Pharmacodynamic Models of Nitroglycerin-Induced Hemodynamic Tolerance in Experimental Heart Failure

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Pharmacodynamic tolerance during continuous nitroglycerin (NTG) infusion is a significant limitation of nitrate therapy. The mechanism of this phenomenon is not well-understood but may involve physiologic compensation which involves vasoconstriction. We have obtained pharmacodynamic data on NTG-induced hemodynamic tolerance in a rat model of congestive heart failure (CHF), which we have shown to mimic human behavior toward NTG *in vivo*. In this report, we developed two mechanism-based pharmacokinetic/pharmacodynamic models to describe the time-dependent effects of NTG infusion on left ventricular end-diastolic pressures (LVEDP) in CHF rats and compared their abilities to describe the observed hemodynamic data. Both mathematical models introduced a counter-regulatory vasoconstrictive effect as a result of NTG-induced vasodilation and assumed the magnitude of this effect to be driven by the extent of the initial hemodynamic effect produced by NTG. The decay of this counter-regulatory effect was described by a first-order process in both models. A model that assumed vasoconstriction to develop via two sequential first-order processes was statistically superior in describing the data, when compared to one that assumed a single first-order process and a lag phase. Both models provided similar estimates of the half-life for the disappearance of the vasoconstriction ($t_{1/2}$ of vasoconstriction: 128min *vs.* 182min, respectively), and both predicted rebound elevations of LVEDP after abrupt NTG withdrawal. These results are consistent with a counter-regulatory mechanism of NTG-induced hemodynamic tolerance and suggest that such an approach may be useful for modeling other tolerance phenomena as well.

KEY WORDS: nitroglycerin; vasodilator tolerance; pharmacodynamic modeling.

INTRODUCTION

Nitroglycerin (NTG) is an organic nitrate vasodilator which is used in the treatment of various forms of angina and congestive heart failure (CHF). Acute administration of NTG typically provides rapid relief of angina symptoms, beneficial reductions in pulmonary and venous congestion, and improvements in myocardial oxygen consumption[1,2]. Despite these favorable hemodynamic characteristics, prolonged nitrate administration leads to the development of pharmacodynamic tolerance, which has been observed in both angina and heart failure therapy[2,3,4]. The mechanism of nitrate tolerance is currently unknown, but it may arise from physiologic compensatory mechanisms and/or a reduction of vascular metabolic activation[5]. Intermittent NTG dosing using a 12 hr on/12 hr off schedule has been shown to prevent or reduce the development of tolerance[6] and is the currently recommended dosing strategy[3]. However, this approach is less than ideal since the patient is left unprotected during the "off" interval, and abrupt NTG withdrawal may precipitate events of hemodynamic rebound[7,8].

We have recently shown a rat model of CHF to be useful for the investigation of the mechanisms of nitrate tolerance[9] and for the examination of the hemodynamic action and tolerance properties of other nitro-vasodilators[10]. This animal model has been shown to mimic many of the physiologic changes which exist in human heart failure[9,11]. We have previously shown that intravenous infusion of NTG to the CHF rat produced similar hemodynamic effects to those observed in CHF patients, viz. significant reductions in left ventricular end-diastolic pressure, with little change in heart rate or arterial pressures, but these initial effects were not maintained throughout a continuous 10-hr infusion[9,10]. This tolerance development was also similar to that observed in CHF patients receiving NTG via either the intravenous[12] or transdermal[13] routes. It therefore appears that this animal model may be useful in developing a better understanding of the phenomenon of nitrate tolerance and for the development of useful strategies to avoid this problem, either through pharmacologic means or by improvements in dosage regimen design.

While the phenomenon of NTG-induced hemodynamic tolerance is well established, no mathematical model has appeared in the literature which attempts to describe the time-courses of the kinetic events relating to tolerance development and its subsequent decay. Availability of such a mathematical model, or models, might permit additional insight into the system and should provide a more rational approach toward devising better dosing regimens for nitrates. In this report, we developed two mechanism-based pharmacodynamic models to describe *in vivo* nitrate tolerance and compared their ability to describe the observed data.

MATERIALS AND METHODS

HEMODYNAMIC DATA IN RATS WITH HEART FAILURE:

Left ventricular pressure tracings were recorded in CHF rats via a catheter placed in the left ventricle, as previously described[9,10], thus allowing the measurement of left ventricular end-diastolic pressure (LVEDP). This parameter is a useful index of venous pressures (preload) and is significantly elevated in CHF rats and patients[1,2,10,11]. Since NTG is considered a predominant venodilator[1,2], changes in LVEDP represent meaningful indices of therapeutic efficacy.

