Nitrate Tolerance in Hypertension
New Insight Into a Century-Old Problem

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Nitrate tolerance to a vascular physiologist is the intriguing insensitivity or loss of responsiveness to drugs whose actions mimic the powerful endogenous vasodilator and platelet inhibitor nitric oxide (NO). To the clinician, it is a serious limitation in the use of an otherwise highly promising and widely used therapeutic class of drugs collectively referred to as nitrates. Nitroglycerin (NTG) and organic nitrates are used in the therapy of ischemic heart disease and heart failure. Clinical trials such as the ISIS-4 trial showed a 20% reduction in 24-hour postinfarct mortality with the use of an oral nitrate such as isosorbide mononitrate (ISMN). Sublingual NTG is the drug of choice for chest pain and acute angina attacks, and intravenous NTG is the drug of choice in acute coronary syndromes. Oral long-acting nitrates are effective in the treatment of chronic stable angina.

Brief History of Nitrate Use and Nitrate Tolerance

The promise for nitrates in medicine dates back to the initial observation by Sobrero in Turin in 1847 noting “violent headaches” produced by small quantities of NTG on the tongue. Brunton in Edinburgh recognized the clinical promise for the use of nitrate in angina in 1867.1 Almost concomitant with his recognition of the strong vasodilatory and other physiological effects of NTG are his poignant observations on its limitations. Brunton noted that if this remedy for relief of angina pain was used for a long time, “...the dose requires to be increased before the effect is produced,” perhaps the first reference to what we now refer to as nitrate tolerance.1 Marsh and Marsh elegantly compiled the explosive yet “nobel” history of NTG and NO in medicine.3

Nitrate Tolerance: Underlying Mechanisms

Several mechanisms that may underlie the complex nitrate tolerance have been proposed. In a recent editorial, Horowitz3 proposed a theory that involves increased endothelial dysfunction and neurohumoral excess (angiotensin II, endothelin-1, and catecholamines) and pseudo-tolerance may underlie the rebound ischemia on cessation of nitrate therapy, the mechanisms that underlie “true” nitrate tolerance and guidance for ways to therapeutically circumvent the phenomenon remain elusive. True nitrate tolerance may involve impaired enzymatic release of NO from nitrates, and in a recent review Gori and Parker4 proposed a theory that involves increased endothelial generation of superoxide, impairing both responses to nitrates and agents that stimulate NO release from the endothelium.

How Do Nitrates and NO Relax Blood Vessels?

In order to understand and appreciate the significance of the problem of nitrate tolerance, one has to consider the molecular mechanisms by which nitrates and NO dilate arteries. Although complex, there is general agreement on the following sequence of events related to the regulation of arterial membrane potential and hence the opening of the dihydropyridine-sensitive Ca2+ channels (DHPR), that are responsible for arterial constriction.5

NO and nitrates increase levels of cGMP, which primarily activates cGMP-dependent kinase (cGK), and to a lesser extent, depending on the levels achieved, cAMP-dependent kinase (cAK). cGK has been shown to directly phosphorylate the large-conductance Ca2+-dependent potassium channel (BK channel).6,7 BK channels play a key role in the regulation of diameter of resistance arteries that exhibit myogenic tone where they act as a negative-feedback mechanism that opposes vasoconstriction8 and promotes vasodilation.

Molecularly, BK channels constitute the last signaling element in what is now referred to as the Ca2+ spark pathway in smooth muscle.9 Ca2+ sparks result from the coordinated opening of many tightly clustered ryanodine receptor Ca2+ release channels (RyR) in the sarcoplasmic reticulum (SR) of muscle cells. In smooth muscle and intact arteries, Ca2+ spark frequency is a key regulator of BK activity, driven by the Ca2+ filling state of the SR.10 The BK channel activity is “tuned” by the ancillary Ca2+-sensing β1 subunit of the channel that responds to the sparks.11 cGMP and cAMP via their respective kinases greatly increase BK channel activity...
by increasing spark frequency. They achieve this via phosphorylation of phospholamban, thus greatly increasing the pumping activity of the SR Ca\(^{2+}\) pump, and phosphorylation of the RyR channel and thus augmenting the release of Ca\(^{2+}\) from the SR as Ca\(^{2+}\) sparks. Because nitrates and NO are key regulators of cGMP levels and synergistically affect multiple signaling elements of the Ca\(^{2+}\) spark pathway, they are the most potent activators of the Ca\(^{2+}\) spark pathway.

