Background. Organic nitrates such as nitroglycerin and isosorbide dinitrate are useful in the treatment of congestive heart failure (CHF), but tolerance develops rapidly during continuous administration. Because combination therapy of nitrate and hydralazine has been shown to provide both short- and long-term benefit but nitrate alone produces hemodynamic tolerance, we questioned whether hydralazine can preserve the favorable preload effects of nitroglycerin.

Methods and Results. Using an in vivo model of nitroglycerin tolerance in the CHF rat, we examined the effects of hydralazine bolus dosing during continuous nitroglycerin infusion. Continuous infusion of nitroglycerin alone (10 \( \mu \)g/min) produced initial reductions in left ventricular end-diastolic pressure of 40–50%, which returned to baseline by 8 hours (tolerance development). Coadministration of hydralazine (2\( \times \)0.1 mg) maintained the effects of nitroglycerin infusion on left ventricular end-diastolic pressure (45% reduction at 10 hours). This hydralazine dose alone reduced left ventricular peak systolic pressure by approximately 12±3% but had no effect on left ventricular end-diastolic pressure. Hydralazine dosing did not affect steady-state plasma concentrations of nitroglycerin or metabolites, and hydralazine was unable to prevent nitroglycerin tolerance induced in vitro.

Conclusions. The beneficial interaction of hydralazine on the preload effects of nitroglycerin may explain the long-term clinical efficacy of hydralazine/nitrate combination in CHF. Our results also suggest that the mechanism of in vivo nitrate tolerance in CHF may be systemic rather than vascular in origin. (Circulation 1991;84:35–39)

Organic nitrates, such as nitroglycerin and isosorbide dinitrate, are a class of vasodilators useful for the treatment of congestive heart failure (CHF) because of their beneficial reduction of preload and relief of venous and pulmonary congestion often associated with this disease. However, organic nitrate tolerance has been observed as soon as 12 hours during continuous administration (intravenous or transdermal) to patients with heart failure. Intermittent nitrate dosing has been proposed as a method of avoiding nitrate tolerance,\(^1\) but this approach is problematic in nitrate monotherapy because the patient is left unprotected during the “dose-off” period. Therefore, the continuous maintenance of the beneficial hemodynamic effects of nitrates without development of pharmacological tolerance remains a challenge.

Interestingly, another nitrovasodilator, that is, sodium nitroprusside, has also been found useful in the treatment of acute heart failure, but pharmacological tolerance to this compound is not evident.\(^2\) This favorable characteristic has been attributed to the more significant afterload effect of nitroprusside compared with nitroglycerin.\(^2\) A treatment hemodynamically equal to intravenous sodium nitroprusside is the combination of hydralazine and a nitrate, which has been shown to provide beneficial effects after short-term\(^3\) as well as long-term\(^4\) therapy in patients with CHF. The reason for this long-term benefit of the hydralazine/nitrate combination has not been determined. Because the hydralazine/nitrate combination provides sustained benefit but nitrate alone produces hemodynamic tolerance, it is logical to question whether hydralazine can preserve the favorable preload effects of an organic nitrate. So far, no reported study has addressed this clinically important question. Therefore, in the present study, we examined the effect of hydralazine coadministration on the ventricular hemodynamics of intravenous
nitroglycerin in a rat model of CHF. We have previously shown that this animal model can mimic the hemodynamic and temporal behavior of nitroglycerin-induced tolerance in left ventricular end-diastolic pressure (LVEDP) seen in patients. Our strategy was to use doses of hydralazine that, by themselves, did not exert an effect on LVEDP. We then observed the left ventricular hemodynamic interactions between hydralazine and nitroglycerin when they were administered in combination. In separate experiments, we also examined the effects of hydralazine and nitroglycerin vascular tolerance induced in vitro.

