

Editorial Comment

What Causes Tolerance to Nitroglycerin?: The 100 Year Old Mystery Continues*

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No pleasure lasts long unless there is variety in it.
(*Publilius Syrius*)

Organic nitrates are among the oldest and most widely used drugs in cardiovascular medicine. Although short-term therapy with sublingual and intravenous nitroglycerin is effective in the treatment of a variety of cardiac emergencies, attempts to extend the benefits of this drug to the long-term management of patients have been disappointing. Tolerance develops rapidly to the hemodynamic and clinical effects of nitrates when they are administered continuously or at frequent intervals; consequently, controlled trials often have been unable to show that prolonged use of these drugs produces symptomatic improvement. Such observations are not new: tolerance to nitroglycerin was first reported in the clinical setting in 1888 (1). However, despite intensive study during the last 100 years, the cause of nitrate tolerance remains a mystery.

Three mechanisms have been proposed to explain the development of tolerance to nitroglycerin: 1) depletion of sulfhydryl cofactors that are required for the vasodilator action of the drug, 2) activation of endogenous vasoconstrictor mechanisms that limit the drug's circulatory effects, and 3) expansion of intravascular volume that results in loss of hemodynamic efficacy. Whereas previous research in the field has largely focused on one mechanism at a time, the report by Dupuis et al. (2) in this issue of the Journal is the first attempt to explore all three mechanisms simultaneously in a single study. One conclusion emerges clearly from this

work: the development of tolerance is far more complicated than anyone had expected.

Intracellular sulfhydryl depletion as a cause of tolerance. Nitroglycerin exerts its vasodilating effects by stimulating guanylate cyclase inside the vascular smooth muscle cell; the resulting increase in cyclic guanosine monophosphate (GMP) causes relaxation of blood vessels by reducing cytosolic calcium (3-5). Nitroglycerin cannot activate guanylate cyclase directly, however. To do so, the drug must be metabolically activated to form nitric oxide or S-nitrosothiols, but such conversion requires the presence of cysteine (6). Tolerance to the drug's vasodilating effects is accompanied by a decreased rate of activation of guanylate cyclase (4,6,7), which seems to be related to a reduction in the metabolic conversion of nitroglycerin rather than a change in the function of the enzyme (8). On the basis of this model, several investigators have suggested that a depletion of intracellular sulfhydryl cofactors (cysteine) might explain the reduced metabolic conversion of nitroglycerin and thereby the development of tolerance to the drug (3,9).

The sulfhydryl depletion hypothesis has become the focus of considerable controversy. The incubation of tolerant arterial strips with a variety of sulfhydryl cofactors in vitro has produced partial reversal of tolerance in some laboratories (9-11) but not others (12-14). Similarly, the administration of N-acetylcysteine and methionine (which are converted into cysteine in vivo) into animals and patients made tolerant to organic nitrates has reversed tolerance in some studies (15-19) but not in others (20,21). How can the proponents of the sulfhydryl depletion hypothesis explain the failure of sulfhydryl cofactors to reverse tolerance in isolated arterial rings? Veins, rather than arteries, may be the primary site of tolerance reversal by sulfhydryl cofactors because nitroglycerin stimulates the synthesis of cyclic GMP more markedly in veins than in arteries (5,11,22). How do skeptics of the sulfhydryl depletion hypothesis explain the hemodynamic responses seen when sulfhydryl cofactors are given to nitrate-tolerant subjects? Sulfhydryl cofactors may enhance the effects of nitroglycerin (by promoting the formation of S-nitrosothiols) whether or not a tolerant state exists (23,24); according to this view, the effects of N-acetylcysteine in vivo are the result of a nonspecific potentiation rather than a specific reversal of tolerance (20,25). Fung and coworkers (26) have postulated that nitroglycerin and N-acetylcysteine can interact *extracellularly* to form S-nitrosothiols, which, after entering the cell, can stimulate the production of cyclic GMP directly. Presumably this may occur whether or not the metabolic conversion of nitroglycerin is impaired or tolerance exists.