Most of the hemodynamic data used for the pharmacodynamic modeling presented here have been previously published by us in two reports. In the first study ("continuous infusion protocol") we infused NTG to CHF rats at 10 μg/min for 10 hrs and measured left ventricular hemodynamics, including LVEDP, throughout the experiment[9]. We found that the initial hemodynamic effects of NTG infusion were

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not maintained throughout the experiment, indicating tolerance development. In a subsequent study ("stepwise infusion protocol"), we performed a dose-ranging study in which we compared the left ventricular hemodynamic effects and tolerance properties of NTG to those of an experimental S-nitrosothiol compound[10]. Stepwise NTG infusions of 3, 5, and 8 μg/min were carried out for 30 minutes each and then at 10 μg/min for 10 hrs. Left ventricular pressures were measured at 20 min and 30 min during each infusion rate and periodically throughout the 10 hrs at 10 μg/min. Twenty one hemodynamic data sets were fit individually by each pharmacodynamic model (15 rats received the continuous infusion protocol, 6 rats received the stepwise infusion protocol). All experiments were carried out in conscious, unrestrained rats.

NTG CONCENTRATIONS:

Plasma concentrations of NTG and dinitrate metabolites were determined during NTG infusion (stepwise infusion protocol) in a separate group of 5 CHF rats (535-550g, weights similar to CHF rats used in the hemodynamic experiments). NTG was infused via a left femoral vein catheter, and blood samples were taken from a catheter placed at the aortic arch via the right carotid artery. NTG was infused at 3, 5, 8, and 10 μg/min in an identical fashion to the hemodynamic study described above. Blood samples (0.4 ml) were taken at 20 and 30 min during each infusion and also at 3 hr and 8 hr during the 10 μg/min infusion. After blood collection, plasma was rapidly isolated and red blood cells were resuspended in saline and readministered to the rats to avoid extensive loss of blood cells during the experiment. Plasma samples were stabilized with 1N silver nitrate and stored frozen in silanized glass to avoid drug adsorption. Assay of NTG and dinitrate metabolite concentrations was performed using gas chromatography and electron capture detection, as previously described[10,14]. The pharmacokinetic data were used for estimates of the first-order elimination rate constant (k_{el}), plasma clearance (CL) and volume of distribution (V).

PHARMACOKINETIC-PHARMACODYNAMIC MODELING:

Two pharmacodynamic models were examined for their ability to describe the behavior of NTG-induced hemodynamic tolerance during continuous intravenous infusion. These models are shown in schematic form in Figure 1, and they differ primarily in the rate processes of counter-regulation (see later). Both models incorporate a classical one-compartment pharmacokinetic model which describes NTG plasma concentrations during and after intravenous infusion (k_0). Linked to this pharmacokinetic model is a pharmacodynamic model that relates NTG concentrations to the observed pharmacodynamic effect. Both models assume that NTG produces a vasodilatory effect which is directly related to NTG plasma concentration. We have proposed that this NTG-induced vasodilatory effect, rather than NTG concentrations in either the central compartment or a hypothetical compartment, is the driving force which activates the nitrate tolerance mechanism. This assumption implies that the dominant mechanism of NTG tolerance is due to physiologic compensatory mechanisms. Thus, when a vasodilating effect

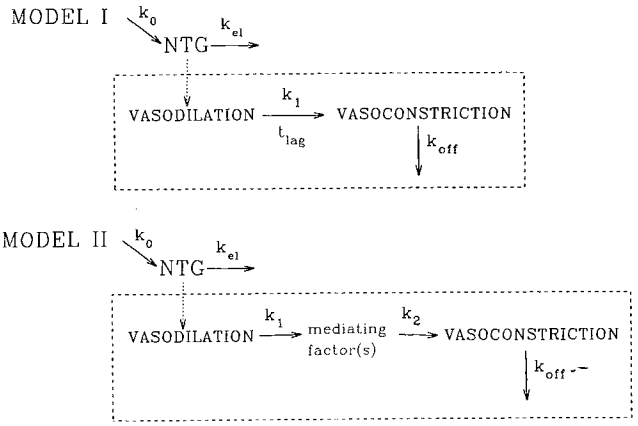

FIGURE 1. Schematic representation of two pharmacokinetic-pharmacodynamic models proposed for describing NTG-induced hemodynamic tolerance.

