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Nitroglycerin dinitrate metabolites

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Nitroglycerin Dinitrate Metabolites:
Pharmacokinetics / Pharmacodynamics in Dogs

by

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DISSERTATION

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Thesis Committee Chair
Dr. Leslie Z. Benet

Frank W. Lee

ABSTRACT

Nitroglycerin Dinitrate Metabolites: Kinetics and Dynamics in Dogs

A simple and sensitive gas chromatography-electron capture detection method which is capable of simultaneously determining the low plasma levels of the antianginal drug nitroglycerin (GTN) and its dinitrate metabolites 1,2-GDN and 1,3-GDN was developed and used in all of the dog studies. GTN exhibited a shorter half-life (T1/2=4 min), higher apparent clearance (CLapp=1440±630 ml/min/kg) and apparent volume of distribution (Vapp=9.87±4.15 L/kg) and lower bioavailability (F=0.015±0.019) than that obtained for 1,2-GDN and 1,3-GDN. The average T1/2, CLapp, Vapp and F for 1,2-GDN were 40 min, 16.4±4.4 ml/min/kg, 0.809±0.192 L/kg and 0.626±0.220, respectively, whereas the same average parameters for 1,3-GDN were 44 min, 15.0±3.7 ml/min/kg, 0.802±0.189 L/kg and 0.680±0.115. The formation of GDNs was rapid and extensive and the GDNs reached levels much higher than GTN after GTN doses. Apparent dose dependent pharmacokinetics of GTN and the GDNs were observed. This seems to be due to concentration dependent tissue uptake, saturable blood metabolism and /or a blood flow decrease and an nonlinear change in the clearance of GTN to 1,2-GDN. The marked change in the 1,2-GDN/1,3-GDN ratio following oral GTN administration suggests the possibility of different enzyme specificity for GTN metabolism to GDNs in different tissues. Generally, 75% of the GTN dose may be accounted for in terms of measurable dinitrate metabolites in the systemic circulation. The GTN pharmacokinetics and pharmacodynamics were not affected by GDNs. There were no marked pharmacokinetic and pharmacodynamic differences between young and old dogs. The systolic blood pressure decrease(SPD) was the most marked hemodynamic response observed with GTN and the GDNs. Comparing maximum net systolic pressure decrease (Dmax) after iv doses, GTN was about 10-12 times more potent than GDNs. Comparing the ratio of AUCspd/dose, 1,2-GDN was about twice as potent as GTN while 1,3-GDN was about

equipotent with GTN. Oral GTN is pharmacologically active. The active dinitrate metabolites contribute a significant portion to GTN pharmacodynamics. However, these hemodynamic measures may not be useful when comparing antianginal efficacy of orally vs intravenously dosed drug.

GLOSSARY

1,2-GDN :	1,2-glyceryl dinitrate.
1,3-GDN :	1,3-glyceryl dinitrate.
1-GMN :	1-glyceryl mononitrate.
2-GMN :	2-glyceryl mononitrate.
A/V :	arterial / venous plasma concentration ratio.
AUC :	area under the curve.
AUCpl :	area under the drug plasma concentration vs time curve.
AUCspd :	area under the systolic blood pressure decrease vs time curve.
AUMC :	area under the moment curve.
AUPRC :	area under the pharmacodynamic response vs time curve.
B.W. :	body weight.
C :	drug plasma concentration.
cGMP :	cyclic 3',5'-guanosine monophosphate.
CLapp :	apparent total body clearance.
CLapp,m1 :	fractional apparent clearance of GTN to 1,2-GDN.
CLapp,m2 :	fractional apparent clearance of GTN to 1,3-GDN.
Clast :	last measurable drug plasma concentration.
CLm1 :	fractional GTN clearance to 1,2-GDN.
CLm2 :	fractional GTN clearance to 1,3-GDN.
Cmax :	maximum plasma level.
Css :	steady-state plasma concentration.
Css,10 :	steady-state plasma concentration following 10 μg/min drug infusion.
Css,20 :	steady-state plasma concentration following 20 μg/min drug infusion.
Css,30 :	steady-state plasma concentration following 30 μg/min drug infusion.
Css,50 :	steady-state plasma concentration following 50 μg/min drug infusion.

Css,70 :	**steady-state plasma concentration following 70 μg/min drug infusion.**
Css,100 :	**steady-state plasma concentration following 100 μg/min drug infusion.**
CV :	**coefficient of variation.**
Dmax :	**net maximum blood pressure decrease.**
DPD :	**net diastolic blood pressure decrease.**
E :	**pharmacodynamic effect.**
EC50 :	**plasma concentration to induce 50% of Emax.**
Emax :	**maximum pharmacodynamic effect.**
F :	**bioavailability.**
fm1 :	**fraction of the GTN dose converted to 1,2-GDN.**
fm2 :	**fraction of the GTN dose converted to 1,3-GDN.**
GC-ECD :	**gas chromatography-electron capture detection.**
GC-MS :	**gas chromatography-mass spectrometry.**
GTN :	**nitroglycerin, glyceryl trinitrate.**
HPLC :	**high-performance liquid chromatography.**
HRC :	**net heart rate change.**
iv :	**intravenous.**
k :	**elimination rate constant.**
MBP :	**mean blood pressure.**
MPD :	**net mean blood pressure decrease.**
n :	**slope factor in a sigmoid Emax model (Hill equation).**
N.S. :	**not significant.**
NO :	**nitric oxide.**
po :	**oral.**
Ro :	**infusion rate.**
S.D. :	**standard deviation.**
SBP :	**systolic blood pressure.**

SPD : net systolic blood pressure decrease.

T1/2 : half-life.

Tlast : sampling time for the Clast.

Tmax : time to reach the Cmax.

Vapp,ss : apparent volume of distribution at steady-state.

CHAPTER I

NITROGLYCERIN AND ITS GLYCERYL DINITRATE METABOLITES

General Background

Nitroglycerin (GTN), a glyceryl trinitrate ester (Figure I-1), was synthesized by Sobrero in 1846 and first used by Dr. William Murrell to treat angina patients in 1879. It remains a principal mode of therapy of this disorder to this day. Nitroglycerin is often administered sublingually for the immediate relief of an acute angina attack. Because of the short duration of effect from a sublingual nitroglycerin dose, long-acting dosage forms such as sustained release oral tablets and topical ointments, gels, and patches have been developed. In recent years, the clinical usefulness of IV infusion nitroglycerin has been demonstrated in the treatment of congestive heart failure, unstable angina and to minimize myocardial damage after acute myocardial infarction.

Mechanism of Action at the Cellular Level

Nitroglycerin is a potent vasodilator which relaxes smooth muscle cells in the walls of veins and arteries directly without neuroendocrine conduction. The mechanism of action at the cellular level has not been well elucidated. However, all organic nitrates seem to exhibit the same mechanism of action.

Three mechanisms of this action have been proposed: a) A theory suggesting the existence of an organic nitrate receptor containing a sulfhydryl functional group was proposed by Needleman and Johnson (1973). In 1965, Needleman and Hunter (1965) and Needleman and Krantz (1965) demonstrated that one micromole of organic nitrate reacts with 2 micromoles of glutathione in the presence of organic nitrate reductase, (glutathione S-transferase)

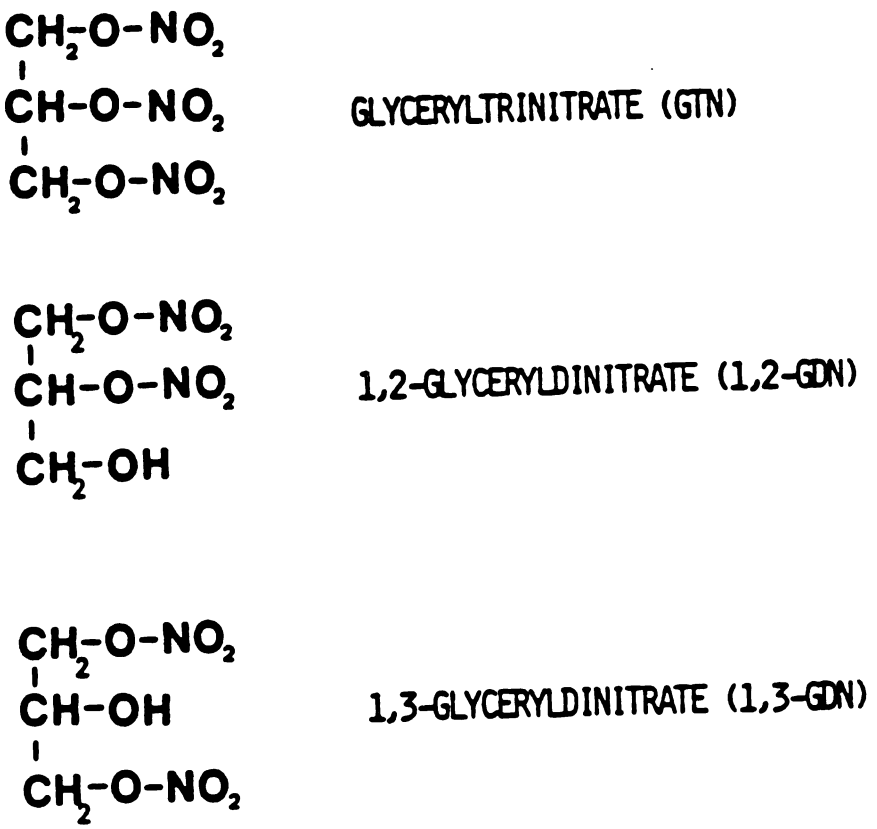

Figure I-1. Chemical structure of nitroglycerin, 1,2-GDN and 1,3-GDN.

suggesting that the action of organic nitrates was mediated via reactions with sulfhydryl groups. In the 1970's, Needleman and Johnson (1973) also demonstrated a positive correlation between the oxidation of titratable tissue sulfhydryl groups and tissue tolerance to organic nitrates. Based upon these observations, Needleman and Johnson proposed that organic nitrates react with a vascular smooth muscle receptor which contains reduced sulfhydryl groups. The organic nitrates oxidize these sulfhydryl groups to form disulfide groups and free inorganic nitrite which results in a vascular smooth muscle relaxation through an undefined link (Figure I-2). They further concluded that this receptor is organic nitrate specific since tolerant tissues were still responsive to other vasodilators (i.e., isoproterenol, cyclic AMP, sodium nitroprusside and papaverine) but not to organic nitrates. The drawbacks to this theory are that no specific receptor sites for organic nitrates on plasma membranes have been located and no specific antagonists have been discovered for organic nitrates.

b) A theory evoking S-nitrosothiol intermediate formation was proposed by Ignarro and coworkers (1981, 1985). These investigators suggested that nitric oxide (NO), formed intracellularly via denitration of organic nitrates, reacts with endogenous sulfhydryl groups to form S-nitrosothiols. These intermediates then activate guanylate cyclase to form cyclic 3',5'-guanosine monophosphate (cGMP). The increase in cGMP induces vascular smooth muscle relaxation. A schematic diagram of the proposed mechanism is shown in Figure I-3. Three key steps are involved in this theory: The first is denitration of nitroglycerin. This step seems to be rate-limiting and requires sulfhydryl groups. The depletion of sulfhydryl may be responsible for the development of nitroglycerin tolerance (Axelsson et al., 1982; Kukovetz and Holzmann, 1983) and the decrease in GTN metabolism (Brien et al., 1986). Tolerance to nitroglycerin is reversible and the cardiovascular effects can be potentiated by the administration of N-acetylcysteine, a sulfhydryl donor (Winniford et al., 1986; Horowitz et al., 1983; Torresi et al., 1985). The second is formation of S-nitrosothiols. S-nitrosocysteine has proved to be the most active intermediate exhibiting vasodilation effects

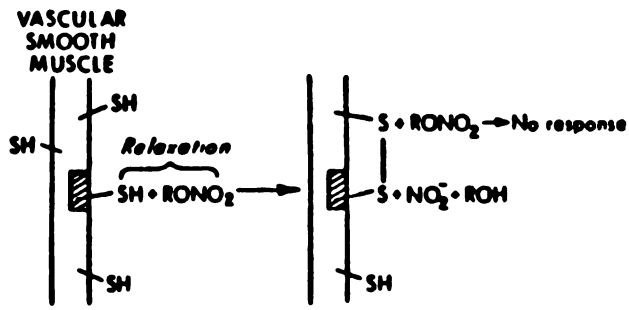

Figure I-2 Schematic diagram of the reaction of GTN with tissue sulfhydryl groups at its vascular receptor. Abbreviations: $RONO_2$, organic nitrate; ROH, denitrated metabolite (Needleman and Johnson, 1973).

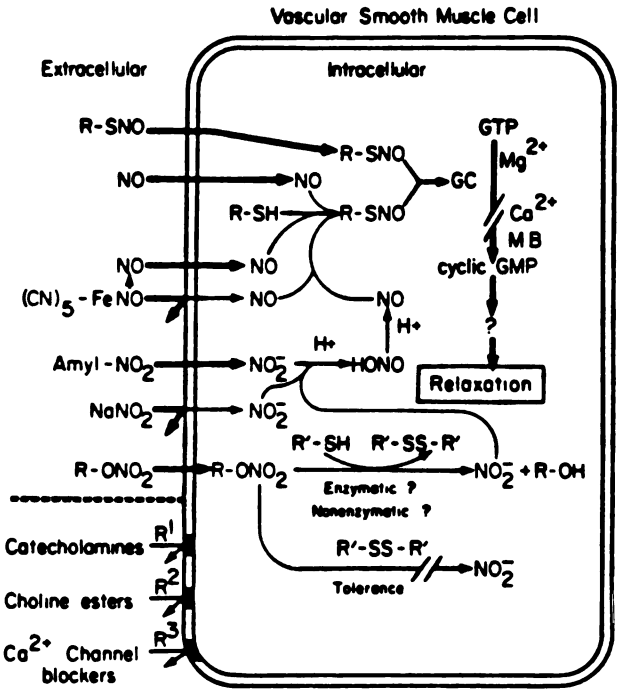

Figure I-3. Schematic diagram of proposed mechanisms by which nitrogen oxide-containing vasodilators relax smooth muscle. Abbreviations: R-SNO, S-nitrosothiol; NO,nitric oxide; HONO, nitrous acid; $(CN)_5$-FeNO, nitroprusside; R-ONO_2, organic nitrate; R-OH, denitrated organic nitrate; R-SH, low or high molecular weight thiol; R'-SH, thiol that is distinct from R-SH; GC, guanylate cyclate; M B , methylene blue; $R^{1,2,3}$, extracellular specific receptors. (From Ignarro et al., 1981).

(Ignarro et al., 1981). Third, an increase of cGMP levels. Murad (1986) demonstrated that various NO-forming compounds, including nitroglycerin, activate guanylate cyclase and increase the concentrations of cGMP in vascular smooth muscle cells.

c) A theory proposing that nitroglycerin acts as a stimulant of prostaglandin synthesis was presented by Levin et al. (1981) and Wallis et al. (1982). These investigators suggested that nitroglycerin may stimulate the prostaglandin system, induce the synthesis of prostacyclin and decrease thromboxane A2 synthesis. However, this proposed pathway has been disproved in more recent studies (Rehr et al.,1984; Thadani and Kellerman, 1983; Pazenback et al., 1984). Nitroglycerin induced coronary dilatation was not related to intracoronary thromboxane concentrations. Bennett et al. (1983) showed that vasodilation of rabbit celiac and mesenteric arteries induced by nitroglycerin was not altered by pretreatment with indomethacin, a prostaglandin synthesis inhibitor.

In summary, the specific binding sites for nitroglycerin proposed by Needleman et al. may not exist; however, the denitration of organic nitrates by sulfhydryl groups coincides with the first step of the mechanism proposed by Ignarro et al. It is most likely that lipophyllic organic nitrates penetrate the cell membrane and are denitrated intracellularly to form nitric oxide which in turn combines with sulfhydryl groups to form S-nitrosothiols. These S-nitrosothiols activate guanylate cyclase and increase the cGMP concentration, resulting in vasodilation. The mechanism by which cGMP induces vasodilation is still not clear.

Physiological Effects

Nitroglycerin at low concentrations acts mainly on the venous system, dilating peripheral veins and the vena cava. As a result, blood is stored in the veins, preload is decreased and right and left ventricular filling pressure is decreased. Arteriolar resistance is not affected and the heart rate is slightly increased. At higher doses, both veins and arteries are dilated

which results in decreased systolic and diastolic pressures as well as cardiac output. Consequently, hypotension occurs and leads to reflex tachycardia and peripheral arteriolar vasoconstriction.

The mechanism by which anginal pain is relived was first attributed to coronary vasodilation which resulted in an increase in coronary blood flow and the oxygen supply to the myocardium (Winbury et al., 1969 and Horowitz et al., 1971). However, Ganz and Marcus (1972) found that direct intra-coronary injection of GTN to patients with coronary artery disease failed to relieve anginal pain. However, the pain diminished after intravenous GTN was given to patients. Therefore, peripheral venous dilation resulting in a decrease in cardiac preload, cardiac work, and consequently, oxygen demand may be the major mechanism for the relief of angina pectoris. Nevertheless, coronary vasodilation may still play an important role in the relief of variant (coronary vasospasm) type angina (Pepine et al., 1982).