Do the data of Dupuis et al. (2) in the present study support or refute the sulfhydryl depletion hypothesis? In this

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study the addition of N-acetylcysteine completely prevented the development of tolerance to the initial effect of nitroglycerin on mean right atrial pressure, partially prevented tolerance to the action of the drug on pulmonary artery and wedge pressures but did not alter the development of tolerance to the drug's effect on mean arterial pressure and systemic vascular resistance. The favorable effects of N-acetylcysteine on right and left ventricular filling pressures might have been even more apparent if the authors had carried out a paired analysis in patients who received two infusions of nitroglycerin (with and without N-acetylcysteine) instead of analyzing the responses to two infusions in the same patient as if they had been given to different people. In any case, their data support the concept that sulfhydryl cofactors are more likely to interfere with the development of nitrate tolerance in veins than arteries; other mechanisms, such as neurohormonal activation, may be responsible for the development of tolerance in arterial resistance vessels (27).

Yet the study by Dupuis et al. (2) also provides a strong argument against the sulfhydryl depletion hypothesis. In their study the coadministration of nitroglycerin and N-acetylcysteine was frequently accompanied by episodes of hypotension, which were not seen in patients who received nitroglycerin alone; similar episodes have been reported by Horowitz et al. (28). These findings indicate that sulfhydryl cofactors can potentiate the hemodynamic effects of nitroglycerin in the nontolerant state; therefore, these data support the existence of a nonspecific interaction. Why did the early hypotension seen by Dupuis et al. (2) disappear after 24 h? Kowaluk et al. (8) have shown that blood vessels may become tolerant to S-nitrosothiols when exposed to them for long periods. The secondary development of nitrosothiol tolerance may explain the apparent lack of sulfhydryl effects in the study of Dupuis et al. (in which N-acetylcysteine and nitroglycerin were infused together for 24 h), whereas favorable sulfhydryl effects have been reported in studies (15,16) in which the two agents were combined for only brief intervals.

Neurohormonal activation as a cause of tolerance. Like other organic vasodilators, organic nitrates may activate endogenous neurohormonal systems (specifically, the sympathoadrenal axis and the renin-angiotensin system); the resulting vasoconstriction and sodium retention may, in turn, limit the pharmacologic effects of these drugs (27,29). These reflex mechanisms become evident as rebound phenomena when, on abrupt discontinuation of a short-acting vasodilator, drug-mediated vasodilation rapidly disappears leaving the reactive neurohormonal forces unopposed. The occurrence of rebound hemodynamic events after the abrupt withdrawal of nitroprusside and nitroglycerin suggests that similar reactive mechanisms can limit the magnitude of drug-mediated peripheral vasodilation and thereby contribute to the development of tolerance (30-32).

Strong evidence in favor of a role for neurohormonal activation in the development of nitrate tolerance has come from our own studies in human heart failure (14). Tolerance produced by the continuous infusion of nitroglycerin for 48 h was paralleled by an increase in heart rate, plasma renin activity and body weight. These neurohormonal events persisted for 2 to 3 h after the discontinuation of nitroglycerin (at which time patients still showed evidence of tolerance) but were no longer apparent 24 h later (when responsiveness to nitroglycerin was restored). Intermittent (12 hourly) dosing with nitroglycerin, which successfully circumvented the development of tolerance, failed to activate endogenous neurohormonal systems, whereas intermittent dosing regimens that produced partial tolerance (4 hourly and 8 hourly) were accompanied by moderate neurohormonal activation (33). Similar neurohormonal responses have been observed in patients with ischemic heart disease (34), although not necessarily in all reports (32,35). The close concordance between neurohormonal activation and the loss of nitroglycerin's hemodynamic efficacy suggests a role for reactive vasoconstriction in the pathogenesis of nitrate tolerance in humans.

The data of Dupuis et al. (2) are consistent with the hypothesis that neurohormonal activation contributes to the development of tolerance to nitroglycerin. In their study tolerance was accompanied by an increase of plasma renin activity after 1 and 6 h of continuous intravenous therapy. The renin-angiotensin system may have been stimulated either by the fall in blood pressure (which activates renal baroreceptors) or by the decline in circulating levels of atrial natriuretic peptide (because the peptide inhibits the secretion of renin by the kidney [36]). Unfortunately, Dupuis et al. performed no hormonal measurements at the time when patients show the greatest development of tolerance—after 24 h of intravenous nitroglycerin therapy. This omission limited their ability to confirm the existence of a close relation between neurohormonal and hemodynamic events. However, it is likely that neurohormonal systems remained activated after 24 h in their study because heart rate was increased significantly at that time. This pattern of response is strikingly similar to that reported in our own studies (15).