is produced, the body produces a counter-regulatory force in an attempt to negate it. According to these models, the overall hemodynamic effect of NTG (E_{NET}) at any time during or after infusion is the sum of the direct NTG-induced vasodilatory effect (E_D) and the opposing compensatory vasoconstrictive effect (E_C). Expressed as percentages:

$$E_{NET} = 100\% - E_D + E_C \quad (1)$$

where E_{NET}, E_D and E_C are effects expressed as percent of the initial baseline LVEDP. Since there are no mass or concentration units associated with the counter-regulatory vasoconstrictive effect, this can be envisioned as a process, the onset and offset of which defines the appearance and disappearance of NTG tolerance. The two models examined differ only in the activation process describing the onset of the indirect vasoconstrictive effect (Figure 1). In model I, this process was described by a first order rate constant (k_1) and a lag time (t_{lag}). In model II, the vasoconstrictive process is believed to evolve through two sequential steps which could be described by two consecutive first-order rate constants, k_1 and k_2, respectively. In both models, the offset of the vasoconstrictive force was described by a first order rate constant, k_{off}. We have assumed the direct vasodilatory effect of NTG (E_D) to be linearly related to NTG plasma concentrations, C_{NTG}, thus:

$$E_D = m \cdot C_{NTG} \quad (2)$$

where m is a proportionality constant. A more complex relationship, such as a sigmoidal Emax (Hill-type) equation, could have been employed but our data sets were not sufficiently extensive to justify the added parameters associated with such an equation. Furthermore, a maximal effect model may be unidentifiable in the presence of tolerance since a plateau of the pharmacologic effect may be due either to reaching a maximal effect or the development of tolerance[15].

For each model the differential equations describing the behavior of the indirect vasoconstrictive effect were solved using Laplace transforms. According to model I, the vasoconstrictive effect at any time, *t*, during NTG infusion can be described as (see Appendix for derivations):

$$E_C=\frac{k_0\cdot k_1\cdot m}{V}\left(\frac{1}{k_{el}\cdot k_{off}}-\frac{e^{-k_{el}(t-t_{lag})}}{k_{el}(k_{off}-k_{el})}-\frac{e^{-k_{off}(t-t_{lag})}}{k_{off}(k_{el}-k_{off})}\right) \quad (3)$$

whereas according to model II the same effect can be described by:

$$E_C=\frac{k_0\cdot m\cdot k_1\cdot k_2}{V}\left(\frac{1}{k_{el}\cdot k_2\cdot k_{off}}-\frac{e^{-k_{el}\cdot t}}{k_{el}(k_2-k_{el})(k_{off}-k_{el})}-\frac{e^{-k_2\cdot t}}{k_2(k_{el}-k_2)(k_{off}-k_2)}-\frac{e^{-k_{off}\cdot t}}{k_{off}(k_{el}-k_{off})(k_2-k_{off})}\right) \quad (4)$$

Data Analysis:

Because of the possible effects of blood sampling on blood pressures in conscious unrestrained rats, the pharmacokinetic and pharmacodynamic data were obtained from different animals. NTG plasma concentrations from the stepwise infusion experiments were used to provide estimates of k_{el}, CL, and V in CHF rats. The pharmacokinetic data from these five CHF rats were simultaneously fitted with Equation 5, using PCNONLIN (version V02, Statistical Consultants, Lexington, KY).

$$C_{NTG}=\frac{k_0}{CL}(1-e^{-k_{el}\cdot t}) \quad (5)$$

Volume of distribution (V) was determined as a secondary parameter and calculated by CL/k_{el}. The resulting mean pharmacokinetic parameter estimates were then used (as constants) to fit the pharmacodynamic models. Hemodynamic data from each individual rat were fitted independently to each pharmacodynamic model (model I and model II). Pharmacodynamic tolerance data were gathered from a total of 15 rats which received a continuous 10 μg/min infusion of NTG and 6 rats which received the stepwise infusion protocol described above (total n=21). At least 10 data points describing LVEDP effects during NTG infusion were available for each individual pharmacodynamic data set. Four parameters were estimated for each of the models of NTG tolerance (model I: m, t_{lag}, k_1, k_{off}, model II: m, k_1, k_2, k_{off}).

RESULTS

Figure 2 shows the plasma NTG concentrations observed in 5 CHF rats during stepwise infusions of NTG at 3, 5, and 8 μg/min, each 30 minutes in duration, followed by constant infusion at 10 μg/min for the next 8 hours. Plasma NTG concentrations were determined at 20 and 30 min during the step-up phase and at 3 and 8 hr during the continuous infusion at 10 μg/min. The line drawn through these data points represents the computer-fitted curve. By fitting all concentration data simultaneously, population estimates of k_{el} (0.127 ± 0.034 min^{-1}), CL (279 ± 9.2 ml/min) and V (2.2 ± 0.53 liters) were obtained. The first-order elimination half-life was estimated to be 5.5 ± 1.4 minutes. These pharmacokinetic parameters showed good agreement with previously reported values for rats[16,17]. Similar estimates were obtained when individual rat data were fit to the pharmacokinetic model (k_{el} 0.170 ± 0.094 min^{-1}, CL 292 ± 13 ml/min, V 2.2 ± 1.0 liters, mean ± SD, n=5).