The coronary vasodilation induced by organic nitrates is beneficial in limiting the area of damage caused by cardiac infarction (Feldman et al., 1982). This may occur because organic nitrates improve subendocardial blood flow by redistributing regional blood flow without causing an increase in total coronary blood flow. The decrease in the left ventricular filling pressure can also increase the subendocardial perfusion.

Intravenous infusions of nitroglycerin are usually administered to chronic heart failure patients at doses which cause dilation of both venous and arterial vessels followed by reduction of both preload and after load and diminished cardiac work. This leads to relief of pulmonary congestion and an increase in cardiac output (Cohn and Franciosa, 1977).

Metabolism and Pharmacokinetics

Nitroglycerin can be extensively and rapidly taken up in almost all tissues of the body and metabolized to two active dinitrate metabolites, 1,2- and 1,3-glyceryl dinitrate (Figure I-4). In initial studies, Needleman and his colleagues used radioisotope labeled nitroglycerin to carry out a series of in vivo and in vitro animal studies and found that nitroglycerin was rapidly degraded by the liver enzyme glutathione-organic nitrate reductase (Needleman and Hunter, 1965; Needleman and Krantz, 1965). In an isolated perfused rat liver study, Johnson and co-workers (1972) found that the elimination half-life of GTN was only two minutes. In other in vivo studies, the systemic clearance of GTN calculated using venous blood concentrations was found to be much higher than liver blood flow and cardiac output in man (Armstrong et al., 1980a) and rat (Fung et al., 1984a). Thus, extra hepatic clearance must contribute significantly to the total body clearance of GTN and, indeed, many tissues in the body can metabolize nitroglycerin. In vitro degradation of GTN after incubation with human blood has been reported by different groups of investigators (Lee, 1973; Armstrong et al., 1980b; Noonan and Benet, 1982; Cossum and Roberts, 1985a). In blood, GTN is rapidly metabolized (T1/2 = 3-15 min) to 1,2-GDN and 1,3-GDN and subsequently to the glyceryl mononitrates, 1-GMN and 2-GMN. Cossum and Roberts (1985b) demonstrated in vitro that nitroglycerin was metabolized in sheep liver, lung, muscle, arterial and venous tissue homogenates. Rat tissue (i.e., vena cava, aorta, abdominal muscle, lung and liver) uptake and metabolism of nitroglycerin has been shown by Fung and co-workers (1984b). Tissue uptake and metabolism were also demonstrated by the arterial/venous nitroglycerin concentration gradient observed in dogs (Fung et al., 1981; Moffat et al., 1984) and humans (Armstrong et al., 1982).

The nitroglycerin metabolic profile after oral and subcutaneous dosing was studied by three groups of investigators using C-14 labeled nitroglycerin (DiCarlo et al., 1968; Needleman et al., 1971, and Hodgson and Lee, 1975). A major portion of the dose (25%) was recovered as

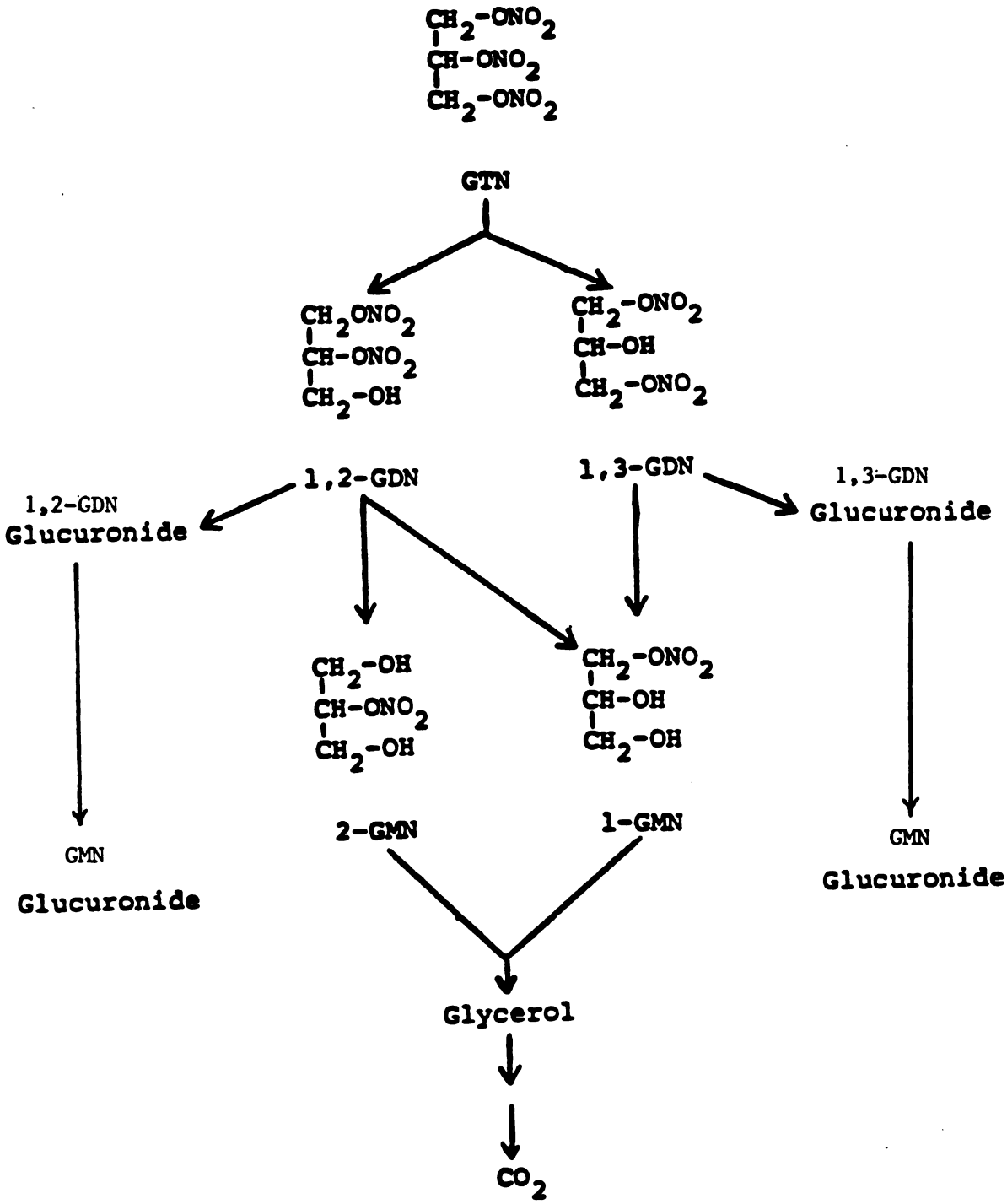

Figure I-4. Metabolic scheme for nitroglycerin.

CO_2 and about 40% of the dose was recovered in urine as 1,2-GDN, 1,3-GDN, GMNs, glucuronides of GDNs and GMNs, and glycerol. The total recovery of the dose was about 70%. The remaining 30% of the radioactivity was probably still retained in the body 24 hours after the nitroglycerin dose.

Controversy Concerning the Efficacy of Oral and Long-Acting Nitrates

Based on early metabolic studies of nitroglycerin in animals, Needleman et al.(1972) wrote, "The biotransformations in rats are catalyzed rapidly by the liver enzyme glutathione-organic nitrate reductase. Human liver biopsy samples have the same enzyme capacity for denitration as the rat liver. These results lead to the conclusion that there is no rational basis for the use of long-acting nitrates (administered orally) in the prophylactic therapy of angina pectoris". He suggested therefore that oral dosing of nitroglycerin should be of no clinical benefit. However, other investigators documented that oral nitroglycerin was clinically effective (Krantz and Leake, 1975; Winsor and Berger, 1975). In 1980, Needleman and Johnson modified their position, "Organic nitrates have been administered orally in an attempt to provide convenient and prolonged prophylaxis against attacks of angina. The effectiveness of such administration was controversial until dosages were adjusted so that active drug would reach the systemic circulation...The hemodynamic effects that are observed after large doses of nitrates are swallowed likely result because of saturation of the capacity of the liver to denitrate the intact molecule. The active agent can thus reach the systemic circulation." Girre et al. (1980) reported that they were able to measure plasma GTN levels (4-5 ng/ml) using a GC method following 7.5 mg oral doses of nitroglycerin. Bashir et al. (1982) also reported high GTN levels after dosing oral sustained release GTN to healthy human volunteers. These results are questionable, since these authors did not indicate whether their assay method was able to separate GTN, 1,2-GDN and 1,3-GDN.

In more recent studies, Noonan and Benet (1986) and Dugger et al. (1983), after oral dosing of 6.5 mg immediate release GTN capsules and 2.5 mg sustained release GTN, respectively, to humans showed that no or very low levels of GTN and high levels of the dinitrate metabolites were detected in plasma. We believe that the controversy concerning the efficacy of oral and long-acting nitroglycerin might be explained in terms of the generation of high levels of the dinitrate metabolites. Although the pharmacological activities of 1,2-GDN and 1,3-GDN have been shown to be 40 and 53 times, respectively, less active than nitroglycerin in lowering guinea pig blood pressure 15 mm Hg (Bogaert et al., 1968) and 10 and 14 times, respectively, less active than nitroglycerin in lowering dog blood pressure (Needleman et al., 1969), a pharmacological responses to the high levels of 1,2-GDN and 1,3-GDN should be expected.

Objectives

In order to shed more light on this controversy, it is necessary to have a thorough understanding of the pharmacokinetics and pharmacodynamics of the glyceryl dinitrates, which is limited at present time. We therefore propose the following objectives:

1. To develop a specific and sensitive capillary gas chromatographic assay method with electron capture detection which is capable of simultaneously measuring nitroglycerin, 1,2-GDN and 1,3-GDN at concentrations to be found in man after the usual sublingual, oral, topical and intravenous doses used to alleviate angina.

2. To study the pharmacokinetics and pharmacodynamics of glyceryl dinitrates after intravenous and oral dosing of GTN, 1,2-GDN and 1,3-GDN to conscious dogs.

During the course of the study other objectives were also proposed to better define the pharmacokinetics and pharmacodynamics of GTN and its dinitrate metabolites. These objectives were:

3. To study the dose dependency of GTN and GDN following graded intravenous infusions in conscious dogs.

4. To study the possibility of end product inhibition by the GDNs on the pharmacokinetics and pharmacodynamics of GTN.

5. To determine whether pharmacokinetic and pharmacodynamic differences between young and old dogs might exist for GTN.

CHAPTER II

SIMULTANEOUS DETERMINATION OF NITROGLYCERIN AND ITS DINITRATE METABOLITES BY CAPILLARY GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

INTRODUCTION

The development of a specific and sensitive assay method for nitroglycerin (GTN) and its dinitrate metabolites (1,2-GDN and 1,3-GDN) has been a difficult task. The low GTN plasma concentrations observed after therapeutic dose administration (Armstrong et al., 1982), adsorption of the drug to plastics (Cossum et al., 1978) and the rapid metabolism in blood (Armstrong et al., 1980b; Noonan and Benet, 1982) are problems often encountered in measuring GTN . Several assay methods for GTN including gas chromatography (GC) (Rosseel and Bogaert, 1973; Yap et al.,1978; Wu et al., 1982; Sioufi and Pommier, 1985; Sioufi et al., 1987), high-performance liquid chromatography (HPLC) (Spanggord and Keck, 1980) and gas chromatography-mass spectrometry (GC-MS) (Ottoila et al., 1982; Gerardin et al., 1982; Miyazaki et al., 1982; Settlage et al., 1983) have been developed, but very few papers describe the assay of the dinitrate metabolites. The majority of the methods are limited by their lack of low level detectability, the large plasma sample volumes needed and the complexity of the analytical procedure required. In recent years, the more sensitive capillary gas chromatography-electron capture detector (GC-ECD) technique has been widely used. In our laboratory, Noonan et al. (1984) were able to measure 25 pg of GTN in 1 ml of plasma by using a GC-ECD system equipped with an on-column injector and a fused silica capillary column. Sioufi and Pommier (1985) reported a similar method with a quantitation limit of 50 pg for GTN in 1 ml of plasma. During the course of this work, Noonan et al. (1985) and Sioufi et al. (1987) reported methods for measurement of the dinitrate

metabolites, using a separate analytical procedure from that for GTN. This chapter presents a simple, selective and sensitive capillary GC-ECD method which is capable of simultaneously measuring picogram levels of GTN, 1,2-GDN and 1,3-GDN in 1 ml plasma, thereby not requiring a second analytical procedure for the metabolites as had been used previously (Noonan et al., 1985; Sioufi et al., 1987). This assay can be readily adopted by any laboratory having capillary GC instrumentation.

EXPERIMENTAL

Chemicals and Reagents

GTN (Nitro-bid IV) was purchased from Marion Laboratories, Inc. (Kansas City, MO). Dinitrate metabolites of nitroglycerin (1,2-GDN and 1,3-GDN) were provided by Marion Laboratories as pure chemicals (>99%) and were used as provided. 2,6-Dinitrotoluene was obtained from K&K Laboratories, ICN (Hollywood, CA). Methylene chloride (HPLC grade, J.T. Baker Chemical Co., Phillipsburg, NJ) was treated with charcoal and redistilled before use. Butyl acetate and pentane (HPLC grade) were obtained from Burdick & Jackson Laboratories (Muskegon, MI).

Instruments

A Varian model 3700 gas chromatograph (Walnut Creek, CA) equipped with a Varian Ni-63 electron capture detector (ECD), a J & W on-column injector (J & W Scientific, Rancho Cordova, CA) and a Hewlett-Packard model 3390A integrator (Palo Alto, CA) were used. Capillary columns with a non-polar polydimethylsiloxane stationary phase bonded to a fused silica column (0.32 mm id, 25-30 m length, 1 m film thickness) were purchased from five different commercial sources: Durabond-1 (DB-1) column from J & W Scientific; RSL-150

column from Alltech Associates, Inc. (Los Altos, CA); SPB-1 column from Supelco, Inc. (Supelco Parks, Bellefonte, PA); HP-1 column from Hewlett Packard, and Quadrex 007 column from Quadrex Corporation (New Haven, CT). Each of these columns was tested for applicability in this assay. Hydrogen (zero grade, Liquid Carbonic, Chicago, IL) at a flow-rate of 15 ml/min was used as the carrier gas. Nitrogen (99.997%, Liquid Carbonic) at a flow rate of 30 ml/min was used as the make-up gas. The column temperature program was set at 96°C initially and was held for 9 min then increased to 126°C at 4°C/min. After each assay the column temperature was raised rapidly to 280°C and maintained for 5 min to wash out plasma residues.

Sample Preparation

All glassware was silanized with a 10% (v/v) dimethyl dichlorosilane solution in toluene to prevent adsorption. After soaking for 30 min in the silanization reagent, glassware was immediately rinsed with toluene and methanol before being dried. One ml plasma was transferred into a test tube (16x150 mm) containing 100 µl(2 ng) of internal standard (2,6-dinitrotoluene) and known amounts of GTN, 1,2-GDN and 1,3-GDN and was immediately extracted once with 10 ml of methylene chloride/pentane solvent (3/7,v/v). The samples were mixed well on a mechanical rotator (24 rpm) for 20 min and centrifuged at 1500 xg for 10 min. The organic phase was combined and evaporated under nitrogen at room temperature. The sample vials were removed from the nitrogen stream before they were completely dry. The residue was then reconstituted into 50 µl n-butyl acetate. An aliquot of 0.1-0.5 µl was injected onto the gas chromatograph using an on-column injection syringe.

Standard Curves

Standard solutions containing GTN, 1,2-GDN and 1,3-GDN were prepared as aqueous solutions. Suitable aliquots were added to test tubes containing 1 ml blank plasma to prepare standard samples. Concentrations of GTN ranged from 0.03 to 10 ng/ml, while concentrations of 1,2-GDN and 1,3-GDN ranged from 0.1 to 10 ng/ml. Calibration curves were obtained by regressing weighted peak height ratios (nitrates/internal standard, weight = $1/y^2$) versus plasma concentrations for each of the three compounds. The following equations were used to calculate slope, intercept and correlation coefficient (r):

$$\text{Slope} = [\Sigma Wi \Sigma WiXiYi - \Sigma WiXi \Sigma WiYi]/[\Sigma Wi(\Sigma WiXi^2) - (\Sigma WiXi)^2]$$

$$\text{Intercept} = [\Sigma WiYi(\Sigma WiXi^2) - \Sigma WiXi \Sigma WiXiYi] / [\Sigma Wi(\Sigma WiXi^2) - (\Sigma WiXi)^2]$$

$$r = [\Sigma Wi \Sigma WiXiYi - \Sigma WiXi \Sigma WiYi]/ \{[\Sigma Wi(\Sigma WiXi^2) - (\Sigma WiXi)^2]^{1/2} * [\Sigma Wi(\Sigma WiYi^2) - (\Sigma WiYi)^2]^{1/2}\}$$

where Wi is the weight for the ith measurement, Xi is the spiked concentration, and Yi is the peak height ratio.

