Pharmacodynamics of *in Vivo* Nitroglycerin Tolerance in Normal Conscious Rats: Effects of Dose and Dosing Protocol

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Purpose. We examined the effects of dose and dosing protocol on the pharmacodynamics of *in vivo* nitroglycerin (NTG) tolerance in conscious rats. Mechanism-based pharmcokinetic/pharmacodynamic (PK/PD) models were tested for their ability to describe the observed data.

Methods. Rats were infused with 1, 3, or 10 μg/min of NTG or vehicle for 10 h. Peak mean arterial pressure (MAP) response to an hourly 30 μg i.v. NTG challenge dose (CD) was measured before, during, and at 12 and 24 h after infusion. In separate experiments, the MAP effects of repeated bolus doses of NTG were compared to those after a continuous infusion, both at a total dose of 510 μg NTG.

Results. NTG tolerance was indicated by a decrease in peak MAP response to the CD, relative to the preinfusion peak MAP response. Tolerance toward the MAP effects of bolus CD was observed within 1 h of 10 μg/min of NTG infusion (26.8 ± 2.8% vs. 10.6 ± 0.4% for 0 and 1 h, respectively, $p < 0.001$), and the rate and extent of tolerance development increased with the infusion dose. No apparent MAP tolerance was observed when NTG was given as multiple bolus doses whereas significant MAP tolerance was observed when this dose was infused continuously. PK/PD modeling indicated that a cofactor/enzyme depletion mechanism could adequately describe the composite data.

Conclusions. Our data showed that *in vivo* nitrate tolerance was dose- and dosing protocol-dependent. The pharmacodynamics of tolerance development are consistent with depletion of either critical enzymes or cofactors that are necessary to induce vasodilation.

KEY WORDS: dosing protocol; mean arterial pressure; modeling; nitroglycerin tolerance; pharmacodynamics.

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ABBREVIATIONS: k_{12}, first-order rate constant for drug distribution from distributional to central compartment; k_{el}, first-order rate constant for NTG elimination, obtained from Ref. 13; k_{in}, zero-order rate constant for cofactor production or enzyme generation, chosen arbitrarily via trial and error after initial simulation runs; k_{off}, first-order rate constant for the elimination of NTG at the effect site; k_{on}, second-order rate constant for NTG concentration at the effect site; k_{out}, first-order rate constant for cofactor elimination or enzyme inactivation, value was fixed based on the observed time-course of tolerance washout; K_m, NTG concentration producing 50% of cofactor/enzyme elimination; NTG, nitroglycerin; $R(t)$, NTG infusion rate; V_{max}, maximum cofactor/enzyme elimination rate; V_x, volume of distribution for compartment X; $Y(t)$, the overall observed hemodynamic effects of NTG.

INTRODUCTION

Organic nitrates such as nitroglycerin (NTG) and isosorbide 5-mononitrate are widely used in the treatment of various cardiovascular diseases, including stable and unstable angina pectoris, acute myocardial infarction, and congestive heart failure (1). Although these drugs are effective when used acutely, their use in chronic therapy is limited due to the development of pharmacological tolerance, which generally occurs within several hours of continuous therapy. The phenomenon of nitrate tolerance was first observed in 1888 (2), and today, more than a century later, the underlying mechanisms are still not fully understood.

Clinical nitrate tolerance has been observed with all nitrate dosage forms such as transdermal patch, intravenous infusion, and immediate and sustained-release tablets (1). Although the phenomenon of nitrate tolerance is well documented, the dynamics of tolerance development in response to various infusion or transdermal doses of NTG are not well characterized. DeMots and Glasser (3), for example, reported that acute tolerance to transdermal NTG was not highly sensitive to dose (5 to 20 mg/24 h). Moreover, the effect of rate of drug input on *in vivo* nitrate tolerance has not been examined. We have previously shown that patients who had become tolerant to transdermal NTG still responded to a sublingual dose of the drug (4). The relative pharmacodynamic effects of repeated bolus dosing vs. continuous drug input are not well understood.

Human studies addressing these questions are difficult to justify ethically because patients are subjected to repeated episodes of coronary ischemia (the testing end-point) within a short period. Various animal models, including *in vitro*, *ex vivo,* and *in vivo* preparations, have been developed to study nitrate tolerance (5–10). More recently, Booth *et al.* (11) had characterized another model of *in vivo* NTG tolerance in normal conscious rats. These authors demonstrated that hemodynamic tolerance to a NTG i.v. challenge dose was observed within 8 h of NTG infusion at 10 μg/min while the antiplatelet activity of NTG was still maintained. These behaviors well mimic those observed for NTG in patients with angina pectoris. This normal animal model is also more accessible than the congestive heart failure model that we reported earlier (6) because the surgery involved is much less demanding.