Methods

Heart failure was produced in Sprague-Dawley rats (300–325 g) by ligating the left coronary artery to produce a myocardial infarct at the left ventricular free wall and apex and allowing the animals to recover for at least 6 weeks. Compared with control rats, rats with large infarcts have elevated venous pressure and reduced cardiac output, which is similar to the hemodynamic changes observed in patients with heart failure. Left ventricular hemodynamics were measured in CHF rats via a catheter implanted in the left ventricle 1 day before the infusion studies. Catheters were also placed in the left femoral and right jugular veins for nitroglycerin infusion and hydralazine administration, respectively. All animals were conscious and unrestrained throughout the experiments, and procedures were approved by the Laboratory Animal Care Committee of the State University of New York at Buffalo.

Preliminary experiments with hydralazine dosing in CHF rats suggested that two separate bolus doses of 0.1 mg (each in 0.1 ml saline) separated by 30 minutes produced a hemodynamic profile in left ventricular peak systolic pressure (LVPSP) similar to that seen with a 10-μg/min infusion of S-nitroso N-acetylpenicillamine (SNAP). We have previously shown that this dosage regimen of SNAP produced significant reductions in both LVPSP and LVEDP and, more important, no tolerance induction in LVEDP after 10 hours of infusion. We therefore titrated the hydralazine dose to produce the same afterload effect (as determined by changes in LVPSP) as that exerted by SNAP while having minimal effects on LVEDP. Bolus dosing of saline vehicle produced no significant hemodynamic effects.

Two separate groups of CHF animals were infused with either nitroglycerin (10 μg/min) or a combination of the same nitroglycerin dose with hydralazine, and hemodynamic parameters were measured. Nitrate plasma concentrations were determined by gaschromatographic analysis. Statistics were performed by one-way analysis of variance and Duncan’s multiple-range test; a probability of less than 0.05 was considered significant.

In the in vitro relaxation experiments, isolated rat aortic rings were preincubated for 1 hour in either nitroglycerin (0.22 mM), nitroglycerin plus hydralazine (0.22 and 1.0 mM, respectively), or Krebs-bicarbonate buffer. The hydralazine concentration used was calculated to be similar to the peak plasma concentration of hydralazine anticipated in the in vivo experiments, based on reported pharmacokinetic data in the rat. Fresh incubating solutions were exchanged every 20 minutes, and no spectroscopic evidence of hydralazine degradation was observed. After 1-hour equilibration, the mounted ring segments were precontracted with phenylephrine (0.5 μM), and cumulative nitroglycerin concentration/response curves were determined. Ring segments isolated from the same animal were paired for testing in at least two of the three preincubation treatments. Log molar EC50 and Emax values were obtained for each individual run by fitting the four-parameter logistic function to the concentration/relaxation data using PCNONLIN. This methodology has been used previously in our laboratory for the examination of vascular tolerance toward nitrates.

Results

When hydralazine alone was administered as two 0.1-mg bolus doses 30 minutes apart, a significant reduction in LVPSP was observed in this rat model of CHF (Figure 1). The magnitude of LVPSP changes (12±3% maximal reduction) were comparable to those seen during SNAP infusion (15±3% reduction). Using this dosing strategy of hydralazine alone, LVPSP remained depressed for approximately 4 hours, whereas LVEDP remained unchanged throughout. Heart rate increased slightly during hydralazine administration, although the difference from baseline was not statistically significant.

Infusion of nitroglycerin in CHF rats produced prompt initial reductions in LVEDP of 46±3% but no significant changes in LVPSP (Figure 2). These ventricular hemodynamic effects are consistent with the predominantly venous action of this drug. When nitroglycerin infusion was continued, these initial hemodynamic effects were not maintained, and LVEDP returned to near-baseline values within 6 hours, indicating tolerance development. We have previously shown that this rat model of CHF behaves similarly to CHF patients with respect to nitroglycerin effects and tolerance properties and that this apparent tolerance is not a result of any changes in the availability of the drug during infusion.