RESULTS AND DISCUSSION

Instrumentation

It has been reported that nitroglycerin may be adsorbed on the surfaces of capillary injectors, glass columns and detectors. This is also true for the glyceryl dinitrates, making picogram quantification on conventional GC equipment impossible. Therefore, the on-column injection

technique was used to deliver the sample directly into the inert fused-silica capillary column. Optimization of the position of the column exit inside the ECD is an important factor so as to balance the loss of and detectability for all three nitrates. The position of the column exit was optimized according to the method reported by Noonan et al. (1984). Among the five commercially available capillary columns tested, DB-1, RSL-150 and SPB-1 showed poor resolution and detectability (Fig. II-1) for the three nitrates whereas HP-1 and Quadrex-007 demonstrated good separation and detectability (Fig. II-2). We used the HP-1 column in all of the assays reported in this work. Other capillary columns with the stationary phase more polar than dimethylsiloxane were also tested and failed to separate 1,2-GDN and 1,3-GDN.

Solvent Extraction and Recovery

Ethyl acetate, ether and chloroform were tested as extraction solvents. With these solvents, all three nitrates could be extracted from plasma; however, polar contaminants which interfere with nitrate peaks and shorten column life were also extracted. This is demonstrated in Figure II-3 when ether was used as the extraction solvent. 1,2-GDN and 1,3-GDN appeared on the chromatogram after the first injection; however, they disappeared after the second injection due to the adsorption of contaminants brought into the column from the first injection. A mixture of methylene chloride/pentane (3/7, v/v) was found to be a significantly better extraction solvent. The methylene chloride was treated with charcoal and redistilled before use. The recovery of ^{14}C-1,2-GDN using this extraction procedure was determined as listed in Table II-1. The percent extractable 1,2-GDN was about 50% at low (0.025 ng/ml) and high (20 ng/ml) concentrations when the ^{14}C-1,2-GDN spiked 1 ml plasma samples were extracted once with 10 ml of methylene chloride/pentane (3/7, v/v). Although the extractability with the ratio 3/7 mixture was lower than that for the ratio 5/5 mixture, it was still sufficient to allow measurements of low levels for all three glyceryl nitrates. The ratio 3/7 mixture was chosen since useful column life was maintained significantly longer

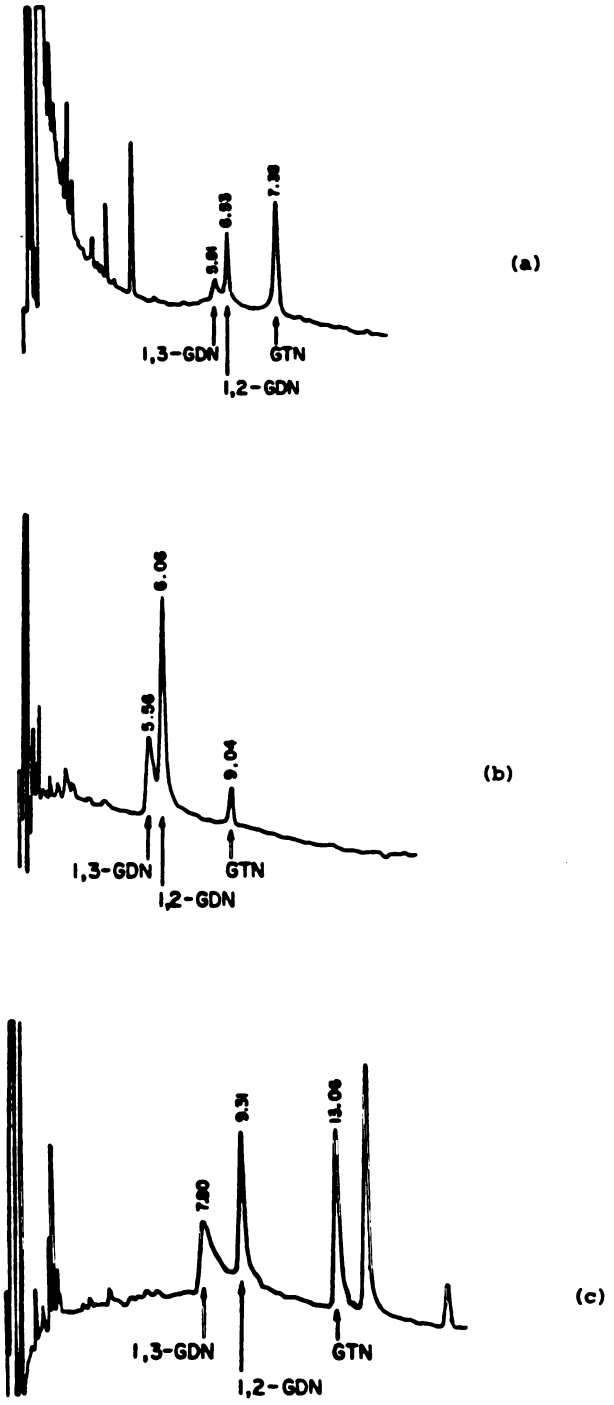

Figure II-1. Gas chromatograms of GTN, 1,2-GDN and 1,3-GDN after the injection of a mixed standard onto (a) DB-1, (b) RSL-150 and (c) SPB-1 capillary columns.

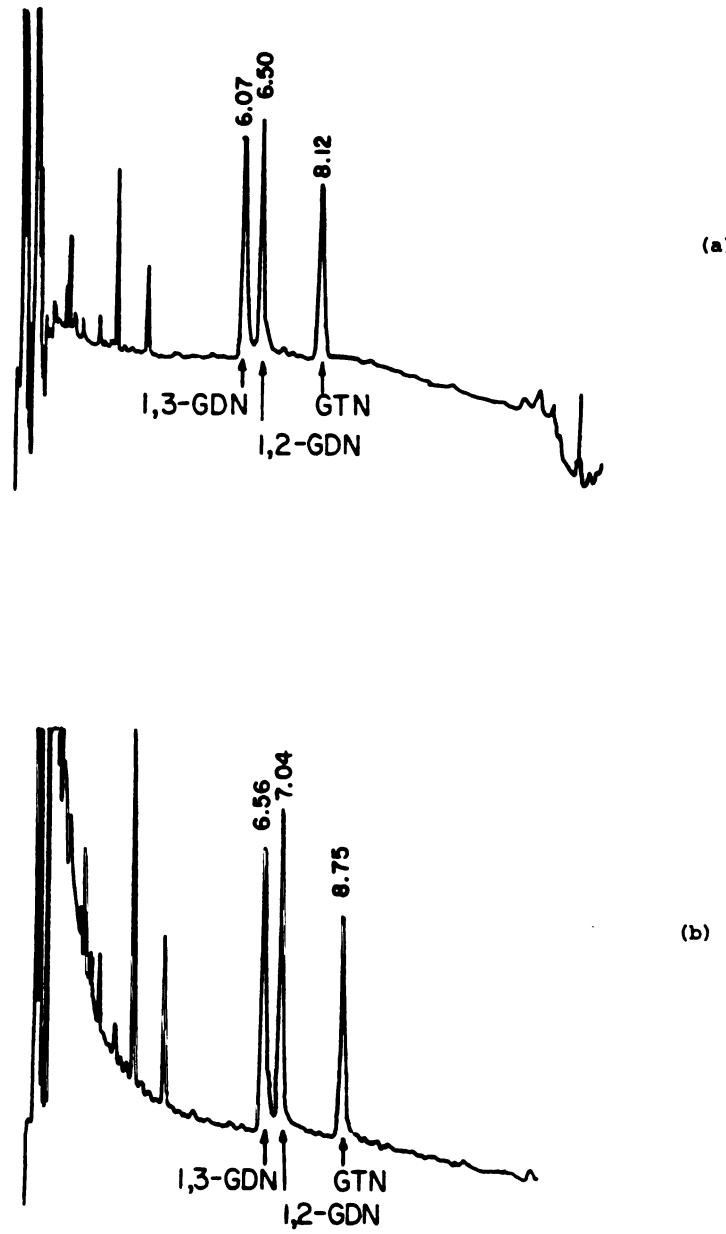

Figure II-2. Gas chromatograms of GTN, 1,2-GDN and 1,3-GDN after the injection of a mixed standard onto (a) HP-1 and (b) Quadrex-007 capillary columns.

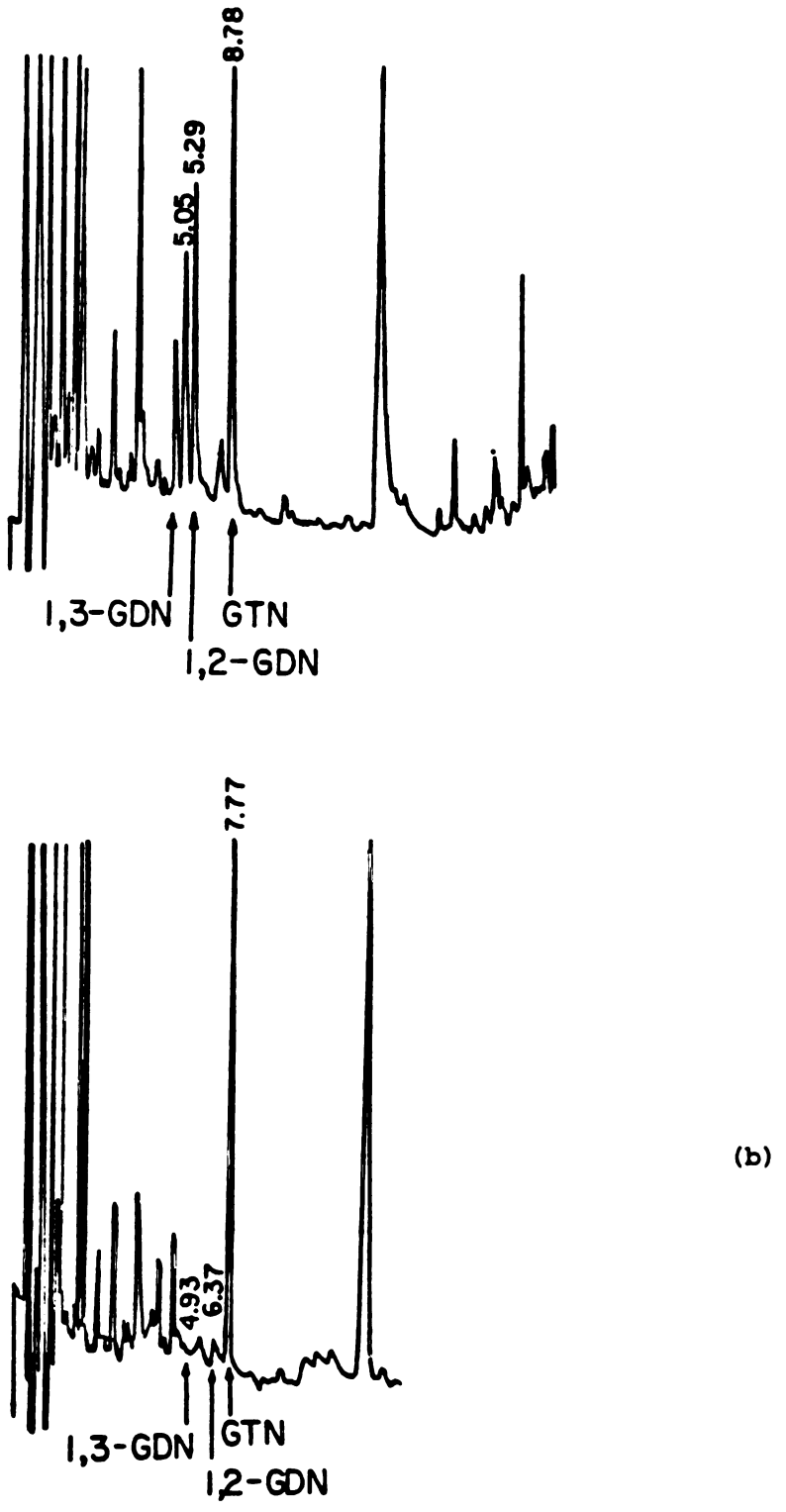

Figure II-3. Gas chromatograms of repeated injections, (a) first injection and (b) second injection, of the same plasma sample spiked with 1 ng/ml each of GTN, 1,2-GDN and 1,3-GDN when ether was used as the extraction solvent.

Table II-1: Recovery of methylene chloride/pentane extractable 1,2-GDN from [C-14]-1,2-GDN spiked human plasma[a]

Methylene Chloride/ Pentane Ratio (v/v)	% Extractable Radioactivity (n = 2)	
	Low Concentration (0.025 ng/ml)	High Concentration (20 ng/ml)
One Extraction[b]		
50/50	63.2	63.7
40/60	54.3	50.9
30/70	49.5	45.1
Two Extractions		
50/50	90.0	84.4
40/60	77.0	75.5
30/70	63.7	66.1
Three Extractions		
50/50	85.9	87.2
40/60	93.7	94.5
30/70	89.0	89.6

a One ml plasma was used.
b Ten ml of solvent was used in each extraction.

compared to the ratio 5/5 mixture. The chromatogram for a 1 ml blank plasma sample extracted with 10 ml of methylene chloride/pentane (3/7, v/v) is presented in Figure II-4, while Figure II-5 depicts that for a plasma sample spiked with 1 ng/ml of GTN, 1,2-GDN and 1,3-GDN, each.

Within-Day Precision (Repeatability)

The within-day precision of this method was checked by analysis of six replicate plasma samples to which known amounts of GTN, 1,2-GDN and 1,3-GDN were added. The results are listed in Table II-2. Coefficients of variation for GTN at the concentrations 0.03, 0.1, 1, and 10 ng/ml and for 1,2-GDN and 1,3-GDN at the concentrations 0.1, 1, and 10 ng/ml were all 5% or less. Accuracy of all samples was within 4% of the actual spiked concentration.

Between-Day Precision (Reproducibility)

The between-day precision was checked by analysis of four concentrations of GTN (0.03 0.1 , 1 and 10 ng/ml) and three concentrations of GDNs (0.1 , 1 and 10 ng/ml) over 6 days. The results are presented in Table II-3. The coefficients of variation were <5% for all concentrations. Accuracy of all samples was within 2% of the actual spiked concentration.

Linearity

Standard curves of GTN and GDNs were not linear over the entire concentration range of 0.02 to 10 ng/ml. As described by Noonan et al. (1984), this is not due to variation of extraction efficiency since the extraction recovery is the same for low and high concentration standards. When standard curves were limited to approximately a 10 fold concentration range, three linear standard curves were obtained for GTN (0.02 to 0.1 ng/ml, 0.1 to 1 ng/ml

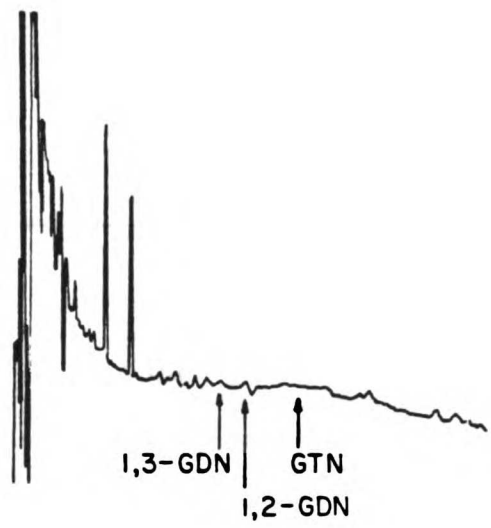

Figure II-4. Gas chromatogram of a blank plasma sample.

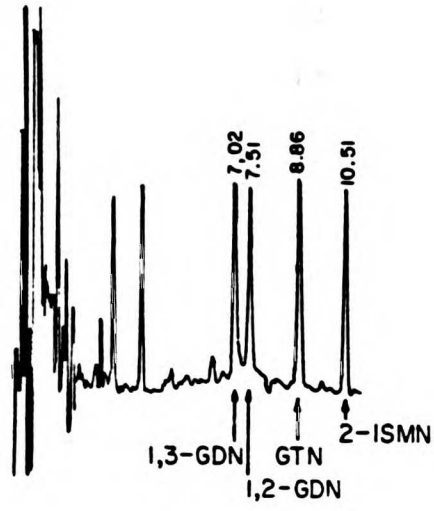

Figure II-5. Gas chromatogram of plasma sample spiked with 1 ng/ml each of GTN, 1,2-GDN and 1,3-GDN. 2-Isosorbide mononitrate (2-ISMN) was used as the internal standard.