The goal of the current study, therefore, was to further characterize the *in vivo* model of nitrate tolerance described by Booth *et al.* (11), by examining (a) the pharmacodynamics of NTG-induced hemodynamic tolerance, (b) the effects of NTG dose and dosing protocol on tolerance development, and (c) the suitability of a mechanism-based pharmacokinetic/pharmacodynamic (PK/PD) model involving cofactor/enzyme depletion to describe the observed results.

MATERIALS AND METHODS

Surgical Procedures

All procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee, University at Buffalo. Male Sprague-Dawley (Harlan, NY, USA) rats weighing 300–350 g were used. Animals were

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anesthetized by intramuscular injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). A polyethylene tubing (PE-50) was inserted approximately 4 to 5 cm into the left femoral artery for blood pressure measurements. A second PE-50 cannula was inserted into the left femoral vein for bolus injection of NTG (Schwarz Pharma, Germany), and a third cannula was implanted in the right jugular vein for continuous NTG infusion. Rats were allowed at least 24 h to recover from surgery before the start of the experiment.

NTG Tolerance Induction

Four groups of animals with 5–6 animals in each group were used. Rats were chosen randomly to receive 1 μg/min, 3 μg/min, or 10 μg/min of NTG or vehicle (5% dextrose, D5W) infusion for 9.75 h using an electronically controlled infusion pump (Harvard Instruments, South Natick, MA, USA). The same infusion volume of 10 μl/min was used in all treatment groups. A schematic representation of the dosing and testing schedules is shown in Fig. 1. Systolic and diastolic blood pressures were recorded continuously via the left femoral artery cannula using a Statham pressure transducer (Ohmeda, Murray Hill, NJ, USA) and a Gould RS3400 recorder (Gould, Cleveland, OH, USA). Basal blood pressure (BP) was recorded for 15–30 min to assure stability in these values prior to initiation of the experiment. An i.v. bolus dose of 30 μg NTG was given initially to determine the baseline hemodynamic response. Baseline mean arterial pressure (MAP) was calculated using BP data recorded 15–30 s before NTG injection whereas MAP after NTG dosing was calculated using BP data obtained about 10–20 s after NTG injection. The peak MAP was then determined, and subsequent responses were expressed as percentage change in peak MAP. NTG or D5W vehicle was continuously infused for 9.75 h starting 15 min after the initial 30 μg NTG i.v. bolus injection. To determine the pharmacodynamics of NTG tolerance, a 30 μg NTG i.v. bolus challenge dose (CD) was given hourly, and the hemodynamic response produced by the hourly challenge dose was compared to that produced at baseline. A decrease in the peak MAP response of the bolus dose in the presence of NTG infusion signaled the development of nitrate tolerance.

Effect of Dosing Protocol on Nitrate Tolerance

NTG hemodynamic tolerance was further determined in two different dosing protocols [repeated short bolus (n = 9) vs. i.v. infusion (n = 5)] using the same total dose of 510 μg NTG over an hour, as shown in Fig. 2. In the i.v. infusion group, animals received two 30 μg i.v. bolus (at 0 and 1 h) and

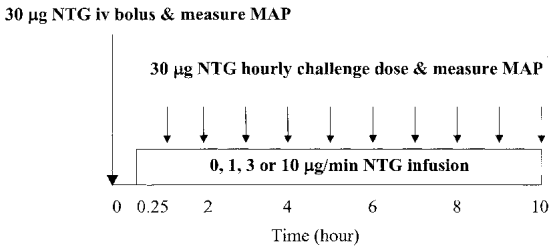

Fig. 1. Schematic representation of the dosing schedule used in our animal model to induce NTG tolerance. Animals received hourly 30 μg NTG i.v. bolus dose in the presence of 1, 3, or 10 μg/min NTG or D5W vehicle infusion starting 15 min after the first NTG bolus.

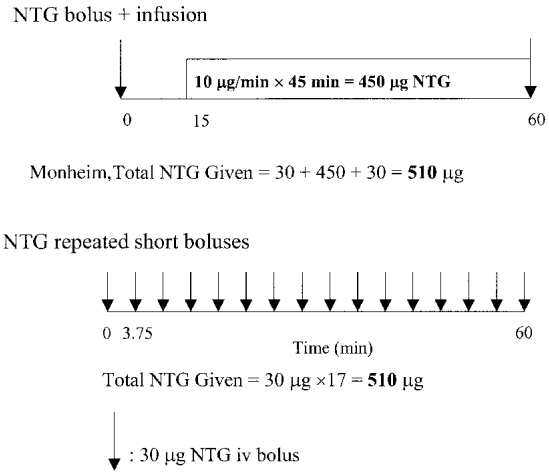

Fig. 2. Schematic representation of the dosing schedules used in the dosing protocol study (i.e., continuous i.v. infusion vs. repeated bolus dosing). The same total dose of 510 μg was used in both studies. ▾ indicates the time of 30 μg NTG i.v. bolus injection.