Figure 3 shows the LVEDP data from 2 representative

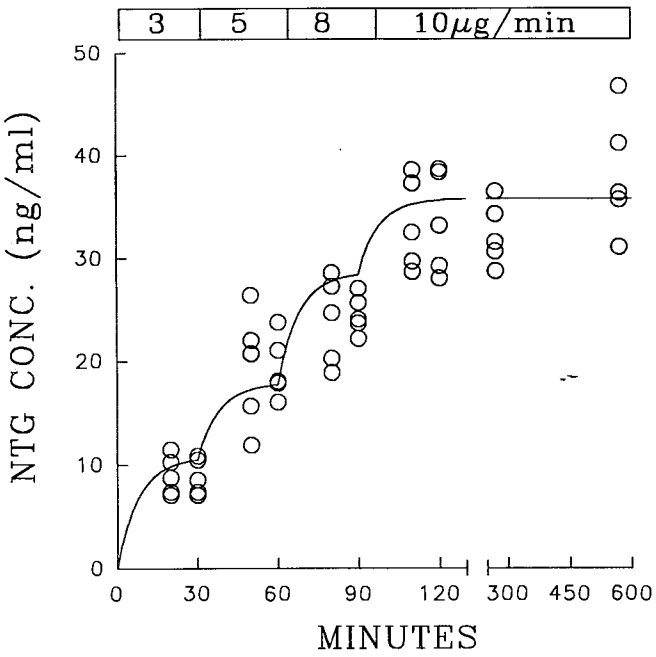

FIGURE 2. Plasma NTG concentrations observed in 5 CHF rats during stepwise infusions of NTG. NTG was infused at 3, 5, and 8 μg/min for 30 minutes each and then at 10 μg/min for 8 hr. Plasma concentrations for each animal are shown as well as the fitted line describing the data according to equation (5). See text for parameter values.

rats (one rat from the stepwise infusion protocol, one rat from the continuous infusion protocol) and the fitted curves from model I (dotted line) and model II (solid line). These results showed that both models were able to describe the time-course of the observed hemodynamic data. Coefficients of variation of the individual parameter estimates during the fitting process ranged from 5-15% for m (both models), 10-20% for t_{lag} (model I), 30-70% for k_1 and k_2 (both models) and 20-40% for k_{off} (both models). In 19 of the 21 sets of hemodynamic data examined, a direct comparison of the sum of squared residuals from each model showed that model II provided lower residual error and a qualitatively better fit. The differences in the sum of squared residuals between the two models were found to be statistically significant (mean ± standard deviation, model I:756 ± 484, model II:710 ± 496, $p<0.01$, paired t-test). This comparison appeared valid since the number of parameters was the same in the two models examined.

Table 1 shows the mean values of the parameter estimates, and the population 95% confidence intervals of the parameters, obtained from fitting data from each individual animal (n=21 rats) to models I and II. Although model II consistently provided a slightly superior fit to that of model I, the mean parameter estimates of m and k_{off} were similar when calculated from either model (m values of 1.45 *vs.* 1.42, and k_{off} values of 0.00381 *vs.* 0.00542, for models I and II respectively). The mean disappearance half-life of the vasoconstrictive force (k_{off}) was estimated to be 182 min and 128 min from model I and II respectively.

Figure 4 shows a computer simulation of the various hemodynamic effects described in the pharmacodynamic