Figure 2 also shows the effects of hydralazine and nitroglycerin coadministration on left ventricular pressures. The hydralazine doses (2×0.1-mg bolus injections) were administered at 1.5 and 2.0 hours during nitroglycerin infusion. LVPSP was not significantly different from baseline during nitroglycerin infusion before hydralazine administration, but it promptly decreased after the first bolus dose and remained depressed throughout the 10-hour nitroglycerin infusion period. Importantly, LVEDP tolerance to nitroglycerin infusion alone, as demonstrated in Figure 2, was apparently avoided by hydralazine coadministration. The initial reductions of LVEDP
were maintained throughout the 10-hour nitroglycerin infusion period.

In a separate experiment, six CHF rats were handled similarly, and blood samples were taken via the left ventricular catheter before and after hydralazine dosing for the determination of plasma concentrations of nitroglycerin and dinitrate metabolites. Steady-state nitroglycerin plasma concentrations were not different at 0.5 and 2.5 hours after hydralazine dosing (19.1±5.2 and 19.6±5.6 ng/ml, respectively) compared with the before-hydralazine measurement (17.6±3.4 ng/ml). No significant changes in plasma concentrations of dinitrate metabolites were observed before and after hydralazine dosing.

Experiments were then carried out to examine whether hydralazine could reduce nitroglycerin-induced vascular tolerance observed in vitro (Figure 3). Preincubation of isolated rat aorta with nitroglycerin produced a significant rightward shift of the concentration/response curve (log molar EC$_{50}$ values: Krebs buffer, −7.40±0.45; nitroglycerin, −4.79±0.36; $p<0.05$). However, this development of vascular tolerance was not affected by inclusion of hydralazine in the incubation solution (nitroglycerin plus hydralazine incubation: log molar EC$_{50}$, −5.08±0.54; $p=NS$ compared with incubation with nitroglycerin alone but statistically different from Krebs control).

**Figure 1.** Plots of effects of hydralazine alone on left ventricular hemodynamics in rats with congestive heart failure (n=6). Bolus doses of hydralazine (0.1 mg) were administered at 0 and 0.5 hours (H). *Statistically significant differences from baseline (p<0.05). Left ventricular peak systolic pressure (LVPSP) was significantly reduced for 4 hours, but no significant effect on left ventricular end-diastolic pressure (LVEDP) was observed. Heart rate increased initially but was not statistically different. Mean±SEM values are shown.

**Figure 2.** Plots of effects of nitroglycerin infusion alone (○, n=7) or in combination with hydralazine (▼, n=8) on left ventricular hemodynamics in rats with heart failure. Nitroglycerin was infused at 10 μg/min in both groups. Hydralazine was administered at 1.5 and 2.0 hours (0.1-mg bolus) (H) during nitroglycerin infusion. Nitroglycerin alone produced initial reductions in left ventricular end-diastolic pressure (LVEDP) but no effect on left ventricular peak systolic pressure (LVPSP). LVEDP returned to baseline by 8 hours, indicating tolerance development. Hydralazine caused significant reductions in LVPSP, which was maintained during remainder of nitroglycerin infusion. Hemodynamic tolerance to nitroglycerin did not occur during coadministration. Mean±SEM values are shown. *Statistically significant differences from baseline ($p<0.05$).

**Figure 3.** Plot of effect of hydralazine (HYD) on nitroglycerin (NTG) tolerance induced in vitro. Preincubation of rat aortic rings with NTG (0.22 mM) caused a significant shift in concentration/effect curve to the right. This development of vascular tolerance was not affected by presence of HYD (1.0 mM) in preincubation solution. No differences between NTG and the NTG plus HYD groups were detected.
Discussion