45 min of 10 μg/min NTG infusion. In the repeated bolus group, animals received 30 μg NTG i.v. bolus at every 3.75 min for 1 h.

Effect of a Nitrate-Free Period on NTG Tolerance

To determine the ability of animals to regain their sensitivity toward NTG, NTG tolerance was induced in animals by infusing 10 μg/min NTG for 10 h (n = 4). The peak MAP response to a 30 μg NTG i.v. bolus challenge dose was measured at 0 h and 10 h during the infusion and at 12 and 24 h after terminating the infusion.

PK/PD Modeling

Initially, two mechanistic models (antagonist generation vs. cofactor/enzyme depletion) of *in vivo* NTG tolerance were tested for their ability to describe the data. In both of these models, NTG was presumed to distribute rapidly into a distributional compartment [namely, the vasculature, as shown in our previous studies (12)], before it arrived at a well-mixed central compartment. Thus, noninstantaneous mixing of the drug in the central (blood) compartment was assumed during the first few minutes of drug dosing, when the drug concentration in the distributional compartment was much higher than that of the central compartment. In these models, it was further assumed that the NTG concentration in the distributional (vascular) compartment, instead of the central compartment, provided the driving force for NTG-induced vasodilatory effect. Both PK/PD models also incorporated a first-order elimination rate process for NTG from the central compartment.

The antagonist generation model has previously been used by Bauer and Fung (13) to describe the hemodynamic tolerance observed with NTG infusion in a rat model of congestive heart failure. However, the current data were poorly described by this model, in that the rapid return to baseline MAP after each CD could not be readily accommodated. Construction of more complex models based on this mechanism was not pursued further.

In comparison, description of the observed data was adequately accomplished with a cofactor/enzyme depletion model (Fig. 3). In this PK/PD model, continuous NTG dosing was presumed to lead to a decrease in some cofactor or enzyme concentration that was critical for its metabolic activation and/or action. Reduced metabolic activation of NTG to nitric oxide has been reported with nitrate vascular tolerance (14), and this reduction could be related to reduced sulfhydryl availability, either as a cofactor (15) or on the metabolic enzyme site itself (16–18). Recent data from our laboratory also showed that NTG can inactivate its metabolic enzyme glutathione-*S*-transferase (18), and past data have shown a decrease in vascular NTG metabolism after tolerance development (19). The cofactor/enzyme compartment is similar to the indirect response model IV by Dayneka *et al.* (20) where the interaction of NTG and the cofactor/enzyme provided an additional pathway for the elimination or inactivation of the cofactor/enzyme. In this model, NTG and the cofactor/enzyme reacted under saturable kinetics in the distributional compartment. The pertinent mathematics for this PK/PD model are presented in the Appendix.

Data Analysis

MAP was calculated as [diastolic pressure + 1/3 (systolic pressure − diastolic pressure)]. Data are presented as mean ± SD. PK/PD modeling was performed using ADAPT II software (Biomedical Simulations Resource, University of Southern California, Los Angeles). Comparison between groups was performed, where applicable, by Student's *t* test, two-way analysis of variance (ANOVA), or ANOVA with Newman–Keuls post-hoc test. Statistical significance was declared at $p < 0.05$.

RESULTS

Effect of Dose and Dosage Regimen on Nitrate Tolerance

Figure 4 shows the representative 1-h blood pressure tracings obtained from rats infused with either 10 μg/min NTG or 10 μl/min D5W control. In this conscious animal model, continuous NTG infusion had no discernable effect on MAP; however, a reproducible decrease in peak MAP was observed with the 30 μg NTG i.v. bolus challenge dose. This bolus dose caused an immediate decrease in peak MAP of approximately 30%, which returned to baseline within seconds. In the presence of 10 μg/min NTG infusion, the second challenge dose of NTG produced an attenuated response (top tracing at right). In the control animal (bottom tracing), the second challenge dose of NTG produced essentially the same response as the first challenge dose.