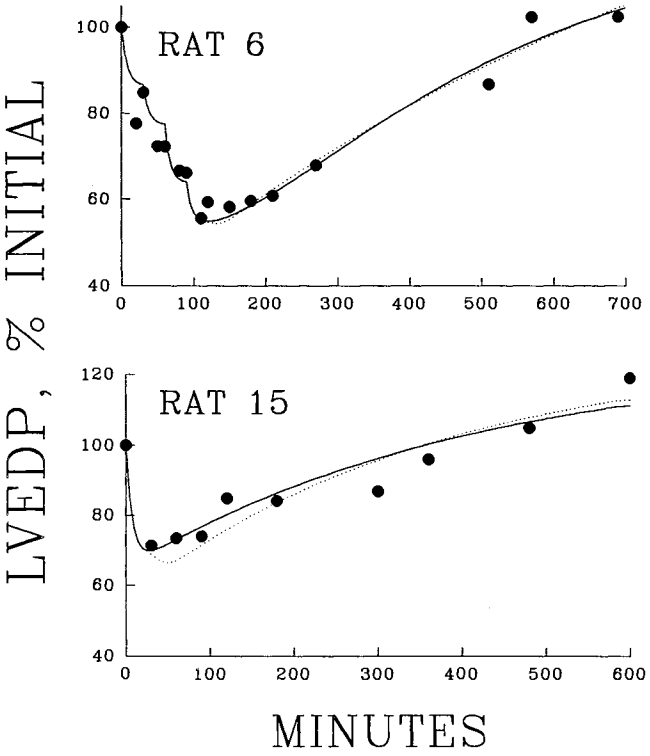

FIGURE 3. Changes in LVEDP during NTG infusion in two representative CHF rats. Rat 6 received a stepwise infusion regimen (see methods), while rat 15 received a continuous NTG infusion at 10 μg/min for 10 hrs. The fitted curves are shown for model I (dotted line) and model II (solid line).

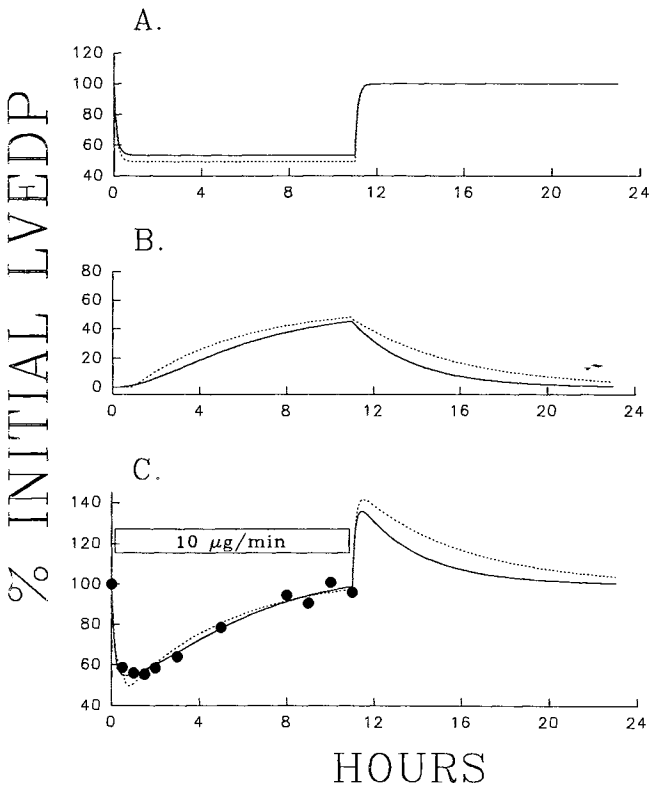

FIGURE 4 Computer simulation of the various hemodynamic effects described by the two pharmacokinetic-pharmacodynamic models, when applied to mean hemodynamic data (solid circles in Panel C). Panel A: direct vasodilation (E_D), Panel B: indirect vasoconstriction (E_C), Panel C: net hemodynamic effect (E_{net}). Model I, dotted line; model II, solid line.

models. The time-courses of the two opposing effects (direct vasodilation and indirect vasoconstriction) during and after NTG infusion are shown separately (panels A and B, respectively). These opposing effects are combined to provide the overall effect, as shown in panel C (Figure 4). The data points shown are the mean values from 15 rats that received a continuous infusion of NTG at 10 μg/min. As evident from Figure 4, both models predict a gradual development of the vasoconstrictive effect during NTG infusion, while the vasodilatory effect of NTG remains constant. The overall behavior provided by the two models during infusion are, by and large, similar. Upon stopping the NTG infusion both models predict a rebound increase in LVEDP, although model I predicted a larger and slightly longer rebound than that predicted by model II. The presence of hemodynamic rebound can be explained by the longer disappearance half-life of the vasoconstriction effect (predicted by both models), relative to the rapid disappearance of the NTG vasodilatory effect, which, in turn, is due to the short pharmacokinetic half-life of NTG.

TABLE 1. Mean values and 95% confidence intervals of the parameter estimates determined from individual rat data (n=21). Statistical comparison of residuals showed that model II provided a significantly better fit ($p<0.05$, see results section).