Table II-2: Within-day precision

Spiked Concentration (ng/ml)	Calculated Concentration (mean±S.D., n=6) (ng/ml)	Coefficient of Variation (%)
GTN		
0.03	0.031 ± 0.001	3.23
0.1	0.101 ± 0.004	4.24
1	0.994 ± 0.039	3.89
10	9.98 ± 0.07	0.72
1,2-GDN		
0.1	0.099 ± 0.005	4.70
1	1.040 ± 0.047	4.58
10	9.82 ± 0.23	2.37
1,3-GDN		
0.1	0.101 ± 0.004	4.00
1	1.02 ± 0.05	5.14
10	10.1 ± 0.29	2.87

Table II-3: Between-day precision

Spiked Concentration (ng/ml)	Calculated Concentration (mean ± S.D., n=6) (ng/ml)	Coefficient of Variation (%)
GTN		
0.03	0.030 ± 0.002	4.97
0.1	0.101 ± 0.002	1.98
1	1.01 ± 0.02	1.86
10	9.93 ± 0.31	3.07
1,2-GDN		
0.1	0.100 ± 0.001	1.00
1	1.01 ± 0.03	2.67
10	9.82 ± 0.18	1.83
1,3-GDN		
0.1	0.101 ± 0.002	1.98
1	1.00 ± 0.02	1.80
10	10.0 ± 0.13	1.25

and 1 to 10 ng/ml) and two for the GDNs (0.1 to 1 ng/ml and 1 to 10 ng/ml) using weighted linear regressional analysis (weight = $1/y^2$) (Table II-4).

Pharmacokinetic Studies

This assay method has been used to determine simultaneously the plasma levels of GTN, 1,2-GDN and 1,3-GDN after various dosing routes for GTN in man and dogs. Figure II-6 shows a gas chromatogram of a dog plasma sample obtained 2 min following a 0.025 mg/ml iv bolus GTN dose. The plasma concentration versus time plots for parent drug and its dinitrate metabolites following a 0.3 mg iv dose and a 6.5 mg oral dose in a dog are shown in Figures II-7 and II-8, respectively. The maximum plasma concentrations for GTN, 1,2-GDN and 1,3-GDN following the iv dose were 4.39 ng/ml, 14.4 ng/ml and 2.70 ng/ml, respectively; following the oral dose they were 0.78 ng/ml, 34.7 ng/ml and 17.1 ng/ml, respectively. The half-lives determined for GTN, 1,2-GDN and 1,3-GDN after the iv bolus dose were 2.5 min, 53 min and 68 min, respectively. These values are very close to those reported previously (Miyazaki et al., 1982; Settlage et al., 1983).

CONCLUSIONS

A simple, sensitive GC-ECD method for the simultaneous determination of the antianginal drug nitroglycerin and its dinitrate metabolites was developed. The limits of detection of this method for GTN, 1,2-GDN and 1,3-GDN in plasma are 0.025, 0.1 and 0.1 ng/ml, respectively, which allows quantitation of the low levels of nitroglycerin and its dinitrate metabolites observed following therapeutic doses of nitroglycerin. This method has many advantages compared to other reports. The method does not require two separate assays to measure GTN and GDN plasma levels as reported by Noonan et al. (1985) and Sioufi et al. (1987). The methylene chloride/pentane (3/7) solvent employed here extracts less

Table II-4: Linearity of representative calibration curves

	GTN		1,2-GDN		1,3-GDN	
Spiked Conc. (ng/ml)	Peak Height Ratio	Calc. Conc. (ng/ml)	Peak Height Ratio	Calc. Conc. (ng/ml)	Peak Height Ratio	Calc. Conc. (ng/ml)
0.02	0.029	0.019	-	-	-	-
0.03	0.049	0.032	-	-	-	-
0.05	0.078	0.050	-	-	-	-
0.07	0.106	0.067	-	-	-	-
0.10	0.160	0.101	-	-	-	-
Intercept		-0.002				
slope		1.60				
r^2		0.9952				
0.1	0.160	0.099	0.075	0.100	0.081	0.102
0.3	0.307	0.306	0.192	0.296	0.163	0.293
0.5	0.441	0.495	0.313	0.498	0.249	0.494
0.7	0.600	0.719	0.441	0.712	0.340	0.707
1.0	0.783	0.977	0.612	0.997	0.471	1.013
Intercept		0.090		0.015		0.038
Slope		0.709		0.599		0.428
r^2		0.9991		0.9998		0.9995
1.0	0.783	1.01	0.612	0.988	0.471	0.991
3.0	1.76	2.89	1.63	3.08	1.28	3.06
5.0	3.01	5.30	2.66	5.19	2.07	5.06
7.0	3.87	6.96	3.46	6.82	2.88	7.12
10.0	5.37	9.85	4.91	9.79	3.88	9.64
Intercept		0.261		0.130		0.081
Slope		0.519		0.488		0.393
r^2		0.9978		0.9986		0.9990

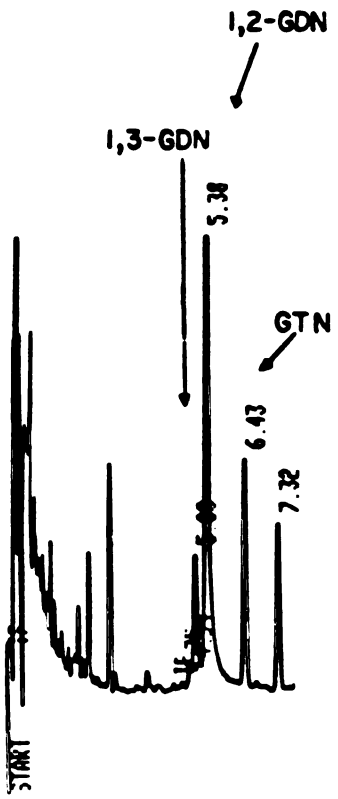

Figure II-6. Gas chromatogram of plasma sample (2 min) after a single intravenous dose (0.025 mg/kg) of GTN to a dog.

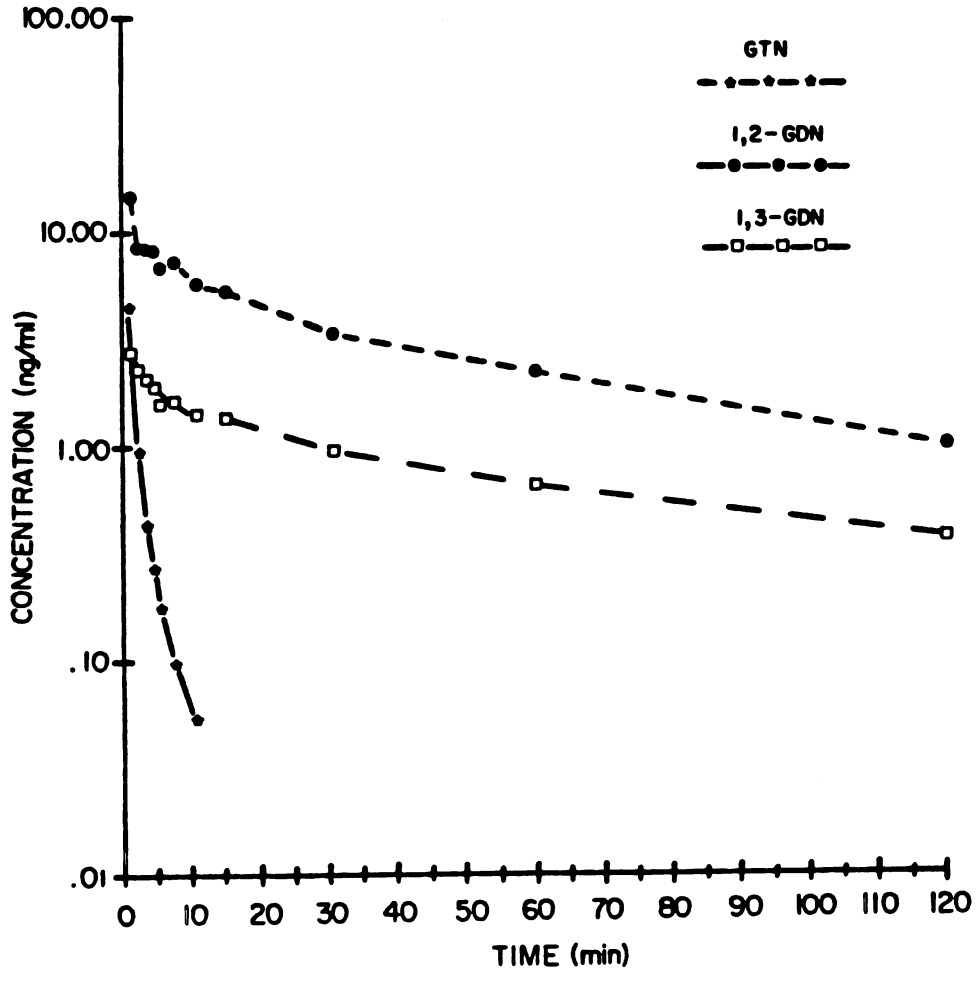

Figure II-7. Venous plasma concentrations of GTN, 1,2-GDN and 1,3-GDN following a single iv bolus dose of GTN (0.3 mg) to dog #2.

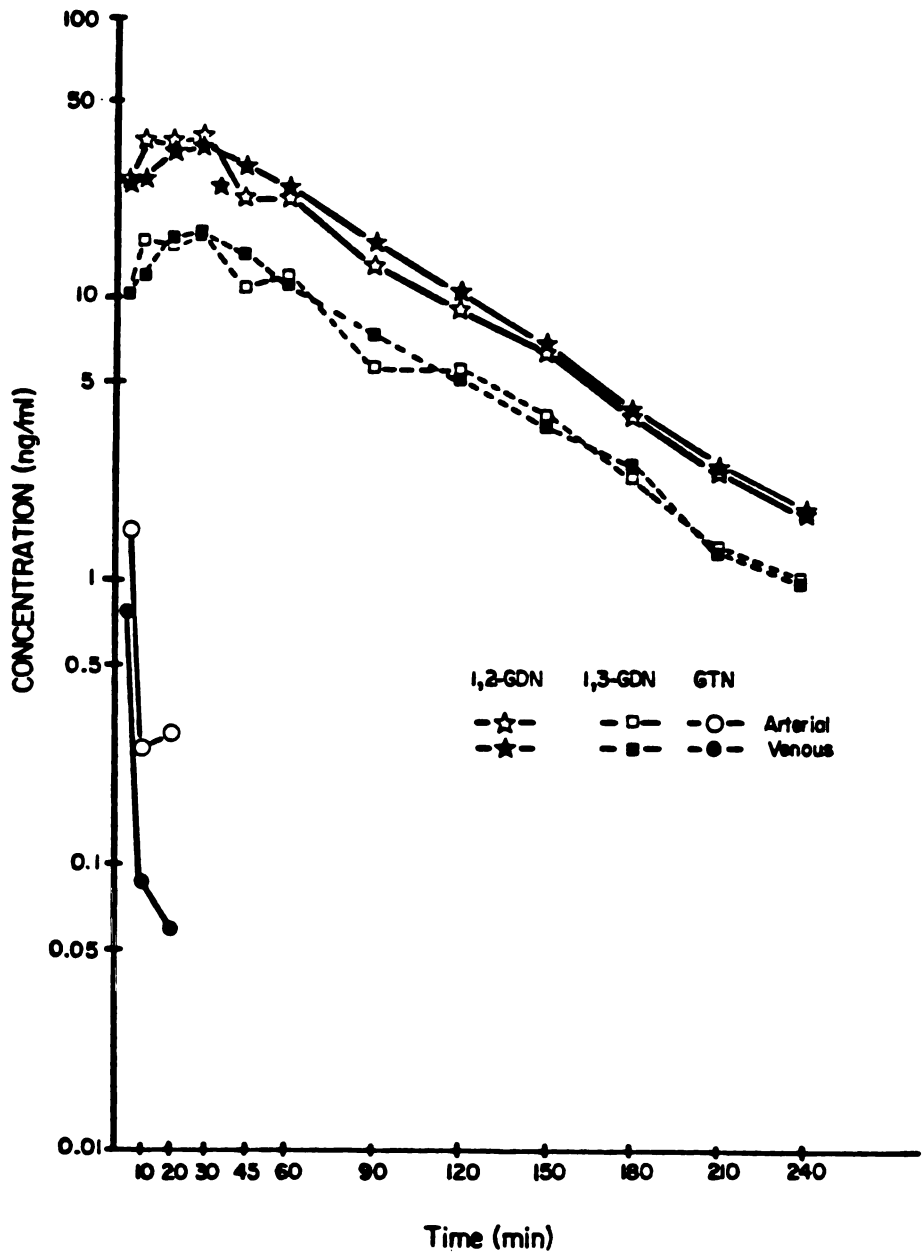

Figure II-8. Arterial and venous plasma concentrations of GTN, 1,2-GDN and 1,3-GDN following a single oral dose of GTN (6.5mg) to dog #2.

contaminants from plasma than other solvents, resulting in cleaner chromatograms and prolonged column life. Although the GC-MS method reported by Settlage et al. (1983) is more sensitive than our method, its cost is certainly higher, and its sensitivity is not necessary to adequately define the pharmacokinetics of GTN and its GDN metabolites. The required manual sample injection is the major disadvantage of our method. The newly developed automatic on-column injector should overcome this disadvantage.

CHAPTER III

PHARMACOKINETICS AND PHARMACODYNAMICS OF GLYCERYL TRINITRATE (GTN), 1,2-GLYCERYL DINITRATE (1,2-GDN) AND 1,3-GLYCERYL DINITRATE (1,3-GDN) IN CONSCIOUS DOGS

INTRODUCTION

The purpose of this study was two fold: 1) to examine the dinitrate metabolites levels and hemodynamic response after the intravenous and oral administration of GTN to conscious dogs; 2) to determine the pharmacokinetic parameters and hemodynamic response after the intravenous and oral administration of 1,2-GDN and 1,3-GDN to conscious dogs.

MATERIALS AND METHODS

Organic Nitrates

Nitroglycerin (Tridil IV) was purchased from Du Pont Critical Care (McGaw Park, IL). 1,2-GDN and 1,3-GDN were provided by Marion Laboratories, Inc. (Kansas City, MO) as pure chemicals (>99%) and used without further purification.

Animals

Six conditioned mongrel dogs, 2 males (dogs #6 and #7) and 4 females (dogs #3, #4, #5 and #8), weighing 25-30 kg were used. The conditioning schedule involved a month of observation under veterinary care before experimentation to insure a good state of health.

Dogs were housed individually in a room lighted from 0700 to 1800 hr and maintained at 22°C and 70% humidity. Dogs were given a ration of dry chow (Purina) daily at 1400 hr and had water available ad libitum. Body weight, body temperature and complete blood counts (CBC) were checked weekly for each dog to insure that the healthy condition of each dog was maintained.

Surgical Procedures

In order to facilitate the blood sampling and hemodynamic measurements, chronic catheters were implanted into the femoral artery and vein of each dog. The surgical procedure was conducted under aseptic conditions. Acepromazine was given subcutaneously as a pre-anesthetic agent (0.5 mg/kg), following which the dogs were anesthetized with sodium pentobarbital (25 mg/kg), intubated, and artificially ventilated. A Tygon catheter (ID 0.05 in, OD 0.09 in) was placed in the femoral artery, and the tip of the catheter was advanced into the abdominal aorta at the level of the renal arteries. Another catheter was placed into the femoral vein and the tip of the catheter was advanced into the abdominal vena cava. Catheters were routed subcutaneously and exteriorized between the shoulder blades. All catheters were wrapped with gauze and protected by placing them in a pocket, which was fastened inside a jacket (Alice King Chatham Medical Arts, Los Angeles, CA). Dogs were given penicillin (20,000 U/kg) combined with streptomycin (25 mg/kg) for 5 days and allowed at least 2 weeks to recover from the surgical procedure. Patency of vascular catheters was maintained by flushing with saline and filling with heparin (1000 U/ml) on alternate days. The Tygon catheters were previously shown to cause no adsorption of nitroglycerin from plasma samples.

Experimental Protocol

Food was withheld from the dogs overnight and throughout each study. Water was available ad libitum but not during the studies. The dogs were randomly dosed intravenously and orally with GTN, 1,2-GDN and 1,3-GDN at a dose level of 0.25 mg/kg. A lower intravenous dose of GTN at 0.025 mg/kg was also given to each dog. These dose levels are similar to the doses administered to dogs by Needleman et al. (1969). Generally, 5 days separated studies in each dog. Blood samples (5 ml) were collected through the venous catheter at 0, 1, 2, 3, 4, 5, 7, 10, 15, 30, 60, 90, 120, 150 and 180 min after bolus intravenous administration and at 0, 1, 2, 4, 5, 7, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min after oral administration. The blood volume loss due to blood sampling was replaced with normal saline following each blood sample withdrawal. Intravenous doses were administered through a front leg vein using an Angiocath (20 g, 1 1/4 in, Deseret Medical, Inc. Sandy, UT) and oral doses were given through a Teflon stomach tube. Arterial blood pressure was monitored with a Gould Statham p23db transducer (Oxnard, CA) connected to a Grass model 7 polygraph (Quincy, MA). All experiments were conducted in a quiet room with the dog in a sling (Alice King Chatham Medical Arts) which provided support but minimal restraint. The dogs were trained for one week to acclimatize them to this environment. Before each study the arterial blood pressure was monitored for one hour as the control period.

The blood samples were centrifuged using an Eppendorf centrifuge (Brinkmann Instruments, Westbury, NY) immediately after collection. The plasma was separated and frozen using dry ice. Plasma samples were assayed within a month of collection.