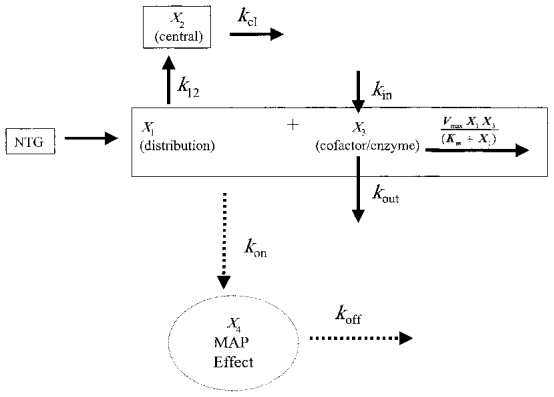

Fig. 3. Schematic representation of a PK/PD model of NTG tolerance based on cofactor/enzyme depletion.

Figure 5 (symbols) shows the observed peak MAP responses to bolus challenge doses of NTG at hourly intervals after infusion with vehicle control (D5W) and various NTG doses. When D5W was infused over 9.75 h, repeated bolus challenge doses of 30 μg NTG produced a consistent drop in peak MAP of about 25–30% (Fig. 5A), and none of the values observed during the infusion period was different from that observed prior to infusion (zero time point). In contrast, when rats were treated with infusion doses of 1, 3, or 10 μg/min NTG, the peak MAP response toward the challenge NTG dose was attenuated to varying degrees, and this attenuation was shown to be both time- and dose-dependent, by two-way ANOVA. Post-hoc analyses showed that the percentage change in peak MAP response of all three-infusion doses of NTG tested were different from those produced by the control infusion ($p < 0.05$). NTG infusion at 1 μg/min (Fig. 5B) did not appear to exhibit altered peak MAP response to bolus NTG challenge doses over most of the dosing period, but at 10 h, the peak MAP response (21.0 ± 4.0 mm Hg) was significantly attenuated vs. baseline (26.0 ± 5.1 mm Hg, $p < 0.05$) and vs. the time-matched D5W control (29.6 ± 2.2 mm Hg, $p < 0.01$), suggesting tolerance development. At the infusion rate of 3 μg/min NTG (Fig. 5C), attenuation in peak MAP response was more apparent. Finally, at the infusion rate of 10 μg/min NTG, attenuation in peak MAP response to the challenge dose was clearly evident, even after just 45 min of infusion. This tolerance effect was sustained throughout the entire infusion period, and no further exacerbation of response was observed. The mean peak MAP response of the hourly challenge doses over the entire infusion period was 27.4 ± 2.2, 25.9 ± 3.2, 21.9 ± 3.7, and 12.6 ± 5.0 mm Hg for D5W control, 1, 3, and 10 μg/min NTG infusion doses, respectively. ANOVA with Dunnett post-hoc test revealed that values from the 3 and 10 μg/min infusion groups were significantly different from control ($p < 0.01$ for both), but the 1 μg/min infusion group was not different from control ($p > 0.05$).

The effects of NTG dosing protocol on NTG tolerance development are shown in Fig. 6. When the same total dose of 510 μg NTG was given as multiple short boluses of 30 μg each over 1 h, the peak MAP lowering effect of NTG bolus was more sustained than that seen after the infusion dose, although a trend toward a slight decrease in peak MAP response was observed during the first 20 min of testing. ANOVA analysis of the data observed from the repeated bolus protocol did not, however, reveal any time-dependent difference in peak MAP response. When this response was compared to that observed from the infusion protocol (of the same total dose), it was clear that the extent of tolerance produced in the multiple bolus dosing, if any, was much smaller than that observed from the infusion. Figure 6B showed that in the presence of 10 μg/min NTG infusion, a 30 μg NTG i.v. bolus given at 1 h only produced 10.6 ± 0.4% decrease in peak MAP whereas in the repeated bolus group, a peak MAP decrease of 21.6 ± 6.7% was observed at 1 h ($p < 0.01$). D5W-infused animals exhibited 24.7 ± 2.7% decrease in peak MAP at 1 h, which was not significantly dif-