PARAMETER	MEAN VALUE	95% CONFIDENCE INTERVAL
	MODEL I	
m (%/ng/ml)	1.45	1.33–1.57
t_{lag} (min)	43.8	38.9–48.6
k_1 (min^{-1})	0.00436	0.00306–0.00566
(t½, min)	(159)	(122–227)
k_{off} (min^{-1})	0.00381	0.00266–0.00495
(t½, min)	(182)	(140–261)
	MODEL II	
m (%/ng/ml)	1.42	1.29–1.55
k_1 (min^{-1})	0.00703	0.00523–0.00884
(t½, min)	(99)	(78–133)
k_2 (min^{-1})	0.19392	0.07976–0.30808
(t½, min)	(3.6)	(2.3–8.7)
k_{off} (min^{-1})	0.00542	0.00409–0.00675
(t½, min)	(128)	(103–170)

DISCUSSION

We have previously shown the CHF rat to be a useful model for the study of NTG tolerance[9,10,18]. This apparent tolerance cannot be explained by changes in drug pharmacokinetics, since steady-state plasma concentrations remain unchanged during NTG infusion[10,13]. Here we have attempted to describe the process of NTG-induced tolerance, observed during continuous infusion in the CHF rat, by means of two similar pharmacokinetic-pharmacodynamic

models. These models were examined to determine their capabilities in describing our observed data.

Pharmacodynamic tolerance can be defined as the reduction of a drug-induced effect during repeated or continuous drug exposure. This phenomenon may arise via desensitization of the target site (e.g. down regulation or alterations of receptor populations, uncoupling of receptor-effector systems, depletion of cofactors or messengers)[19,20]. Alternatively, the apparent tolerance may be due to systemic adaptations by physiologic systems (e.g. neurohormonal counter-regulation)[21]. In the modeling of our data, we assumed that NTG produced a direct vasodilatory or hypotensive effect which was unchanged during infusion so long as the plasma concentration remained constant. We also assumed that a counter-regulatory vasoconstrictive force was responsible for the development of NTG tolerance and that this vasoconstriction was driven by the vasodilating effect of NTG administration. These assumptions appeared to be physiologically relevant, since a variety of neurohormonal systems (for example, the adrenergic and renin-angiotensin systems) involved in hemodynamic regulation are often activated as a result of blood pressure reduction via pharmacologic or mechanical means[21]. Our reported data on the tolerance-sparing effect of hydralazine on NTG-induced hemodynamic tolerance are consistent with the view that *in vivo* NTG tolerance is mediated through physiologic neurohormonal compensation, rather than specific vascular desensitization[18].

Estimation of NTG pharmacokinetic parameters was performed using data from 5 CHF rats receiving a stepwise infusion protocol. Computer curve fitting provided pharmacokinetic estimates of k_{el}, CL, and V which were in good agreement with previously reported values in normal rats[16,17]. This concordance suggested the presence of CHF did not cause an observable difference in these parameters, at least as measured from arterial blood.

In modeling the observed NTG tolerance we assumed that metabolites do not contribute materially to the LVEDP effects and the degrees of tolerance developed. The dinitrate metabolites are vasodilators *in vitro,* with potencies much less (10 to 100-fold) than the parent NTG[22,23,24]. Gumbleton and Benet have also demonstrated pharmacologic activity of 1,2-GDN and 1,3-GDN after oral administration to normal volunteers[25]. Therefore, there is good reason to believe that under acute NTG dosing metabolites may contribute to the observed pharmacologic activity, particularly at later time points when metabolite concentrations are high[22]. However, in this study continuous NTG dosing caused metabolite concentrations to rise while the pharmacologic activity declined toward baseline. Under these conditions any positive activity of the metabolites is overcome by the tolerance mechanism produced, not only by the parent drug, but also by the metabolites themselves. If the positive effects of the metabolites were to be included in the current models, then their "negative" effects (viz: those involved in tolerance induction) should be included as well. Unfortunately, such a comprehensive approach is not presently feasible because the individual metabolite activity in lowering LVEDP and the tolerance dynamics of each of the four potentially active metabolites (2 dinitrates and 2 mononitrates) have not been determined. Such determination is not possible to achieve using our current experimental conditions; preliminary experiments with a lower potency nitrate, isosorbide-5-mononitrate, suggested that large volumes of fluid, or an organic solvent vehicle, would have to be introduced to allow for the greater amount of drug needed to produce a similar hemodynamic effect. Infusion of greater fluid volumes (or cosolvent vehicle) would be difficult in these already hemodynamically compromised animals, and would necessitate a completely new set of control experiments. Thus, the pharmacokinetic-pharmacodynamic models, though theoretically imperfect because they ignore the contribution of metabolites, are the simplest mechanism-based models that can satisfactorily describe the current set of data.

The two models examined here differ only in the description of the relationship between the direct hypotensive effect of NTG and the vasoconstrictive counter-regulation. Model I assumed that there was a single first-order process between these two effects and that a lag time was required prior to the significant development of counter-regulation. Model II assumed, on the other hand, that two sequential first-order processes are involved in the stimulation of the vasoconstrictive mechanism. Both models appeared to describe the data well, although model II was shown to be superior statistically. The better performance of model II is likely to be due to the use of a triexponential equation (as opposed to the biexponential for model I) to describe the escalation of the vasoconstrictive effect during NTG infusion, allowing a more gradual sigmoidal shape of the tolerance curve (see Fig 4). Intuitively, it also appeared more physiologically "reasonable" to consider the vasoconstriction process as a series of sequential steps, since many hemodynamic regulatory systems, such as the sympathetic nervous system and the renin-angiotensin system, typically include a cascade of steps prior to activation and vasoconstriction.