Although nitrate tolerance is a well-recognized clinical problem, the underlying mechanism of tolerance development has not been fully defined. In vitro studies have shown that incubation of vascular segments with high concentrations of nitroglycerin causes a reduced relaxation response.\textsuperscript{10} Based on a number of in vitro studies, Needleman and Johnson\textsuperscript{11} in their classic "sulfhydryl depletion" hypothesis, first proposed that nitrate tolerance is a result of depletion of critical intracellular sulfhydryl pools, leading to a loss of vasodilatory response. However, conflicting data exist that argue against the validity of this hypothesis.\textsuperscript{12} Reduction in the vascular metabolic activation of nitrates\textsuperscript{12} and/or diminished guanylate cyclase activation\textsuperscript{13} have also been proposed as mechanisms of specific vascular tolerance. Based on the literature, these hypotheses of nitrate tolerance, based primarily on in vitro data, have gained wide acceptance. However, the concentrations used to produce in vitro vascular tolerance vastly exceed the typical plasma concentrations of nitrates observed in patients with heart failure. In addition, these in vitro findings were obtained in the absence of systemic regulatory mechanisms, which could play an important role in modulating vascular activity. Thus, the applicability of these in vitro mechanisms of nitrate tolerance to the in vivo situation can be questioned.

Another possible hypothesis for the mechanism of nitrate tolerance suggests that chronic nitrate administration evokes the activation of compensatory processes responsible for regulating systemic hemodynamics, involving, for example, the sympathetic nervous system and/or the renin-angiotensin-aldosterone axis. Studies in CHF patients have shown that various neurohormonal changes can occur during long-term nitrate therapy. Elevation in plasma renin activity, decreases in hematocrit, and increases in body weight have all been observed during continuous long-term nitroglycerin therapy, suggesting that nitrate tolerance in vivo may result, at least in part, from systemic compensatory changes.

The successful development of an animal model to study in vivo nitrate tolerance in heart failure affords an opportunity to examine tolerance mechanisms without clinical restrictions and complications. Such complications include those incurred by the coadministration of diuretics and digitalis preparations, which can confound data interpretation, and difficulties encountered in performing invasive hemodynamic monitoring for extended durations. An animal model also provides the opportunity to conduct in vitro studies with isolated blood vessels in parallel. In this article, we show that nitrate tolerance in left ventricular hemodynamics can be induced by an infusion regimen of 10 \(\mu\)g/min (or approximately 18 \(\mu\)g/kg/min) of nitroglycerin. Although this dose is extrapolated to be approximately 1.3 mg/min for a 70-kg person, the hemodynamic effects produced in this animal model (i.e., about 40–50\% reduction in LVEDP without substantial change in heart rate) are similar to those seen with nitroglycerin therapy in CHF patients.\textsuperscript{3} The difference in the doses used is probably a result of interspecies differences in hemodynamic sensitivity toward nitrates.

Intravenous administration of hydralazine alone to CHF rats produced significant reductions in LVSP and no change in LVEDP. These results are consistent with investigations in heart failure patients\textsuperscript{15} and in an identical rat model of heart failure,\textsuperscript{16} showing that hydralazine causes reductions in blood pressure and increases in cardiac output with little or no effect on the venous circulation. Although hydralazine is commonly administered to CHF patients orally, we chose to use intravenous administration, thus allowing the titration to a predetermined effect. The effects of oral hydralazine administration in our animal preparation have not been determined.

We have shown earlier\textsuperscript{7} that SNAP, an S-nitrosothiol nitrovasodilator that produced significant effects on LVSP and LVEDP, did not exhibit tolerance in LVEDP in CHF rats after 10 hours of continuous infusion. Consistent with this finding, the hydralazine/nitroglycerin combination, which produced both afterload and preload effects, also appeared to avoid the development of nitrate tolerance in vivo. The results of these studies may suggest that balanced arterial and venous actions of a vasodilator, or a vasodilator combination, could be an important determinant of both subchronic and long-term efficacy. This view is supported by clinical evidence that the combination treatment of hydralazine plus isosorbide dinitrate\textsuperscript{4} or enalapril alone\textsuperscript{17} can provide long-term improvements in mortality and morbidity in CHF patients. We have not performed any experiments examining the effects of repeated dosing or more prolonged infusions in this animal preparation to determine the duration of this favorable hydralazine/nitroglycerin drug interaction.