Assay Method

A capillary GC-ECD method was used to simultaneously determine the plasma concentrations of GTN, 1,2-GDN and 1,3-GDN. The detailed procedure was described in Chapter II.

Statistical Analysis

One-way analysis of variance (ANOVA) with repeated-measures was used to compare the potency between drug treatments (i.e., GTN, 1,2-GDN and 1,3-GDN) via the same route of dose administration (i.e., iv or oral). If a significant difference was found then the Newman-Keuls multiple range test was used to isolate the individual pairs exhibiting the significant difference. Paired Student's t test (two tailed) was used to compare the potency between routes of administration for the same drug treatment.

RESULTS

A. Pharmacokinetics

1) Intravenous and Oral Administration of Nitroglycerin

Low Dose of Intravenous Nitroglycerin

Plasma concentrations of GTN, 1,2-GDN and 1,3-GDN versus time plots after 0.025 mg/kg intravenous bolus doses of GTN are shown in Figures III-1 to III-3, respectively. Pharmacokinetic parameters are listed in Table III-1. The terminal half-lives were estimated by linear regression of the linear portion of the terminal phase of the log drug

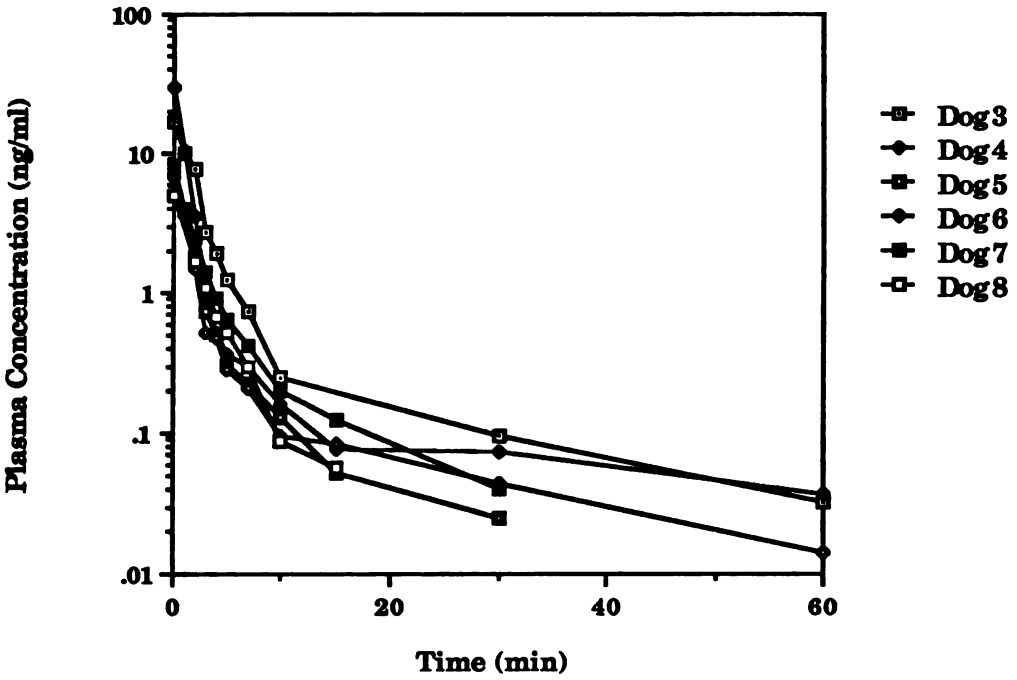

Figure III-1: GTN plasma concentrations vs time plots following iv doses of GTN (0.025 mg/kg) to dogs

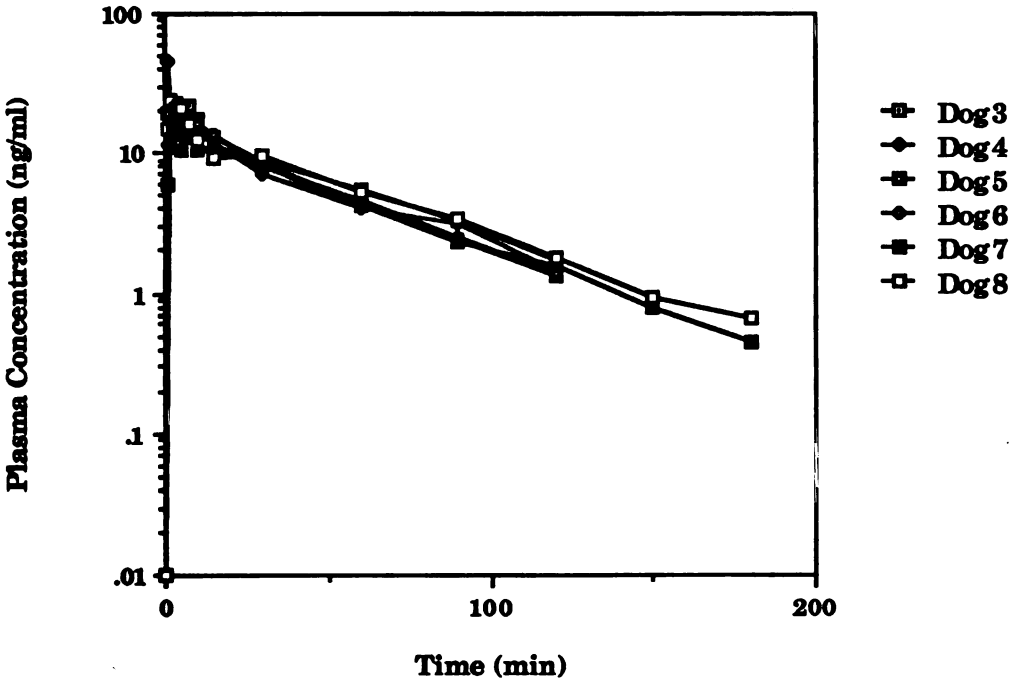

Figure III-2: 1,2-GDN plasma concentrations vs time plots following iv doses of GTN (0.025 mg/kg) to dogs

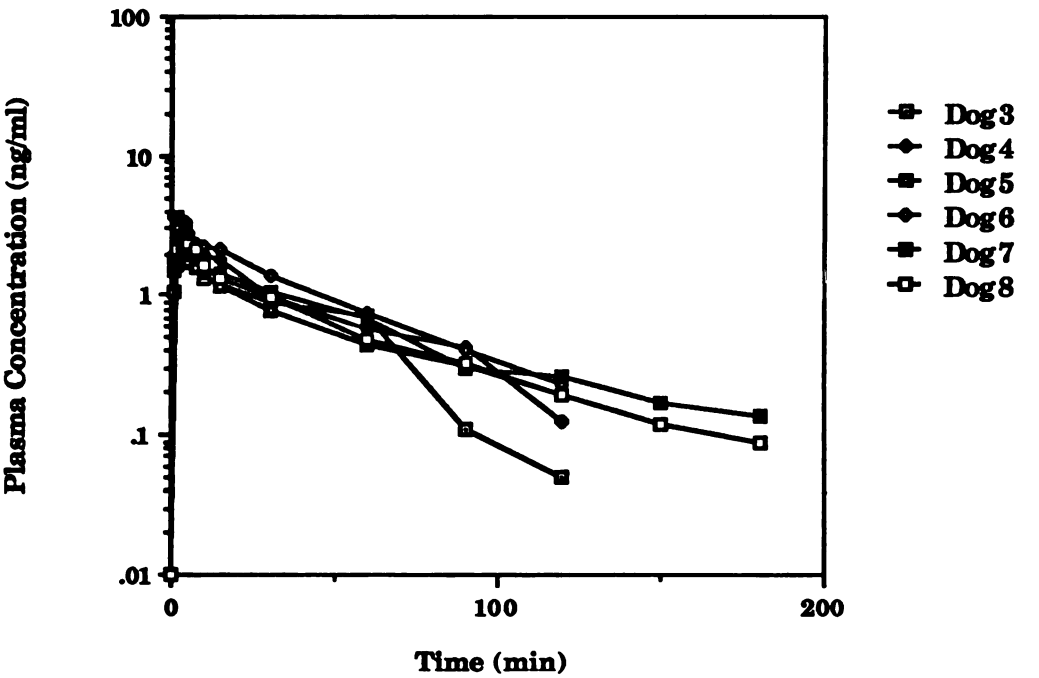

Figure III-3: 1,3-GDN plasma levels vs time plots following iv doses of GTN (0.025 mg/kg) to dogs

Table III-1: Pharmacokinetic parameters of nitroglycerin (GTN) following intravenous dosing of GTN (0.025 mg/kg) to conscious dogs

Dog #	Cmax (ng/ml)	Tmax (min)	Total AUC (ng*min /ml)	Total AUMC (ng*min^2 /ml)	$T_{1/2}$ (min)	CLapp (ml/min /kg)	Vapp,ss (L/kg)
GTN							
3	9.88	1	39.4	259	17	635	4.17
4	10.3	1	34.7	376	39	720	7.81
5	4.00	1	13.1	51.2	9.3	1900	7.46
6	3.83	1	13.0	106	18	1920	15.7
7	3.77	1	17.8	149	16	1410	11.8
8	1.68	2	12.1	72.1	6.8	2060	12.3
Mean	5.58	1	21.7	169	18	1440	9.87
S.D.	3.60	0	12.2	125	11	630	4.15
1,2-GDN							
3	22.8	2	912	46429	37		
4	45.7	1	862	48434	46		
5	23.2	3	819	37009	34		
6	21.6	5	808	37984	35		
7	15.3	5	713	38204	36		
8	24.6	2	888	49646	38		
Mean	25.5	3	834	42951	38		
S.D.	10.4	2	71	5822	4		
1,3-GDN							
3	3.68	2	80.4	2825	22		
4	3.63	1	105	4669	35		
5	1.95	3	86.7	5279	47		
6	3.03	2	129	6235	35		
7	2.27	3	113	7759	50		
8	2.35	5	97.9	5675	44		
Mean	2.82	3	102	5407	39		
S.D.	0.74	1	18	1644	10		

plasma concentration versus time plots as 0.693/(-slope). The total AUC was estimated from 0 to infinite time. The trapezoidal method was used to calculate the area from time 0 to the last time point. The area from the last time point to infinite time was estimated as the last measurable plasma concentration divided by k. The parameter k is the elimination rate constant which has the same value (-slope) mentioned above. The trapezoidal method was also used to calculate AUMC from time 0 to the last time point. The area under the moment curve from last time point to infinity was estimated using the method proposed by Benet and Galeazzi (1979) as follows: Area (Tlast to infinity) = (Tlast *Clast/k) + ($Clast/k^2$).

Longer GTN terminal half-lives (mean $\pm$ SD = 17.7 $\pm$ 11.4 min) than found in man (Armstrong et al., 1979, 1982; Noonan et al., 1985) were observed in all six dogs and ranged from 6.8 to 39 min. This longer half-life was also observed by Fung et al. (1984a) in rats and Miyazaki et al. (1982) in dogs. It is probably due to the rate limited redistribution of GTN from tissue back to plasma. Since the AUC corresponding to this slow decline phase contributed less than 20% of total AUC, this longer half-life does not represent the great majority of the GTN clearance. The T1/2 corresponding to the rapid decline phase which represents the majority portion of the AUC was about 4 min. This is similar to the values reported previously in humans (Armstrong et al., 1979, 1982; Noonan et al., 1985), and rats (Yap et al., 1978). The apparent clearance, CLapp, calculated as dose/AUC varied between dogs and was always greater than cardiac output (~120 ml/min/kg). Such high clearance can be attributed to the extensive metabolism of GTN in blood (Noonan and Benet, 1982) and liver (Needleman et al., 1972) and the uptake and metabolism by blood vessels (Fung et al., 1984b). The mean value of clearance after the low GTN dose (0.025 mg/kg) was 1440 ml/min/kg (S.D. = 630) and ranged from 635 to 2060 ml/min/kg. The apparent volume of distribution at steady-state, Vapp,ss, calculated as Dose*$AUMC/AUC^2$ (Benet and Galeazzi, 1979), also varied between dogs and ranged from 4.17 to 15.7 L/kg (mean $\pm$ S.D. = 9.87 $\pm$ 4.15). The formation of 1,2-GDN and 1,3-GDN was rapid and extensive (Figure III-4). The

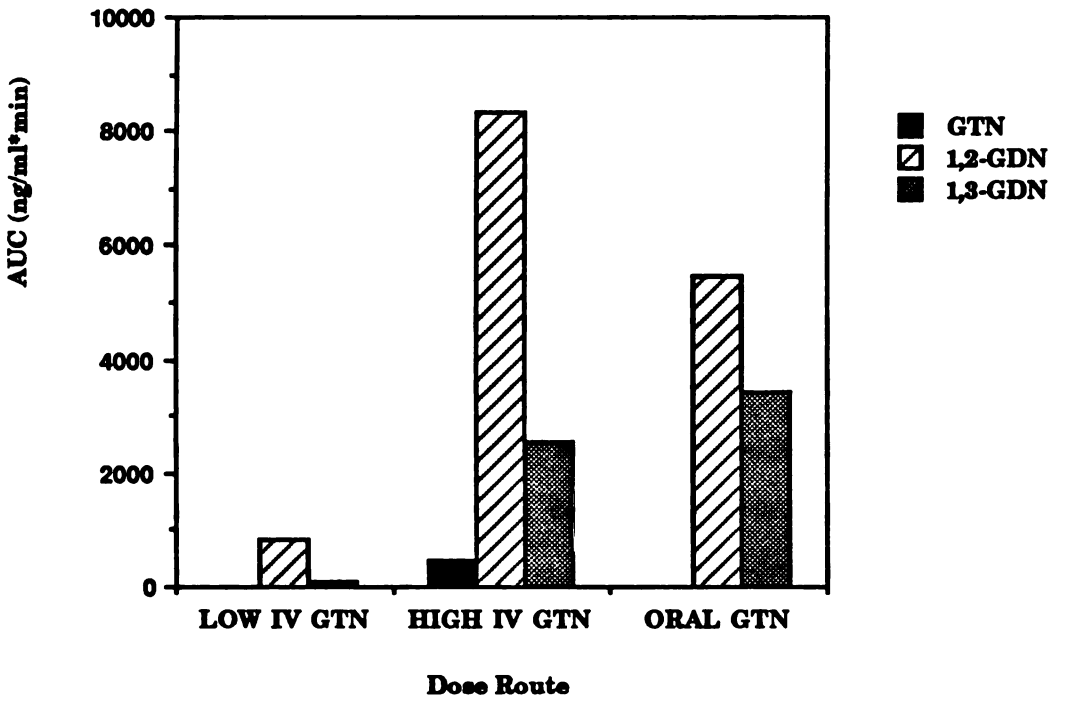

Figure III-4: AUC of GTN, 1,2-GDN and 1,3-GDN plasma vs time curves following various GTN administrations

average Tmax values were 3 min (S.D. = 1.7) and 3 min (S.D. = 1.4) for 1,2-GDN and 1,3-GDN, respectively, whereas Cmax was 25.5 ng/ml (S.D. = 10.4) and 2.82 ng/ml (S.D. = 0.74), for the two metabolites, respectively. The half-lives were 38 min (S.D. = 4) and 39 min (S.D. = 10) for 1,2-GDN and 1,3-GDN, respectively. The ratio of AUCs, 1,2-GDN to 1,3-GDN, was calculated to be 8.44 (S.D. = 1.94).