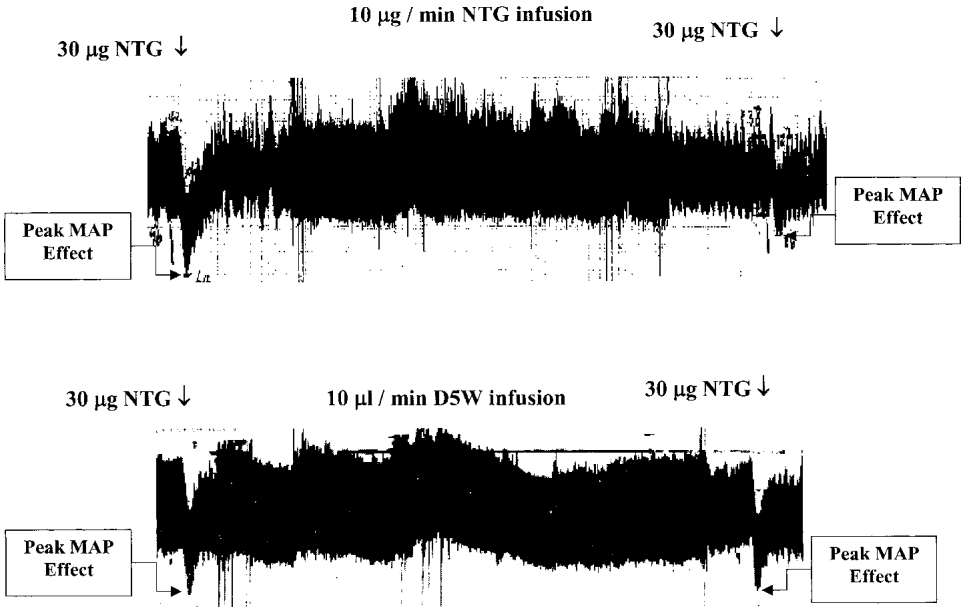

Fig. 4. Representative 1-h blood pressure tracings obtained from NTG (upper panel) or D5W (lower panel) infused animal.

ferent from the corresponding result observed after repeated bolus dosing ($p > 0.05$).

Effect of a Nitrate-Free Period on NTG Tolerance

Figure 7 (symbols) shows the peak MAP response toward the 30 μg NTG challenge dose in rats that were infused with 10 μg/min NTG for 10 h, followed by a drug-free period in the next 24 h. During the infusion period, peak MAP response was significantly attenuated, consistent with data observed in other parts of this study. At 12 and 24 h of the drug-free period, animals regained full responsiveness to the NTG bolus challenge dose, producing 27.3 ± 3.0% and 26.4 ± 4.8% decrease in peak MAP, respectively. These values were similar to that observed before NTG infusion (30.2 ± 3.4%, $p > 0.05$) and significantly different from the response observed at the end of the 10-h infusion (10.0 ± 1.1%, $p < 0.001$).

PK/PD Modeling

Because of the discontinuous nature of the PK/PD profile, it was found necessary, in fitting the data, to insert values of 0% decrease in peak MAP between the challenge doses. An arbitrary number (15) of these values (i.e., every 3.8 min) was chosen in the PK/PD fitting. This maneuver was consistent with the observation that the NTG infusion, by itself, did not produce any hypotensive effects.

Figure 5 (lines) shows the computer fit of the data with the cofactor/enzyme depletion model, using parameter values shown in Table I. The NTG elimination rate constant (k_{el})

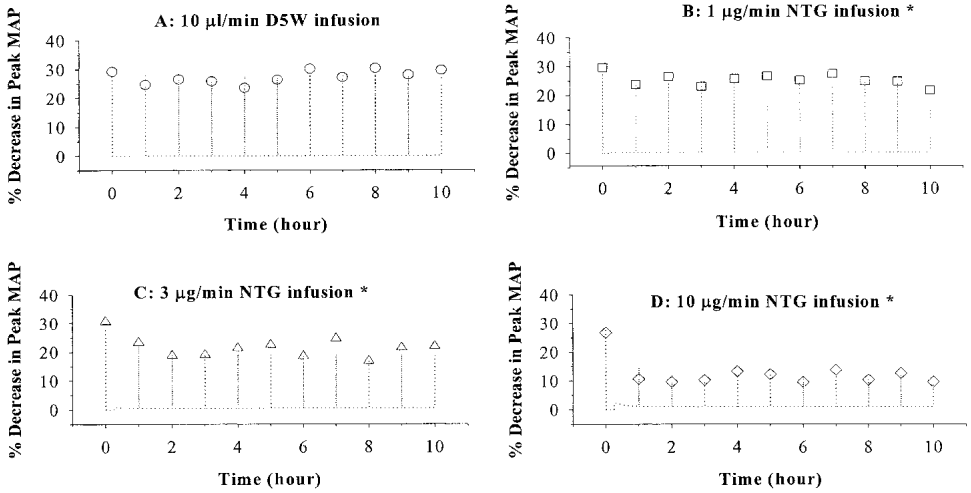

Fig. 5. The effect of NTG infusion dose on the peak MAP response of the hourly 30 μg NTG i.v. bolus challenge. (○) 10 μl/min D5W control infusion; (□) 1 μg/min NTG infusion; (△) 3 μg/min NTG infusion; (◇) 10 μg/min NTG infusion. Mean data are presented, n = 5 to 6. * $p < 0.05$ vs. D5W, two-way ANOVA with Duncan's test . Lines are fitted results based on the PK/PD model outlined in Fig. 3.