The neurohormonal activation hypothesis raises the possibility that tolerance to nitroglycerin could be avoided if the drug were given together with a neurohormonal antagonist. Indeed, Levy et al. (37) reported that nitrate tolerance in normal volunteers can be circumvented by the concurrent administration of an angiotensin-converting enzyme inhibitor. With respect to the sulfhydryl depletion hypothesis, it is noteworthy that tolerance was prevented most successfully in their study when a sulfhydryl-containing angiotensin-converting enzyme inhibitor was employed.

Intravascular volume expansion as a cause of tolerance. The finding that the prolonged administration of nitroglycerin is accompanied by a decrease in the hematocrit (37,38)

has led to the hypothesis that tolerance may be related to an increase in intravascular volume, which would limit the ability of the drug to decrease ventricular filling pressures. Physicians have generally assumed that this hemodilution is a consequence of the sodium retention that accompanies activation of the renin-angiotensin system but this assumption has never been critically assessed. The study of Dupuis et al. (2) in this issue of the Journal represents the first meticulous evaluation of nitroglycerin-induced hemodilution in the clinical setting. These investigators noted that the increase in intravascular volume seen during the infusion of nitroglycerin was *not* accompanied by an increase in body weight. This finding suggests that hemodilution is not the result of generalized sodium retention but is caused by a shift of fluid from the extravascular into the intravascular space. Such a shift might be related to preferential venodilation (which would reduce the extravasation of fluid from the capillaries to the extravascular space) or to the decrease in circulating levels of atrial natriuretic peptide (because the peptide can cause hemoconcentration [36]).

Can internal hemodilution explain the development of tolerance to nitroglycerin? Hemodilution may explain why tolerance develops more readily to the effects of nitroglycerin on ventricular filling pressures than on cardiac output or systemic vascular resistance (2,15,27,31). On the other hand, most of the fluid shift noted by Dupuis et al. (2) was observed during the 1st h of the infusion when the hemodynamic effects of nitroglycerin were most marked; in contrast, little additional hemodilution was observed between 1 and 6 h, although the effects of nitroglycerin on mean arterial pressure became significantly attenuated during this time. Nonetheless, the careful measurements of Dupuis et al. provide the basis for a provocative hypothesis that needs to be thoroughly evaluated in future studies. If their hypothesis is confirmed, diuretics may prove to be a useful intervention in preventing tolerance to nitroglycerin in a fashion similar to their utility in preventing tolerance to other vasodilator drugs (39,40).

Conclusions. The available evidence suggests that multiple mechanisms interact to promote the development of tolerance to nitroglycerin. To make matters more complicated, the relative importance of one pathway versus another may not be constant but may depend on the status of the patient, as well as the dose, duration and route of administration of the nitrate being utilized. How can such a complex interaction be investigated? Future work should emphasize both basic and clinical investigations. Molecular pharmacologic techniques can be applied to elucidate the pathways utilized for the metabolic activation of nitroglycerin. Such *in vitro* studies should not be relied on too heavily, however, because experiments carried out in isolated vascular rings may not be applicable to the clinical setting where both neurohormonal activation and intravascular fluid shifts may play important roles. The interactions

are probably so complex that the solution is not likely to emerge for many years.

What should physicians do in the meantime to prevent the development of tolerance? Should they routinely use N-acetylcysteine, converting enzyme inhibitors or diuretics to interfere with the three primary mechanisms that have been implicated in this phenomenon? Although future studies may eventually support the utility of these interventions, the only worthwhile way to circumvent the development of tolerance at the present time is to administer nitroglycerin *intermittently*. Unfortunately, the intermittent use of nitrates cannot produce a continuous therapeutic effect and is frequently associated with intolerable side effects and rebound clinical events (41,42). These important limitations of intermittent therapy make it imperative that we solve the mystery of nitrate tolerance as quickly as possible.

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