High Dose of Intravenous Nitroglycerin

Generally, all dogs demonstrated a similar plasma level versus time relationship. Therefore, only a representative plot from a single dog is presented for the following studies. Representative plasma levels of GTN, 1,2-GDN and 1,3-GDN versus time plots after a single 0.25 mg/kg bolus intravenous nitroglycerin dose to dog 3 are shown in Figure III-5. Pharmacokinetic parameters in all 6 dogs are summarized in Table III-2. The terminal half-lives after the high dose intravenous GTN were quite long (mean $\pm$ S.D. = 70 $\pm$ 42 min) and ranged from 21 to 121 min. The AUC corresponding to this slow decline phase was substantial and averaged 40%. Therefore, this long half-life may not be insignificant in terms of either pharmacokinetics or pharmacodynamics. The apparent clearance (mean $\pm$ S.D. = 686 $\pm$ 317 ml/min/kg) decreased to about half the value observed for the low dose of GTN ($p<0.05$) while the apparent volume of distribution at steady-state (mean $\pm$ S.D. = 10.6 $\pm$ 5.4 L/kg) did not change. The apparent clearance ranged from 249 to 1140 ml/min/kg whereas volume of distribution ranged from 6.08 to 21.0 L/kg. The Cmax of 1,2-GDN and 1,3-GDN were 150 ng/ml (S.D. = 58) and 37.8 ng/ml (S.D. = 17.1), respectively. The Tmax were 4.8 min (S.D. = 1.3) and 7.0 min (S.D. = 4.1) for 1,2-GDN and 1,3-GDN, respectively. The half-lives of 1,2-GDN and 1,3-GDN were 50 min (S.D. = 5) and 47 min (S.D. = 7), respectively. The ratio of AUCs, 1,2-GDN to 1,3-GDN, was 3.41 (S.D. = 0.90). This value is much lower ($p<0.01$) than the ratio calculated for the low dose GTN studies. When AUCs of 1,2-GDN and 1,3-GDN were compared separately between the high and low dose GTN

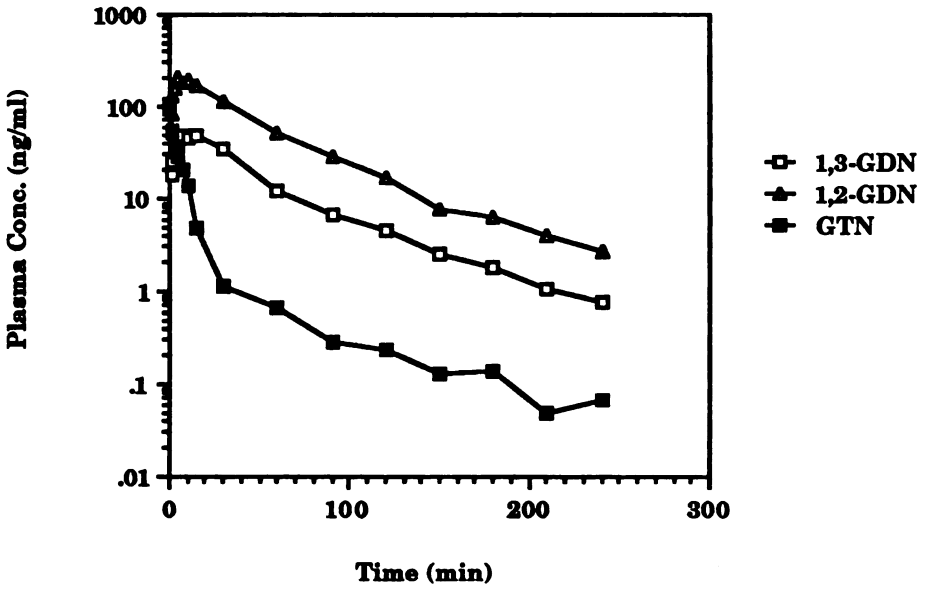

Figure III-5: GTN, 1,2-GDN and 1,3-GDN plasma concentration vs time plots following an iv dose of GTN (0.25 mg/kg) to dog #3.

Table III-2: Pharmacokinetic parameters of GTN following intravenous dosing of GTN (0.25 mg/kg) to conscious dogs

Dog #	Cmax (ng/ml)	Tmax (min)	Total AUC (ng*min /ml)	Total AUMC (ng*min^2 /ml)	$T_{1/2}$ (min)	CLapp (ml/min /kg)	Vapp,ss (L/kg)
GTN							
3	55.8	1	518	9789	61	482	9.1
4	271	1	1005	24580	121	249	6.0
5	59.4	2	409	7222	69	611	10.8
6	60.1	1	359	10824	115	696	21
7	33.1	1	220	1880	21	1140	9.71
8	72.2	1	266	1890	30	940	6.68
Mean	91.9	1	463	9364	70	686	10.6
S.D.	88.7	0	286	8370	42	317	5.4
1,2-GDN							
3	208	5	9791	499415	56		
4	215	5	11005	561441	50		
5	172	3	10297	700709	43		
6	137	4	6618	371097	50		
7	71.4	5	5920	478161	56		
8	101	7	6447	440094	46		
Mean	150	4	8346	508486	50		
S.D.	58.0	1	2256	113410	5		
1,3-GDN							
3	47.2	5	2624	135810	50		
4	68.7	7	4255	257724	51		
5	27.5	4	2121	142432	38		
6	28.5	4	1743	126000	57		
7	24.1	15	2388	205861	48		
8	31.0	7	2169	132117	38		
Mean	37.8	7	2550	166657	47		
S.D.	17.1	4	885	53319	7		

studies the ratio of 1,3-GDN AUC $_{high}$/AUC $_{low}$ was 25.0 while 1,2-GDN AUC $_{high}$/AUC $_{low}$ was 10.0. This indicates a change in the metabolism pattern in which relatively more GTN was converted into 1,3-GDN at the high dose.

Oral Administration of Nitroglycerin

Representative plasma levels of GTN, 1,2-GDN and 1,3-GDN versus time plots after a single oral dose (0.25 mg/kg) of GTN to dog 3 are illustrated in Figure III-6. Pharmacokinetic parameters for all six dogs are summarized in Table III-3. The Cmax and Tmax for GTN following oral doses (0.25 mg/kg) of GTN were 1.50 ng/ml (S.D. = 1.84) and 1.3 min (S.D. = 0.5), respectively. The average half-life of GTN was 4.1 min (S.D. = 2.4) and ranged from 1.6 to 6.9 min. Bioavailability of oral GTN was calculated as 0.015 (S.D. = 0.019) by comparing total AUC obtained after the 0.25 mg/kg oral dose with that obtained after the 0.25 mg/kg intravenous dose of GTN. The Cmax of 1,2-GDN (mean $\pm$ S.D. = 85.4 $\pm$ 53.1 ng/ml) and 1,3-GDN (mean $\pm$ S.D. = 55.7 $\pm$ 27.9 ng/ml) were much higher than that of GTN. The Cmax of 1,2-GDN was about 57 times higher than that of GTN whereas the Cmax of 1,3-GDN was about 37 times higher than that of GTN. The Tmax of 1,2-GDN and 1,3-GDN were 28 min (S.D. = 14) and 27 min (S.D. = 15), respectively, following oral dosing of GTN. Half-lives of 1,2-GDN and 1,3-GDN were 43 min (S.D. = 5) and 46 min (S.D. = 6), respectively. The total AUC of 1,2-GDN (5477 ng * min/ml) averaged 978 times higher than the total AUC of GTN (5.6 ng * min/ml) while the total AUC of 1,3-GDN averaged about 613 times higher than that of GTN. The AUC of 1,2-GDN was 1.56 times (S.D. = 0.20) higher than that of 1,3-GDN. As shown in Table III-4, this ratio is much lower than that obtained after intravenous doses of GTN ($p<0.01$ vs low and high iv doses).

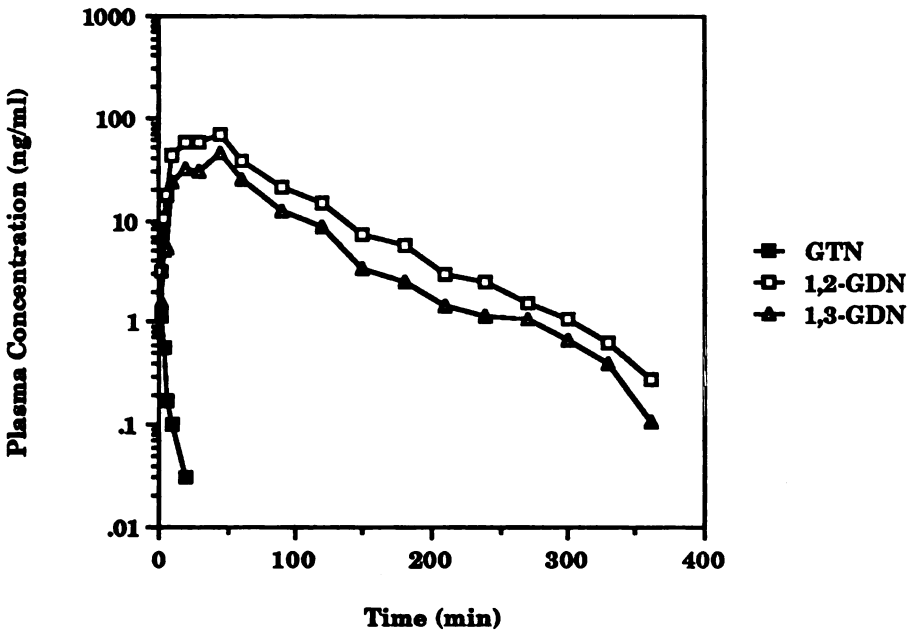

Figure III-6: GTN, 1,2-GDN and 1,3-GDN plasma concentration vs time plots following an oral dose of GTN (0.25 mg/kg) to dog #3

Table III-3: Pharmacokinetic parameters of GTN following oral dosing of GTN (0.25 mg/kg) to conscious dogs

Dog #	Cmax (ng/ml)	Tmax (min)	Total AUC (ng*min /ml)	$T_{1/2}$ (min)	F
GTN					
3	1.17	1	4.43	5.9	0.009
4	0.703	1	3.21	2.0	0.003
5	0.322	2	2.24	5.9	0.006
6	5.20	1	19.1	6.9	0.053
7	0.630	1	2.23	1.6	0.010
8	0.971	2	2.37	2.0	0.009
Mean	1.50	1	5.60	4.1	0.015
S.D.	1.84	1	6.67	2.4	0.019
1,2-GDN					
3	70.5	45	5306	46	
4	97.9	10	5684	49	
5	186	20	9962	41	
6	63.5	45	4335	41	
7	36.8	20	3286	36	
8	57.5	30	4289	43	
Mean	85.4	28	5477	43	
S.D.	53.1	14	2354	5	
1,3-GDN					
3	45.8	45	3060	46	
4	60.2	10	3491	57	
5	110	20	5551	41	
6	40.2	45	2839	46	
7	35.2	20	2566	47	
8	42.5	20	3085	41	
Mean	55.7	27	3432	46	
S.D.	27.9	15	1082	6	

Table III-4: Ratio of AUCs of 1,2-GDN and 1,3-GDN after various doses of GTN to conscious dogs

Dog #	Body Weight (kg)	High IV (0.25mg/kg)	Low IV (0.025mg/kg)	Oral (0.25mg/kg)
3	27	3.73	11.3	1.73
4	30	2.59	8.20	1.63
5	25	4.86	9.46	1.79
6	22	3.80	6.28	1.53
7	29	2.48	6.31	1.28
8	29	2.97	9.07	1.39
Mean	27	3.41	8.44	1.56
S.D.	3	0.90	1.94	0.20

2) Intravenous and Oral Doses of 1,2-GDN

Representative plasma levels of 1,2-GDN versus time plots after intravenous bolus and oral doses of 1,2-GDN (0.25 mg/mg) to dog #3 are shown in Figure III-7. Pharmacokinetic parameters are tabulated in Table III-5 for all six dogs. The mean Cmax after the intravenous dose was 488 ng/ml (S.D. = 133) and ranged from 285 to 682 ng/ml whereas after the oral dose of 1,2-GDN the mean Cmax was 150 ng/ml (S.D. = 47.4) and ranged from 85 to 202 ng/ml. The Tmax averaged 12 min (S.D. = 4) after the oral dose. Mean half-life was 40 min (S.D. = 5) and ranged from 34 to 45 min after the intravenous dose. The T 1/2 was 44 min (S.D. = 6) and ranged from 36 to 50 min after the oral dose. Average oral bioavailability for 1,2-GDN was 0.626 (S.D. = 0.220) and ranged from 0.482 to 1.03. Apparent body clearance of 1,2-GDN (mean $\pm$ S.D. = 16.4 $\pm$ 4.4 ml/min/kg) was much lower than that for the same dose of nitroglycerin (mean $\pm$ S.D. = 686 $\pm$ 317 ml/min/kg) and ranged from 12.8 to 24.9 ml/min/kg. The average apparent volume of distribution at steady-state was 0.809 L/kg (S.D. = 0.192) and ranged from 0.614 to 1.18 L/kg.

3) Intravenous and Oral Doses of 1,3-GDN

Representative plasma levels of 1,3-GDN after intravenous and oral doses (0.25 mg/kg) of 1,3-GDN to dog #4 as a function of time are illustrated in Figure III-8. Dog #4 was chosen as the representative animal here, rather than dog #3 since the latter received a higher than usual oral dose (see Table III-6). Pharmacokinetic data are summarized in Table III-6 for all six dogs. The pharmacokinetic characteristics of 1,3-GDN were very similar to those of 1,2-GDN. The mean Cmax after iv and oral 0.25 mg/kg doses were 518 ng/ml (S.D. = 107) and 138 ng/ml (S.D. = 33.0), respectively. The Cmax ranged from 354 ng/ml to 641 ng/ml after the iv dose and ranged from 89 ng/ml to 185 ng/ml after the oral dose. The Tmax was 28 min (S.D. = 14) after the oral dose and ranged from 10 to 45 min. Half-lives of 1,3-GDN after the

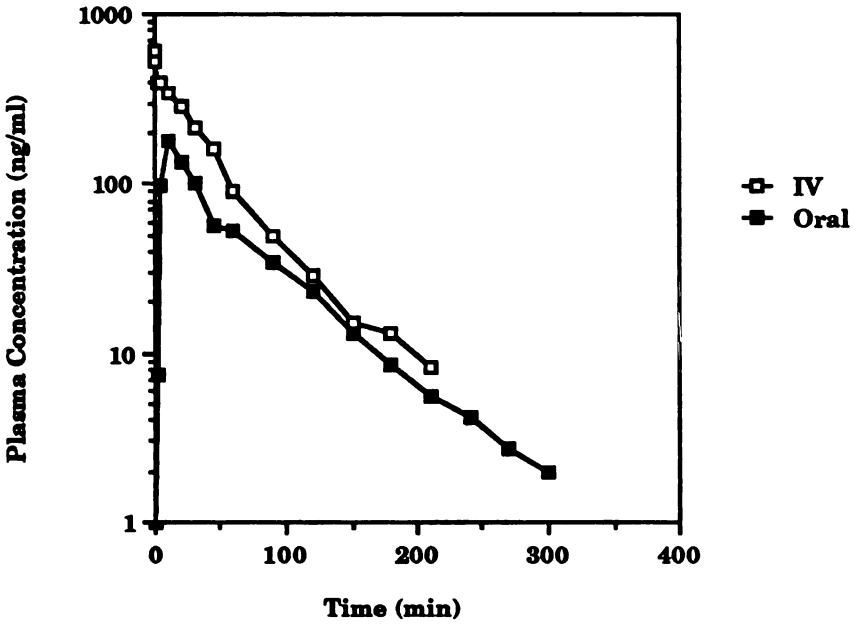

Figure III-7: 1,2-GDN plasma concentration vs time plots following iv and oral doses of 1,2-GDN (0.25 mg/kg) to dog #3.

Table III-5: Pharmacokinetic parameters of 1,2-GDN following intravenous and oral dosing of 1,2-GDN (0.25 mg/kg) to conscious dogs

Dog #	Cmax (ng/ml)	Tmax[a] (min)	Total AUC (ng*min /ml)	Total AUMC (ng*min^2 /ml)	$T_{1/2}$ (min)	CLapp (ml/min /kg)	Vapp,ss (L/kg)	F
Intravenous Dose								
3	530	1	16724	868787	42	14.9	0.776	
4	682	1	19577	1186882	45	12.8	0.774	
5	507	1	14612	646944	38	17.1	0.758	
6	285	4	10057	478133	37	24.9	1.18	
7	404	5	18086	803459	34	13.8	0.614	
8	520	1	16662	834913	45	15.0	0.752	
Mean	488	2	15953	803186	40	16.4	0.809	
S.D.	133	2	3327	237741	5	4.4	0.192	
Oral Dose								
3	182	10	8863	593441	49			0.530
4	135	10	9450	687457	50			0.483
5	202	10	15030	1039601	37			1.03
6	85.0	10	7375	618102	45			0.733
7	188	20	8957	559407	36			0.495
8	110	10	8037	619492	48			0.482
Mean	150	12	9619	686250	44			0.626
S.D.	47	4	2752	178136	6			0.220

[a] The first sampling time was chosen as the Tmax for the iv studies.

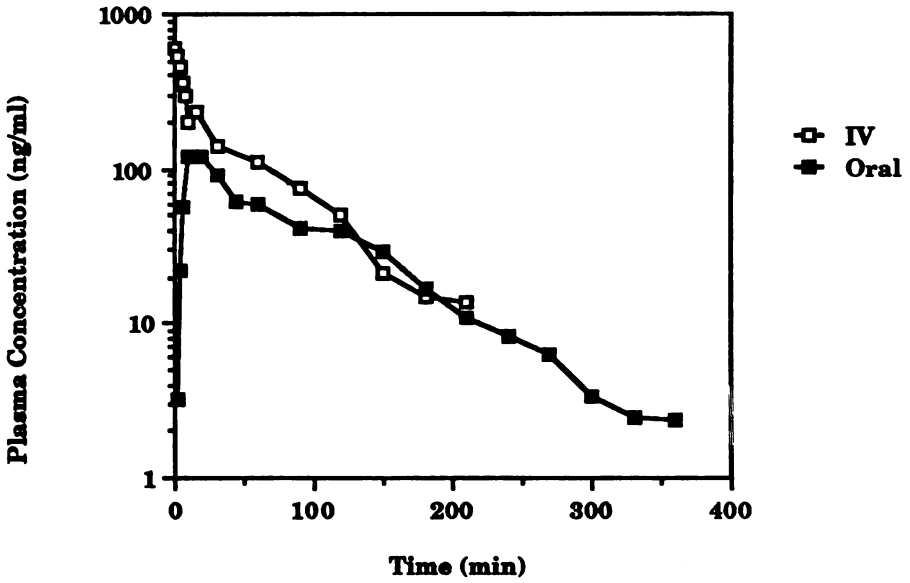

Figure III-8: 1,3-GDN plasma concentration vs time plots following iv and oral doses of 1,3-GDN (0.25 mg/kg) to dog #4.