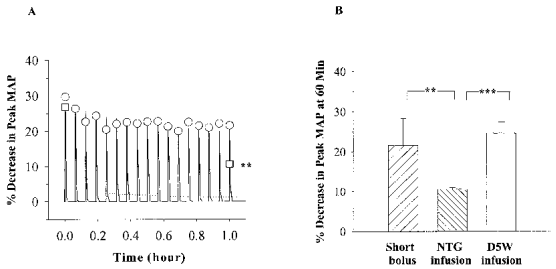

Fig. 6. (A) The peak MAP effect of a 30 μg NTG i.v. bolus after repeated short bolus (○) or 10 μg/min continuous NTG infusion (□). Mean data are presented, n = 5 to 9. ** $p < 0.01$ vs. short bolus at the corresponding time point, by ANOVA with Student–Newman–Keuls post-hoc test. Lines are fitted based on the PK/PD model outlined in Fig. 3. (B) The effects of NTG dosing protocol on hemodynamic tolerance development at 60 min. Data are expressed as mean ± SD, n = 5 to 9. ** $p < 0.01$; *** $p < 0.001$ vs. 10 μg/min NTG infusion, by ANOVA with Student–Newman–Keuls post-hoc test.

was obtained from our previous studies (13). This PK/PD model was also used to describe the data in Fig. 6 (repeated bolus doses vs. infusion). The fitted and observed data agreed well with each other, suggesting that a PD model of cofactor/enzyme depletion was consistent with our results.

The temporal changes for the hypothetical cofactor/enzyme in each NTG treatment group were then simulated for regimens used in this study, and the results are shown in Fig. 8. As the NTG infusion dose was increased, the simulated cofactor/enzyme concentration was shown to decrease, consistent with the higher degree of pharmacological tolerance observed. Figure 8B shows that, based on this PD model, depletion of this critical cofactor/enzyme was also more extensive with the infusion protocol vs. the repeated bolus protocol, again consistent with our observed results. Based on these simulations, a cofactor/enzyme concentration above 75% of baseline appeared to be required for a bolus NTG dose of 30 μg to produce its peak MAP effect because this level separated the nontolerant regimens (control and 1 μg/min) vs. the tolerance regimens (3 and 10 μg/min, Fig. 8A). Figure 8B also showed that the less tolerant repeated bolus

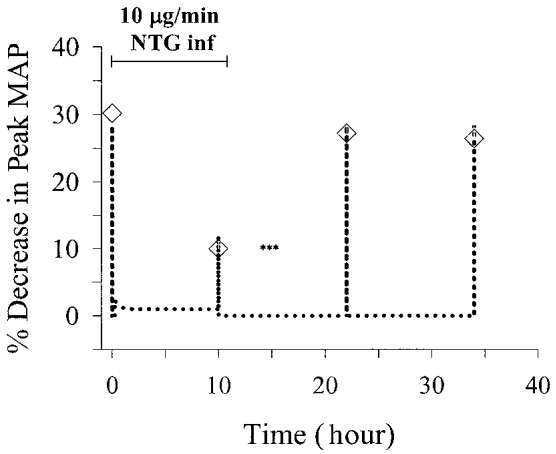

Fig. 7. The effects of a nitrate-free period on hemodynamic tolerance development. Mean data are presented, n = 4. ***$p < 0.0001$ vs. 0, 22, and 34 h; ANOVA with Student–Newman–Keuls post-hoc test. Symbols represent observed data and lines represent computer fitting of the data based on the model presented in Fig. 3.

Table I. PK/PD Parameters of Nitrate Tolerance Based on an Enzyme/Cofactor Depletion Model

Parameter	Value	CV%
k_{12} (h^{-1})	720	82
K_{el} (h^{-1})	12.9	Fixed
k_{in} (μg h^{-1})	20.0	Fixed
k_{out} (h^{-1})	1.00	Fixed
V_{max} (h^{-1})	16.0	88
K_m (μg)	8.00	38
k_{on} (μg^{-1} h^{-1})	89	7
k_{off} (h^{-1})	600	88

regimen was able to sustain cofactor/enzyme level at about this threshold at 1 h whereas the tolerance-producing regimen of 10 μg/min infusion could not.

