism of tolerance. In light of these observations, studies should investigate whether vinpocetine can prevent the development of tolerance during sustained therapy. Such a study has been done with zaprinast and, despite the fact that this agent could reverse tolerance, it had no effect on its prevention.

The hypothesis that PDE1 upregulation plays an important role in the development of tolerance must also confront the presence or absence of cross-tolerance between different NO donors. Indeed, because all NO donors achieve their vascular effects through the increased bioavailability of cGMP, in the presence of upregulation of PDE, cross-tolerance would be expected. However, multiple investigations documented the lack of significant cross-tolerance to other NO donors, particularly when nonorganic NO donors were used. Although the differential effect of individual agents on cGMP dose-response curves does complicate the interpretation of these observations, the convincing absence of cross-tolerance with NTG in some studies is notable.

The report of Kim et al emphasizes the wide range of vascular effects that may result from therapy with nitrates and other NO donors. Despite some uncertainties concerning their findings, these results provide a stimulus for further research into the mechanism(s) of PDE1 upregulation during nitrate therapy. It would be of great interest to determine if this upregulation was causally linked to abnormalities in free radical production by the vasculature, because previous studies suggested that oxidative stress not only impairs NO bioavailability but can also have an important impact on its signaling pathway.

In 1990, an editorial concerning the mechanism of nitrate tolerance concluded that the “100-year mystery continues.” Today, little more than a decade later, great progress has been made. It is only during the past decade that the true biochem-
ical nature of the response to long-term nitrate therapy has been elucidated. The role of the endothelium, changes in free radical production and, as is the subject of the present report, disruption of NO signal transduction, are now being understood in detail. The nature of these observations is bringing us closer to an accurate understanding of the genesis of tolerance. At the same time, the recognition that NO donors have profound effects on vascular function challenges us to carefully consider whether long-term exogenous NO therapy is as safe as was heretofore assumed.

References


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