Table III-6: Pharmacokinetic parameters of 1,3-GDN following intravenous and oral dosing of 1,3-GDN (0.25 mg/kg) to conscious dogs

Dog #	Cmax (ng/ml)	Tmax[a] (min)	Total AUC (ng*min /ml)	Total AUMC (ng*min^2 /ml)	$T_{1/2}$ (min)	CLapp (ml/min /kg)	Vapp,ss (L/kg)	F
Intravenous Dose								
3	431	1	11376	520851	43	22.0	1.01	
4	537	1	18779	1205948	45	13.3	0.855	
5	548	1	19341	923729	34	12.9	0.617	
6	354	1	15550	997884	50	16.1	1.03	
7	598	3	20535	1089012	49	12.2	0.646	
8	641	2	18353	882960	44	13.6	0.655	
Mean	518	2	17322	936731	44	15.0	0.802	
S.D.	107	2	3349	234701	6	3.7	0.189	
Oral Dose								
3[b]	116	30	8750	686844	57			0.769
4	122	20	10701	1001552	57			0.570
5	146	45	15698	1494779	43			0.812
6	148	20	11842	939341	48			0.762
7	89.0	45	11152	1031950	44			0.543
8	185	10	11494	835791	47			0.626
Mean	134	28	11606	998376	49			0.680
S.D.	33	14	2280	273688	6			0.115

a First sampling time was chosen as the Tmax for iv studies.
b Actual dose was 0.32 mg/kg. Cmax, F, AUC, and AUMC were normalized to the target dose of 0.25 mg/kg.

iv dose (mean ± S.D. = 44 ± 6 min) and the oral dose (mean ± S.D. = 49 ± 6 min) were independent of the dosing route. Half-lives ranged from 34 to 50 min after the iv dose and from 43 to 57 min after the oral dose of 1,3-GDN. Oral bioavailability of 1,3-GDN was calculated to be 0.680 (S.D. = 0.115). The average apparent total body clearance was 15.0 ml/min/kg (S.D. = 3.7) and the apparent volume of distribution at steady-state was 0.802 L/kg (S.D. = 0.189). The variabilities of the pharmacokinetic parameters for the dinitrates were much less than that observed for GTN in the same dogs.

4) Fractional GTN Clearance

In order to assess the pattern of GTN metabolism the fractional apparent metabolic clearances of GTN to 1,2-GDN (CLapp,m1) and 1,3-GDN (CLapp,m2) were calculated as follows:

$$CLapp,m = (AUCm / 182) * CLapp(m) / (AUC_{GTN} / 227)$$

where AUCm is the area under curve of metabolite 1,2-GDN or 1,3-GDN after GTN intravenous dosing; CLapp(m) is the apparent clearance of 1,2-GDN or 1,3-GDN as determined following intravenous doses of the metabolites in the same dogs (Tables III-5 and 6); AUC_{GTN} is the area under the curve for nitroglycerin; the values 182 and 227 are the molecular weights of the dinitrate metabolite and nitroglycerin, respectively.

As presented previously, the clearance of GTN is dose dependent, decreasing significantly from an average 1440 ± 630 ml/min for the low iv dose (Table III-1) to 686 ± 317 ml/min for the high iv dose. Therefore, the calculated fractional metabolic clearances are apparent values. The clearances of the metabolites themselves were determined following a single intravenous dose of the metabolites (Tables III-5 and 6). However, as will be discussed in

Chapter IV, the metabolites also exhibit an apparent dose dependence. The calculations presented here thus utilize the apparent clearance of the metabolite, CLapp(m), as determined following 0.25 mg/kg intravenous bolus doses of the metabolites.

The values for CLapp,m1 and CLapp,m2 are presented in Table III-7 for the low and high intravenous GTN doses in each dog. The apparent clearance of GTN to 1,2-GDN decreased significantly ($p<0.04$) in going from the low GTN dose (1030 $\pm$ 620 ml/min/kg) to the high GTN dose (425 $\pm$ 145 ml/min/kg). In contrast the apparent clearance to 1,3-GDN remained unchanged (low dose: 107 $\pm$ 55 ml/min/kg and high dose: 115 $\pm$ 37 ml/min/kg).

The ratio of the sum of the partial metabolic clearances to the dinitrate metabolites divided by the total apparent GTN clearance represents the fraction of the GTN dose accounted for as measured dinitrate metabolites. This is expressed as the percentage of the GTN dose accounted for in Table III-7. This percentage for the low iv dose was 75.7 $\pm$ 19.1%, a value not significantly different than the percent of the high dose accounted for by the dinitrate metabolites, 86.2 $\pm$ 21.2% ($p>0.2$). A similar calculation can be made for the oral dose as the amount of dinitrate metabolite formed is equal to the product of AUCm and CLapp(m). Correcting for molecular weight differences and dividing by the GTN dose yields the fraction of the dose accounted for by each dinitrate metabolite. Such values, expressed as percentages of the dose, are presented in Table III-8 for the oral and intravenous doses. The percentage of the oral GTN dose accounted for by the sum of the GDNs (70.3 $\pm$ 28.5%) was not significantly different from that accounted for the iv doses (one-way ANOVA, $p>0.2$). Considering that the bioavailability of orally dosed GDNs is 0.626$\pm$0.220 for 1,2-GDN and 0.680$\pm$0.115 for 1,3-GDN and realizing that less than 1% of an oral GTN dose is available, one may consider that the GDNs formed via oral GTN dosing would not be completely available. Under these conditions 106$\pm$18% of the GTN dose can be accounted for in terms of

Table III-7: Apparent partial metabolic clearances (ml/min/kg) of GTN to 1,2-GDN (CLapp,m1) and 1,3-GDN (CLapp,m2) following low (0.025 mg/kg) and high (0.25 mg/kg) intravenous bolus doses of GTN as well as the percentage of the GTN dose accounted for by the dinitrate metabolites

Dog #	$CL_{app,GTN}$	$CL_{app,m1}$	$CL_{app,m2}$	%GTN Dose Accounted for
Low Dose				
3	635	430	56.0	76.7
4	720	397	50.2	61.9
5	1900	1330	106	75.4
6	1920	1930	199	111
7	1410	689	96.6	56.0
8	2060	1370	137	73.0
Mean	1440	1030	107	75.7
S.D.	630	620	55	19.1
High Dose				
3	482	351	139	102
4	249	174	69.9	98.5
5	611	537	83.4	102
6	696	573	97.5	96.3
7	1140	463	165	55.3
8	940	453	138	62.9
Mean	686	425	115	86.2
S.D.	317	145	37	21.2

Table III-8: Percentages of intravenous and oral GTN doses accounted for by GTN metabolites

Dog #	1,2-GDN (%)	1,3-GDN (%)	Sum (%)
		Low iv dose (0.025 mg/kg)	
3	67.9	8.8	76.7
4	54.9	7.0	61.9
5	69.8	5.6	75.4
6	100	10.4	111
7	49.1	6.9	56.0
8	66.4	6.6	73.0
Mean	68.0	7.6	75.7
S.D.	17.6	1.7	19.1
		High iv dose (0.25 mg/kg)	
3	72.8	28.8	102
4	70.3	28.2	98.5
5	87.8	13.7	102
6	82.3	14.0	96.3
7	40.8	14.5	55.3
8	48.2	14.7	62.9
Mean	67.0	19.0	86.2
S.D.	18.7	7.4	21.2
		Oral dose (0.25 mg/kg)	
3	39.5	33.6	73.1
4	36.3	23.1	59.4
5	84.8	35.7	121
6	53.9	22.8	76.7
7	22.6	15.6	38.2
8	32.1	21.0	53.1
Mean	44.9	25.3	70.3
S.D.	22.1	7.8	28.5

dinitrate metabolites. It appears that the residual fraction, 30% of the oral GTN dose, was sequentially metabolized in the GI tract and liver.

In comparing percentages of individual metabolites across doses, the 44.9 $\pm$ 22.1% value for 1,2-GDN from the oral dose ($p<0.02$) and the 7.6 $\pm$ 1.7% value for 1,3-GDN from the low iv dose ($p<0.01$) were significantly different than percentages via other routes.

B. Pharmacodynamics

The following physiologic parameters were measured: arterial systolic, diastolic, pulse and mean blood pressures and heart rate. The change in systolic blood pressure was the most marked hemodynamic response observed. The net systolic blood pressure decrease (SPD) was thus used as the index to evaluate the pharmacodynamics of GTN, 1,2-GDN, and 1,3-GDN in our studies.

1) Intravenous and Oral Administration of Nitroglycerin

Low Dose of Intravenous Nitroglycerin

SPD versus time plots following iv doses (0.025 mg/kg) of GTN to the six dogs are illustrated in Figure III-9. A transient sharp drop of systolic pressure immediately post-dose was observed. The duration of systolic blood pressure decrease after a single intravenous dose of 0.025 mg/kg GTN was very short. In most of our dog studies the systolic pressure decrease returned to normal within 15 min post-dose. The maximum systolic pressure decrease (Dmax), time to the maximum systolic pressure decrease (Tmax), the area under the SPD vs time curve (AUCspd), the ratio of AUCspd to the area under the plasma concentration time

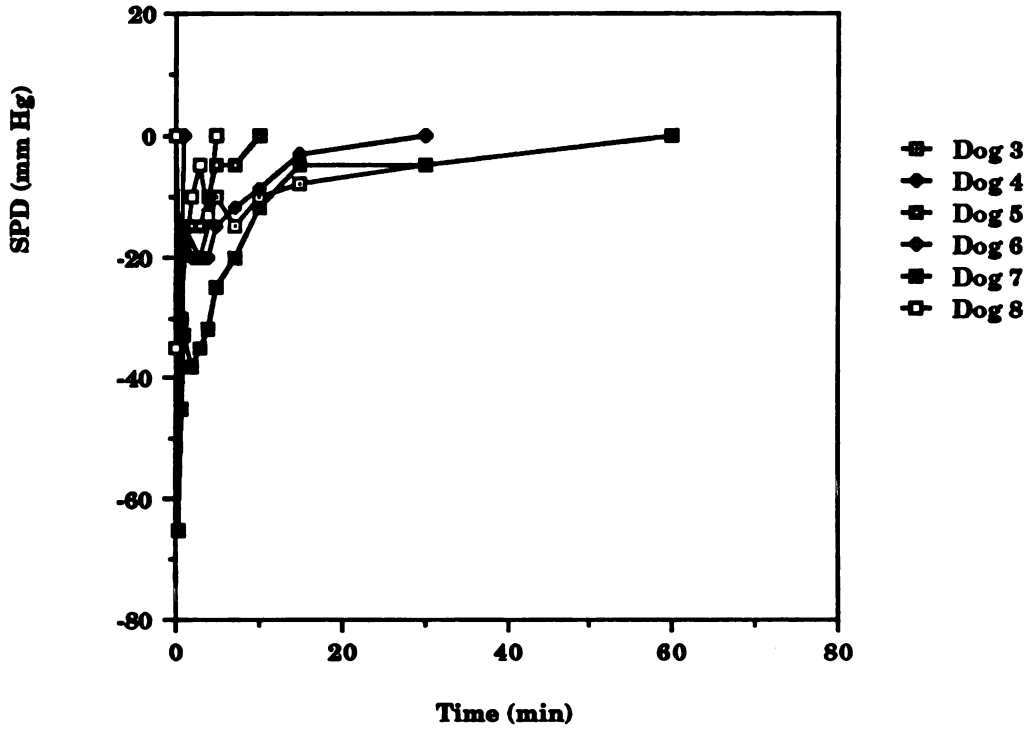

Figure III-9: SPD vs time plots following iv doses of GTN (0.025 mg/kg) to dogs

curve(AUCpl) and the ratio of AUCspd to the dose are listed in Table III-9. The SPD versus GTN plasma level plot for dog #3 is illustrated in Figure III-10.

High Dose of Intravenous Nitroglycerin

Representative plots of systolic blood pressure and SPD versus time after an intravenous high dose (0.25 mg/kg) of GTN to dog #3 are illustrated in Figure III-11. The values for Dmax, Tmax, AUC_{spd}, AUC_{spd}/AUC_{pl} and AUC_{spd}/Dose in all six dogs are listed in Table III-10. The hemodynamic responses were dramatic following this 10 fold higher dose (0.25 mg/kg) of GTN. The response can be divided into four stages which may be better visualized in the SPD versus GTN plasma level plot for dog 3 as shown in Figure III-12: a) systolic blood pressure dropped rapidly and significantly within the first few minutes post-dose. Dmax averaged 63 mm Hg (S.D. = 19) and Tmax averaged 1.0 min(S.D. = 1.1); b) the systolic pressure returned to normal probably due to compensatory sympathetic reflexes; c) systolic blood pressure slowly decreased again over a more prolonged period; followed by d) a slow return to base line.

Oral Dose Administration of Nitroglycerin

Orally dosed nitroglycerin (0.25 mg/kg) did yield significant hemodynamic responses in dogs even though the GTN plasma levels were quit low. The pharmacodynamic parameters for all six dogs are summarized in Table III-11. The Dmax was 34 mm Hg (S.D. = 17) and occurred at 29 min (S.D. = 11) post dose. The AUC_{spd} averaged 3447 mm Hg * min (S.D. = 3670)and ranged from 460 to 10352 mm Hg * min. A representative SPD versus time plot for dog #3 is shown in Figure III-13. The plot of SPD versus the total plasma concentrations of 1,2-GDN and 1,3-GDN (GTN concentrations were negligible compared to the GDN levels) for dog #3 is illustrated in Figure III-14.

Table III-9: Systolic blood pressure decrease following bolus intravenous doses of nitroglycerin (25 μg/kg) to conscious dogs

Dog #	Dmax (mm Hg)	Tmax (min)	AUCspd (mmHg*min)	AUCpl (ng*min/ml)	AUCspd/ AUCpl	AUCspd/ Dose
3	45	0.5	371	39.4	9.42	14.8
4	45	0.5	212	34.7	6.11	8.48
5	30	0.6	85.5	13.1	6.53	3.42
6	30	0.5	15.0	13.0	1.15	0.600
7	65	0.4	462	17.8	26.0	18.5
8	35	0.2	67.2	12.1	5.55	2.69
Mean	42	0.4	202	21.7	9.12	8.08
S.D.	13	0.2	181	12.2	8.67	7.22

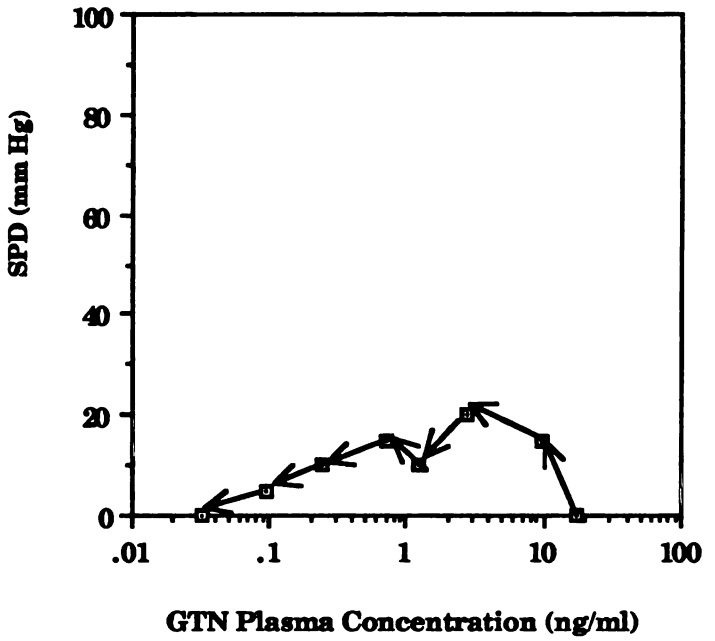

Figure III-10: SPD vs GTN plasma concentration plot following an iv dose of GTN (0.025 mg/kg) to dog #3. Arrows indicate the time order of measurements.