To further test the model, additional simulations were performed to examine the ability of the model to describe the data obtained from the experiment involving a nitrate-free period (Fig. 7). Consistent with earlier fits, the model could describe the loss of tolerance at 12 and 24 h after terminating the NTG infusion (lines in Fig. 7). In addition, the model was able to show the return of the cofactor/enzyme to baseline levels after this drug-free period (Fig. 8C).

DISCUSSION

Under our experimental conditions, NTG infusion (even at the highest dose of 10 μg/min) did not produce any measurable hypotensive effects when compared to the vehicle-induced controls. This observation is consistent with literature reports of the apparent lack of effect of NTG infusion on blood pressure in both conscious rats and rabbits (11,21) and may be related to the existence of compensatory mechanisms when the animal was in its conscious and ambulatory state. On the other hand, a decrease in MAP was reproducibly observed with the i.v. bolus challenge dose of NTG. The use of a bolus challenge dose to demonstrate NTG tolerance in our animal preparation was similar to that used in patients where a sublingual dose of NTG was given to examine nitrate tolerance to continuous NTG infusion or transdermal patch (4,22).

Our results demonstrated, for the first time, a relationship between NTG dose and *in vivo* tolerance development. Hemodynamic tolerance to the MAP response of a NTG i.v. bolus dose was observed within 1 h of 10 μg/min of NTG infusion. The onset and extent of NTG tolerance were faster and larger, respectively, after the 10 μg/min dose than those observed after the 1 and 3 μg/min doses (Fig. 5). The data also showed that NTG tolerance in this animal model was reversed by an intervening nitrate-free period. Animals were completely responsive to NTG 12 h after the last NTG exposure. This pharmacodynamic behavior is consistent with the clinical situation in which patients have been shown to regain their sensitivity to nitrates after an overnight drug-free period (23,24).

One interesting finding in the current study was that total drug exposure was an incomplete determinant of NTG pharmacodynamics. We demonstrated that the extent of NTG tolerance was also dependent on the dosing protocol. The concept that drug-induced effects may be influenced by dosing protocol is commonly known in cancer chemotherapy (for

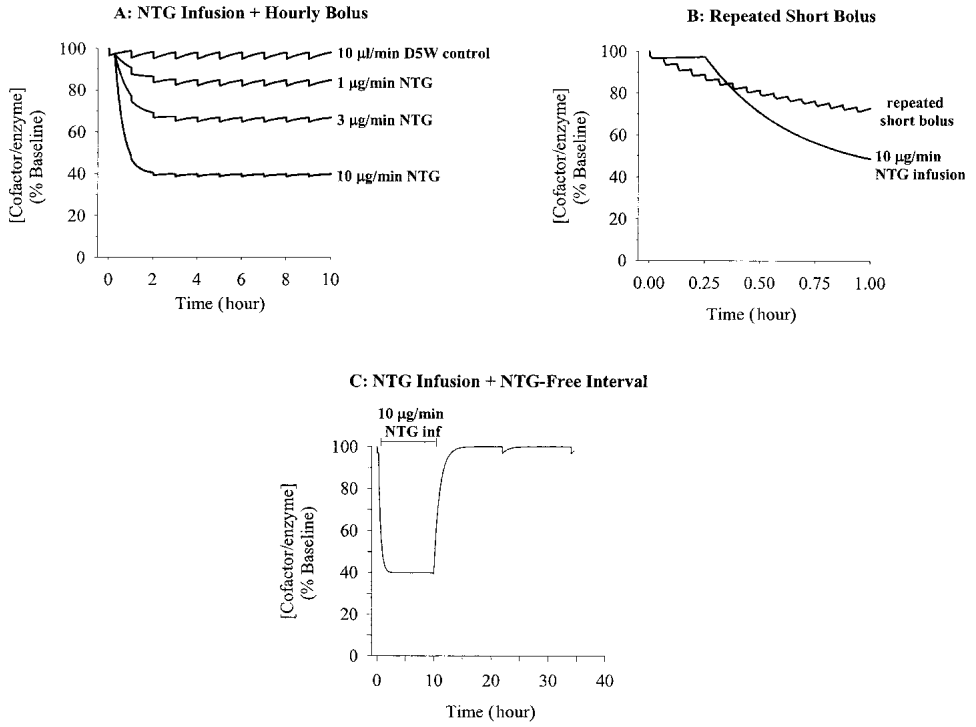

Fig. 8. Simulated cofactor/enzyme concentrations after (panel A) NTG continuous infusion at various rates, (panel B) repeated bolus doses vs. continuous infusion for 1 h, and (panel C) tolerance development and washout after a 10 µg/min infusion.

example, see Ref. 25). In the cardiovascular literature, Kleinbloesem *et al.* (26) have shown that the hemodynamic effects of nifedipine were different when the drug was given as an infusion vs. a bolus dose. In this study, we showed that with the same total dose, hemodynamic tolerance was observed with continuous NTG exposure, but not when NTG was given as repeated multiple bolus doses. This observation may offer some insight in the future design of drug delivery systems that can reduce the tolerance properties of organic nitrates.