Interestingly, both models provided similar estimates for both m and k_{off}. Model I and II estimated the half-life for the disappearance of the vasoconstriction to be 182 and 128 min (3.0 and 2.1 hrs) respectively. Since intermittent NTG therapy is the currently recommended method to avoid or reduce tolerance development[4], this parameter may have importance in determining the optimal dosing intervals. The 2 hr half-life for the vasoconstrictive counter-regulation suggested that after 4-5 lives, or 8-10 hrs, full sensitivity to NTG should be regained. We showed previously that hemodynamic sensitivity to NTG can be regained in this animal model by providing a 12-hr drug-free period[9]. Clinical data also suggested that a 12-hr drug-free interval was capable of restoring NTG responsiveness, but shorter intervals of 4-8 hrs were not[26].

Hemodynamic rebound after abrupt NTG withdrawal is a recognized clinical problem and has been observed in NTG-tolerant CHF patients[6]. Rebound increases in vascular resistence and venous pressures and decreases in cardiac output have been observed in CHF patients after abrupt withdrawal of NTG infusion[7,8]. These hemodynamic rebound events appear rapidly after NTG withdrawal (within 2 hrs after stopping the infusion) and are transient in nature[7]. We have also observed that rebound increases in LVEDP occur in CHF rats after a short-term (90 min) NTG infusion

and that this rebound could be avoided by graded reductions in the NTG infusion rate[27]. Consistent with these experimental and clinical observations is the fact that both pharmacodynamic models examined here predicted rapid but transient rebound elevations in LVEDP after terminating the NTG infusion.

Several pharmacodynamic models describing pharmacological tolerance have appeared in the literature. Using an integrated pharmacokinetic-pharmacodynamic approach, Chow *et al.* have modeled the effects and tolerance development of cocaine on heart rate following intravenous injection[28]. The tolerance process was described by requiring a reduction in the plasma concentration-effect relationship (corresponding to our slope parameter, m) throughout drug exposure. This reduction was achieved by multiplying the slope term by a function with monoexponential decay which had an initial value of 1.0. This model implied that if a steady-state drug concentration was maintained long enough, the pharmacologic effect must disappear completely (i.e., complete tolerance was a requirement). Complete tolerance to NTG was observed in our study, although our proposed models are not limited by this requirement. In addition, Chow's model would not predict the development of rebound after abrupt drug withdrawal, did not allow for different doses or infusion rates during tolerance development, and did not allow for the washout of the tolerance effect when the drug has completely disappeared. Such a model, therefore, could not adequately describe the present data.

Hammarlund *et al.* presented a model similar to that of Chow *et al.* to describe the development of tolerance to the diuretic effect of furosemide[29]. In their model a sigmoidal Emax pharmacologic model was modified to allow the sensitivity (the furosemide excretion rate at half-maximal effect) parameter to decrease exponentially during drug exposure. This model has the same limitations for the present data as those described for the model of Chow *et al.*, although this model does not assume complete tolerance following continuous drug administration.

Zahler *et al.* proposed a model describing the chronotropic effects of cocaine, using a statistical technique known as "distributed lags"[30]. One advantage of this modeling approach is that it is capable of handling a variety of input functions, but its highly empirical nature and the lack of any physiologically relevant parameters are significant limitations.

A physiologic tolerance model has been proposed by Eckblad and Licko, in which the rate of conversion of a hypothetical substance X to a substance Y, is influenced by concentrations of agonist (drug)[31]. The authors suggested that the hypothetical substances can be viewed as unbound receptors being transformed to occupied receptors. The model assumes a constant production of substance X, and first-order degradation of both X and Y (representing degradation of receptors and down-regulation of occupied receptors, respectively). If the pharmacodynamic effect is related to the amount of Y, a transient response will be predicted in the presence of constant drug concentrations. This model is similar to ours, in that it has the ability to handle various drug input rates and also predicts pharmacologic rebound after drug removal. However, the emphasis on receptor down-regulation as the primary mechanism of tolerance is less suited to the present data, since compensatory neurohormonal activation, rather than receptor changes, has been primarily implicated in nitrate tolerance *in vivo*[18].