In this issue of Circulation Research, Gerzanich et al. provide a new molecular insight into the problem of nitrate tolerance. Using the model of angiotensin II–induced hypertension, the authors show that as early as 4 days after the start of angiotensin infusion there is a significant upregulation of cGMP-dependent kinase type Ib (GKIβ). They further show a marked downregulation of the cGKIα isoform. Although GKIα is traditionally considered the principal mediator of cGMP-mediated vasodilation, both isoforms associate with the BK channel protein, and a clear 2-fold increase in BK-GKIβ association was measured by coimmunoprecipitation. So, where is the catch? As elegantly demonstrated by the authors in this study, using the BK channel activity as their marker, GKIβ is less efficacious in stimulating BK channel activity in vascular smooth muscle (VSM) cells. This is probably due to its lesser sensitivity for cGMP compared with the normally dominant GKIα isoforms. Further, the ability of the stable cGMP analogue 8-Br-cGMP to activate BK channels was significantly reduced, as were the effects of the direct NO donor sodium nitroprusside (SNP). The “normal” and “nitrate-tolerance” states after angiotensin-induced hypertension are illustrated in the Figure. Because this newly identified defect lies at the most “downstream” level of nitrate’s mechanism of action on VSM, their findings impact virtually all proposed forms of nitrate tolerance including those associated with the therapeutic use of NO donors such as SNP and NO itself.

**Future Directions and Therapeutic Perspective**

The findings by Gerzanich et al. provide ample opportunity for further investigation into the mechanisms underlying the nitrate tolerance phenomenon and could have important clinical implications as well as provide new leads for treatment of this phenomenon. For example, can we extrapolate these findings to other types of hypertension or to those associated with abnormalities in the renin-angiotensin system? The data in the present study implicate an important role for angiotensin II itself, since the observed effects on the onset of systemic angiotensin-induced hypertension. Other investigators have made a case for a more causal role of the renin-angiotensin system in nitrate tolerance in humans, and angiotensin-converting enzyme (ACE) inhibitors have shown some positive effects. Is a similar mechanism involved in the tolerance associated with increased endothelin-1 or catecholamines? Recent studies have also implicated Ca\(^{2+}\)-activated potassium channels in the mechanisms of endothelial-mediated vasodilation and regulation of endothelial NO release. In nitrate tolerance, are we perhaps dealing with a dual/sequential endothelial/smooth muscle defect?

The findings from the present study, combined with the recent study by Amberg et al. showing that in the same model of angiotensin-induced hypertension there is significant downregulation of the Ca\(^{2+}\)-tuning β1 subunit of the BK channel, point to a key role for this channel not only in blood pressure regulation but also as a potential drug target for the treatment of hypertension and associated nitrate tolerance. As has been shown by Liu et al., the BK channel itself is upregulated in hypertension, yet does not seem to fulfill its promised effect.

In summary, the study by Gerzanich et al. provides a novel insight into a possible mechanism underlying nitrate tolerance. The proposed mechanism for nitrate tolerance involves a shift in expression of splice variants of cGKI and points to a “defect” at the level of translation into vasodilation by the VSM cells. Clearly, for a complex problem as nitrate tolerance, more molecular studies are needed at the endothelial and the VSM level. Lastly, it may be possible to...
reevaluate the potential benefits of use of ACE inhibitors and angiotensin II type 1 receptor blockers on this new therapeutic target and finally unleash the full therapeutic promise of this centuries-old class of drugs called nitrates.

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References

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