Our data confirmed previous findings\textsuperscript{18} that nitrate tolerance is probably not a result of changes in drug pharmacokinetics. Administration of hydralazine did not alter steady-state nitroglycerin concentrations, and the values observed here are similar to those observed when nitroglycerin was infused alone. Plasma concentrations of dinitrate metabolites were also similar to those resulting when nitroglycerin was infused alone\textsuperscript{7} (data not shown).

Our results also suggest that the beneficial effects of hydralazine on nitroglycerin-induced tolerance were not a result of localized vascular interaction between the two drugs. Preincubation of hydralazine with nitroglycerin, at a hydralazine concentration that mimicked the in vivo situation, did not counteract the tolerance-inducing effect of nitroglycerin in vitro. The fact that hydralazine prevented nitroglycerin tolerance in vivo but not in vitro suggests that hemodynamic nitrate tolerance may be better related to systemic neurohormonal compensatory changes than to vascular biochemical alterations. The lack of a localized interaction between hydralazine and nitroglycerin may not be surprising because these two vasodilators operate through two apparently independent biochemical path-
ways. Nitrates are known to undergo metabolic conversion to nitric oxide, which activates guanylate cyclase and leads to the accumulation of cyclic GMP. Sulphydryl compounds are thought to be involved in these reactions. The biochemical mechanism of action of hydralazine is less clear, but its hypotensive action may operate through interaction with pyridoxal.19

Why is a balanced preload-and-afterload effect beneficial in overcoming the development of nitrate tolerance? We do not have a clear answer but can offer a speculation. Leier et al15 have shown that isosorbide dinitrate alone appeared to decrease renal blood flow by an average of approximately 200 ml/min in 15 patients with low-output CHF. Flaim et al20 also showed, in a rat model of heart failure similar to ours, that nitroglycerin infusion (2–8 μg/kg/min) produced decreases in renal blood flow by approximately 20%. This nitrate-induced decrease in renal perfusion may lead to activation of renal neurohumoral factors, including the sympathetic and renin-angiotensin-aldosterone systems, in an attempt to preserve renal blood flow. In the same patient population mentioned above, Leier et al15 showed that hydralazine, when administered alone or in combination with isosorbide dinitrate, increased renal blood flow by about 100 ml/min. This increase in renal blood flow was probably secondary to an increase in cardiac output caused by hydralazine because hepatic and limb blood flow were also increased. A possible explanation for the beneficial effects of hydralazine on nitroglycerin-induced tolerance could then be the preservation of renal perfusion, thus avoiding the activation of renal compensatory neurohumoral adjustments. Interpretation of our data would have been made easier if other central or regional hemodynamic parameters were also available, but our current surgical techniques were not developed to such a stage as to permit these measurements in a conscious and unrestricted small animal. Further study of the role of renal perfusion in the maintenance of nitrate efficacy is required before this speculation can be confirmed.

In conclusion, our results indicate that hydralazine can preserve the preload effects of nitroglycerin in a rat model of CHF without the development of hemodynamic tolerance. This beneficial effect may explain, at least in part, the favorable mortality and morbidity effects of the Veterans Administration Cooperative Study V-HeFT hydralazine/isosorbide dinitrate trial in heart failure,4 despite the fact that isosorbide dinitrate was administered in a dosage regimen that (alone) was likely to produce nitrate tolerance. Further confirmation of the tolerance-sparing effects of hydralazine on nitrates in clinical heart failure is, however, needed before this result in experimental animals can be extended to humans.

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References


KEY WORDS • nitrates • vasodilators • drug interactions
Concurrent hydralazine administration prevents nitroglycerin-induced hemodynamic tolerance in experimental heart failure.

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