The construction of any PK/PD model to fit our data required an accommodation to a basic experimental observation: while the 30 µg NTG i.v. bolus was able to reduce MAP potently in rats, the duration of this effect was short-lived. Within 30 s, blood pressure response returned to baseline. This pharmacodynamic disappearance rate was much shorter than the 4-min plasma half-life of NTG that we previously observed in rats (27). To account for the discrepancy between NTG plasma half-life and the duration of the observed MAP effect, we assumed that NTG concentration in the central (plasma) compartment was not the pharmacodynamic driving force for its hemodynamic effects. Rather, it was assumed that a distributional compartment (most likely the vasculature) was the effect-site compartment. Within the first few moments of drug dosing, the vascular NTG concentration (in the distributional compartment) was anticipated to be higher than that in the central compartment. This assumption is consistent with our previous observation (12), showing that NTG concentration at the local vascular site of injection was over 40-fold higher than the plasma concentration. Without this somewhat uncommon modeling strategy, none of the PK/PD models that we have attempted was able to fit the data.

In Fig. 5 A–D, it was shown that the present PK/PD model could describe two major features of the data: (a) that the peak MAP response decreased dose-dependently with increasing infusion doses of NTG, and (b) that the infusion doses, by themselves, produced little hemodynamic effects. It was evident in Fig. 5D that this model predicted a minor decrease (<5%) in MAP upon the initiation of NTG infusion at 3 and 10 µg/min. This decrease could not be experimentally documented because it was too small and was masked by the underlying variability in the MAP of the animal. More importantly, this PK/PD model could also well describe the PD data obtained after repeated bolus NTG administration vs. continuous infusion over an hour (Fig. 6).

The internal consistency of the model was further examined by simulating the concentration of the hypothetical cofactor/enzyme compartment after different NTG infusion doses and dosage regimens. These simulations (Fig. 8) showed that NTG dose-dependently decreased the cofactor/enzyme concentration. The concept that cofactor/enzyme depletion or inactivation might lead to NTG tolerance was first described by the classic Needleman "sulfhydryl depletion hypothesis" for nitrate tolerance (16). Other literature reports had proposed impaired nitrate biotransformation as an underlying mechanism of nitrate tolerance (19,28,29). Sage *et al.* (30) had also reported decreased production of 1,2-glyceryl dinitrate, a NTG metabolite, from NTG in NTG tolerant human vessels, consistent with our previous observation in rats (19). We showed recently that NTG can inactivate its own metabolizing enzyme, glutathione-*S*-transferase, in a mechanism-based manner (18). The decreased bioconversion mechanism of NTG tolerance is further supported by the ap-

parent lack of cross-tolerance between NTG and *S*-nitroso-*N*-acetylpenicillamine (SNAP, a spontaneous NO donor) observed in this animal model (31).

In summary, we have characterized, for the first time, the time course of NTG hemodynamic tolerance in normal conscious rats. This model of tolerance resembles many aspects of human clinical tolerance. Hemodynamic tolerance was observed with continuous NTG exposure but not intermittent dosing, and nitrate sensitivity was regained after a nitrate-free period. Our studies also revealed that the rate of input of NTG was important in governing the dynamics of nitrate tolerance. PK/PD modeling revealed that a cofactor/enzyme depletion mechanism is consistent with the observed pharmacodynamics of tolerance development. This simple animal model therefore may have utility in studying the underlying *in vivo* mechanisms of acute nitrate tolerance.

APPENDIX

Equations pertaining to the cofactor/enzyme depletion model of nitrate tolerance (Fig. 3):

$$\frac{dX_1(t)}{dt} = -k_{12}X_1(t) + R(t)$$

$$\frac{dX_2(t)}{dt} = k_{12}X_1(t) - k_{el}X_2(t)$$

$$\frac{dX_3(t)}{dt} = k_{in} - k_{out}X_3(t) - \frac{V_{max} \times X_1(t)}{K_m + X_1(t)} \times X_3(t)$$

$$\frac{dX_4(t)}{dt} = k_{on}X_1(t)X_3(t) - k_{off}X_4(t)$$

$$Y(t) = X_4(t)$$

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