More recently, Porchet *et al.* reported a pharmacodynamic model describing the tolerance properties of nicotine[15]. This model employed the generation of a hypothetical "metabolite" of nicotine which acted as an antagonist to the positive chronotropic effects of nicotine. While this model adequately described the development of nicotine tolerance, it assumed that the onset and offset of the tolerance process were governed by a single rate constant. This model also could not accommodate the occurrence of pharmacologic rebound after abrupt withdrawal. We therefore did not consider this model to be appropriate for describing NTG tolerance.

In summary, we have tested two mechanism-based pharmacokinetic-pharmacodynamic models which could describe the behavior of NTG-induced hemodynamic tolerance in the CHF rat. A model linking the vasodilating effect of NTG to the production of vasoconstrictive counterregulation by two sequential first-order steps appeared to describe our observed tolerance data well. This approach is consistent with the known actions of NTG with respect to tolerance development during infusion and hemodynamic rebound upon abrupt drug withdrawal and may be applicable to other vasodilating drugs as well. The general form of this model can accommodate changes in drug input, allowing the flexibility of examining the effects of various dosing schemes on the development of tolerance and perhaps allowing the rational design of better dosage regimens to overcome this phenomenon.

APPENDIX

DERIVATION OF EQUATION (3):

According to the scheme of model I

$$\frac{dE_C}{dt}=k_1 \cdot E_D - k_{off} \cdot E_C \tag{A1}$$

Since $E_D = m \cdot C_{NTG}$, the above equation becomes

$$\frac{dE_C}{dt}=k_1 \cdot m \cdot C_{NTG} - k_{off} \cdot E_C \tag{A2}$$

The Laplace transform of this equation is

$$s\overline{E_C} - E_{C0} = k_1 \cdot m \cdot C_{NTG} - k_{off} \cdot \overline{E_C} \tag{A3}$$

where E_{C0}, the initial vasoconstrictive effect, is assumed to be zero. The Laplace transform for NTG plasma concentrations during infusion is

$$\overline{C_{NTG}} = \frac{k_0}{V(s)(s + k_{el})} \tag{A4}$$

By substituting equation (A4) and rearrangement of equation (A3), we obtain

$$\overline{E_C} = \frac{k_1 \cdot m \cdot k_0}{V(s)(s+k_{el})(s+k_{off})} \tag{A5}$$

Solving for E_C and inserting a lagtime (t_{lag}) gives

$$E_C=\frac{k_0\cdot k_1\cdot m}{V}\left(\frac{1}{k_{el}\cdot k_{off}}-\frac{e^{-k_{el}(t-t_{lag})}}{k_{el}(k_{off}-k_{el})}-\frac{e^{-k_{off}(t-t_{lag})}}{k_{off}(k_{el}-k_{off})}\right) \quad \text{(A6)}$$

DERIVATION OF EQUATION (4):

According to model II

$$\frac{dNH}{dt}=k_1\cdot m\cdot C_{NTG}-k_2\cdot NH \quad \text{(A7)}$$

$$\frac{dE_C}{dt}=k_2\cdot NH-k_{off}\cdot E_C \quad \text{(A8)}$$

where NH is the intermediate neurohormonal effect (caused by mediating factors), as shown in model II, Figure 1. The Laplace transforms of these two equations are

$$s\overline{NH}-NH_0=k_1\cdot m\cdot\overline{C_{NTG}}-k_2\overline{NH} \quad \text{(A9)}$$

$$s\overline{E_C}-E_{C0}=kk_2\overline{NH}-k_{off}\overline{E_C} \quad \text{(A10)}$$

where the initial conditions, NH_0 *and* E_{c0}, are both assumed to be zero. Rearrangement of equation (A9) and substitution of equation (A4) provides

$$\overline{NH}=\frac{k_1\cdot m\cdot k_0}{V(s)(s+k_{el})(s+k_2)} \quad \text{(A11)}$$

Substituting equation (A11) into equation (A10) and rearranging, we obtain

$$\overline{E_C}=\frac{k_1\cdot k_2\cdot m\cdot k_0}{V(s)(s+k_{el})(s+k_2)(s+k_{off})} \quad \text{(A12)}$$

Solving for E_C results in

$$E_C=\frac{k_0\cdot m\cdot k_1\cdot k_2}{V}\left(\frac{1}{k_{el}\cdot k_2\cdot k_{off}}-\frac{e^{-k_{el}\cdot t}}{k_{el}(k_2-k_{el})(k_{off}-k_{el})}-\frac{e^{-k_2\cdot t}}{k_2(k_{el}-k_2)(k_{off}-k_2)}-\frac{e^{-k_{off}\cdot t}}{k_{off}(k_{el}-k_{off})(k_2-k_{off})}\right) \quad \text{(A13)}$$

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