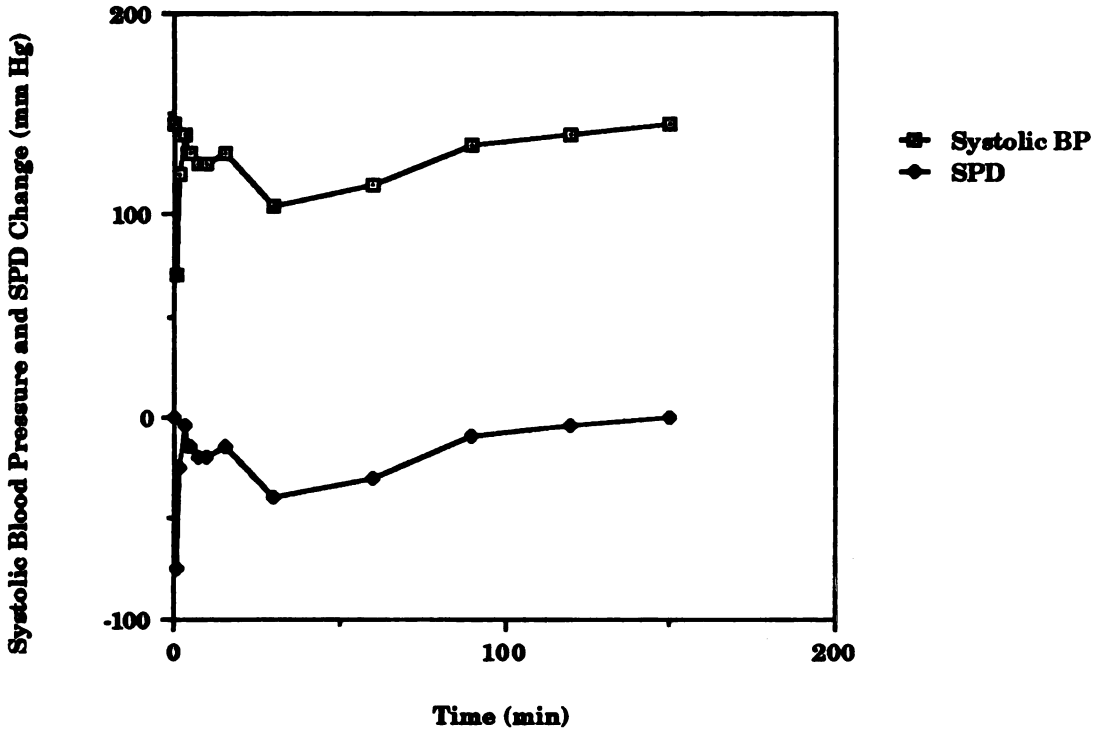

Figure III-11: Systolic blood pressure and its change (SPD) vs time plots following an iv dose of GTN (0.25 mg/kg) to dog #3.

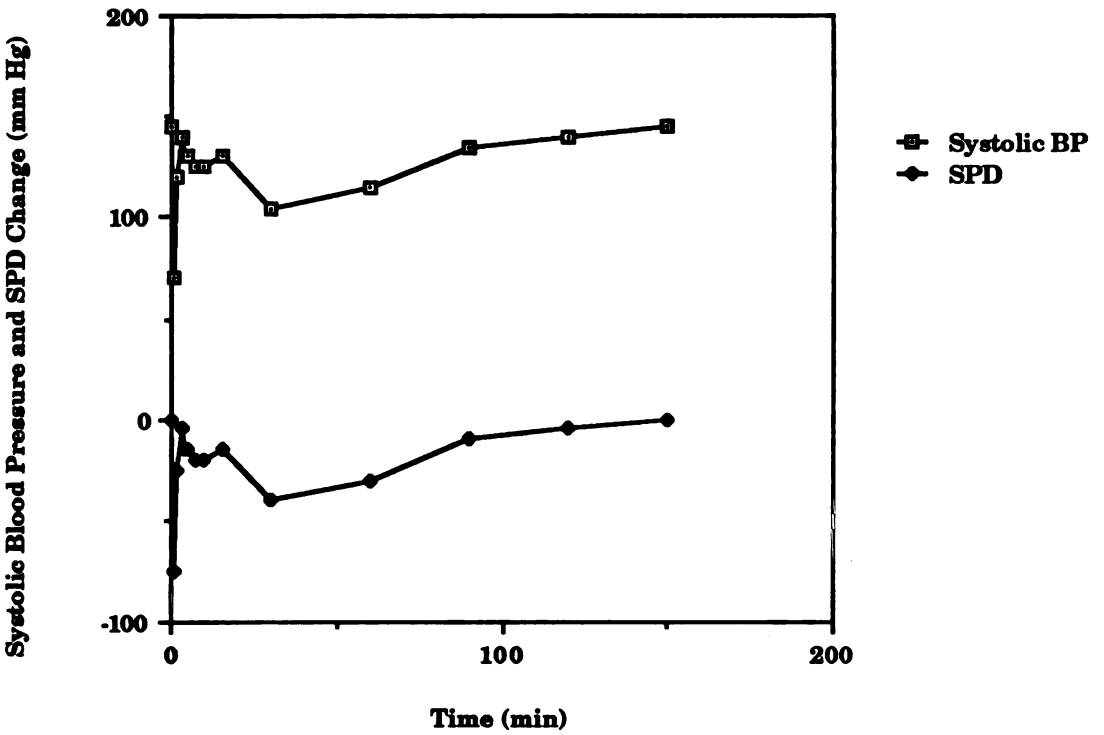

Figure III-11: Systolic blood pressure and its change (SPD) vs time plots following an iv dose of GTN (0.25 mg/kg) to dog #3.

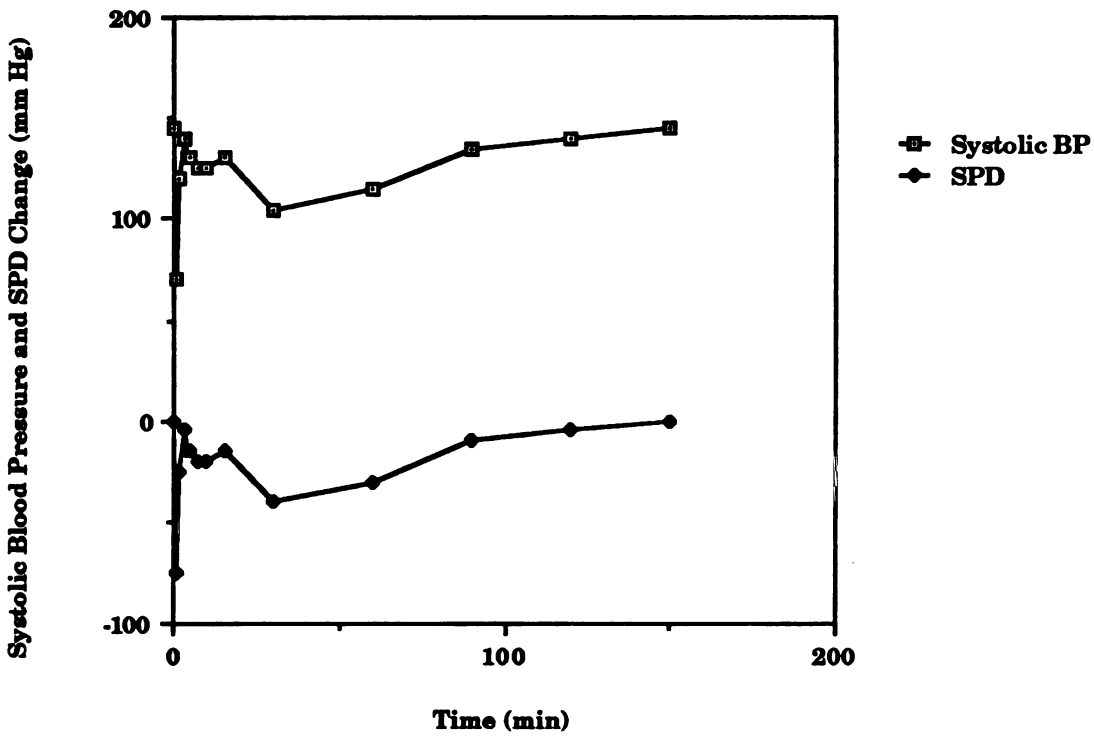

Figure III-11: Systolic blood pressure and its change (SPD) vs time plots following an iv dose of GTN (0.25 mg/kg) to dog #3.

Table III-10: Systolic blood pressure decrease following bolus intravenous doses of nitroglycerin (250 μg/kg) to conscious dogs

Dog #	Dmax (mm Hg)	Tmax (min)	AUCspd (mmHg*min)	AUCpl (ng*min/ml)	AUCspd/ AUCpl	AUCspd/ Dose
3	75	1.0	2673	518	5.16	10.7
4	95	3.0	2470	1005	2.46	9.88
5	50	0.2	1370	409	3.35	5.48
6	50	0.2	1158	359	3.23	4.63
7	61	0.3	646	220	2.94	2.58
8	48	1.0	2373	266	8.92	9.49
Mean	63	1.0	1782	463	4.34	7.13
S.D.	19	1.1	833	286	2.43	3.33

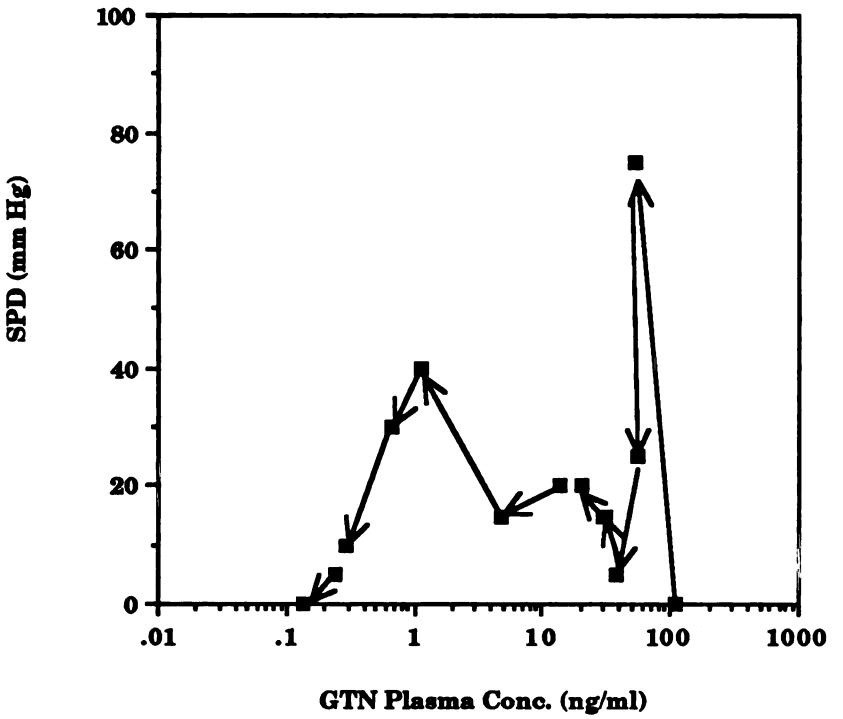

Figure III-12: Systolic blood pressure decrease vs GTN plasma concentration plot following an iv dose of GTN (0.25 mg/kg) to dog #3.

Table III-11: Systolic blood pressure decrease following oral doses of nitroglycerin (250 μg/kg) to conscious dogs

Dog #	Dmax (mm Hg)	Tmax (min)	AUCspd (mmHg*min)	AUCpl (ng*min/ml)	AUCspd/ AUCpl	AUCspd/ Dose
3	65	45	10352	4.43	2340	41.4
4	32	30	4363	3.21	1360	17.5
5	20	30	895	2.24	400	3.58
6	40	30	2933	19.1	154	11.7
7	20	30	1678	2.23	752	6.71
8	26	10	460	2.37	194	1.84
Mean	34	29	3447	5.60	867	13.8
S.D.	17	11	3670	6.67	849	14.7

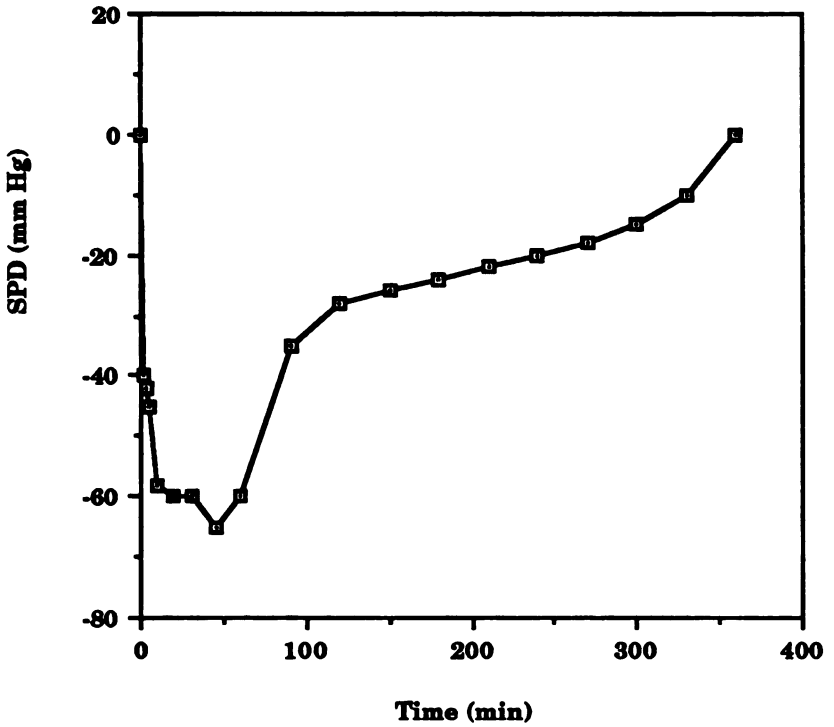

Figure III-13: Systolic blood pressure decrease vs time plot following an oral dose of GTN (0.25 mg/kg) to dog #3.

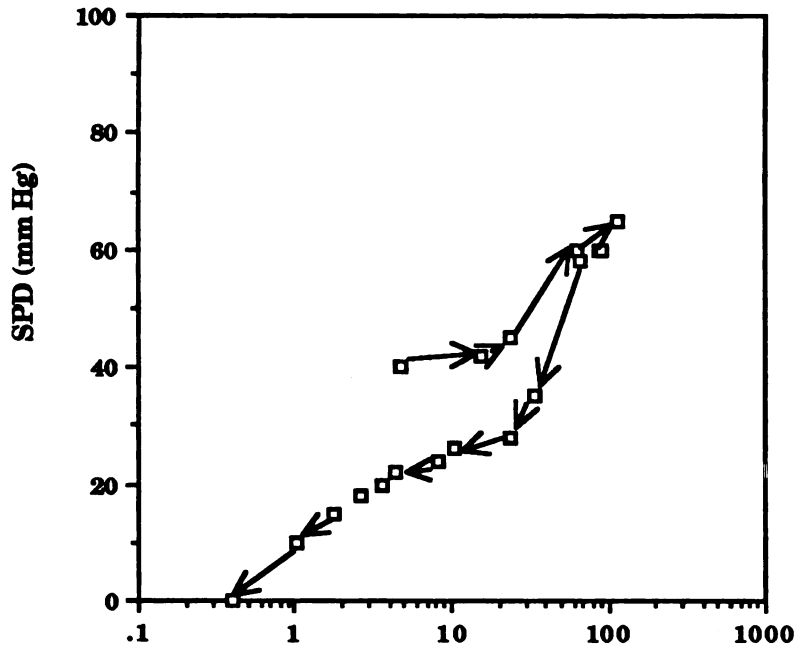

Figure III-14: Systolic blood pressure decrease vs total plasma concentration of 1,2-GDN and 1,3-GDN following an oral dose of GTN (0.25 mg/kg) to dog #3.

2) Intravenous and Oral Doses of 1,2-GDN

A significant systolic blood pressure decrease was observed after the administration of 1,2-GDN (0.25 mg/kg) via both intravenous and oral routes. Representative SPD versus time plots after intravenous and oral doses of 1,2-GDN to dog #3 are illustrated in Figure III-15. The pharmacodynamic parameters for all six dogs are summarized in Tables III-12 and III-13 for intravenous and oral doses, respectively. Representative SPD versus plasma concentrations of 1,2-GDN plots after iv and oral doses of 1,2-GDN in dog #3 are shown in Figure III-16. The Dmax and Tmax values after the intravenous dose averaged 41 mm Hg (S.D. = 9) and 7 min (S.D. = 5), respectively. After the oral dose of 1,2-GDN, the Dmax and Tmax averaged 40 mm Hg (S.D. =13) and 18 min (S.D. =8), respectively. The AUC_{spd} was 3665 mm Hg * min (S.D. = 1894) and 3116 mm Hg * min (S.D. = 1423) whereas the ratio of AUC_{spd}/AUC_{pl} was 0.233 (S.D. = 0.112) and 0.346 (S.D. = 0.180) for the intravenous and oral doses, respectively.

3) Intravenous and Oral Doses of 1,3-GDN

The hemodynamic responses in dogs after iv and oral doses (0.25 mg/kg) of 1,3-GDN were similar to that observed following 1,2-GDN dosing. Representative SPD versus time plots for dog #4 are presented in Figure III-17 after iv and oral doses. The pharmacodynamic parameters are summarized in Tables III-14 and III-15 for iv and oral doses, respectively. Mean Dmax values after iv and oral administration of 1,3-GDN were 36 mm Hg (S.D. = 16) and 31 mm Hg (S.D. = 5), respectively. Average Tmax was 6 min (S.D. = 3) after an iv dose and 27 min (S.D. = 17) after oral dosing. The AUC_{spd} after iv doses averaged 1997 mm Hg * min (S.D. = 1513) and ranged from 185 to 3856 mm Hg * min. The AUC_{spd} after oral doses averaged 3597 mm Hg * min (S.D. = 1043) and ranged from 2369 to 5078 mm Hg * min. The average ratio of AUC_{spd}/AUC_{pl} was also calculated giving a value of 0.130 (S.D. = 0.121)

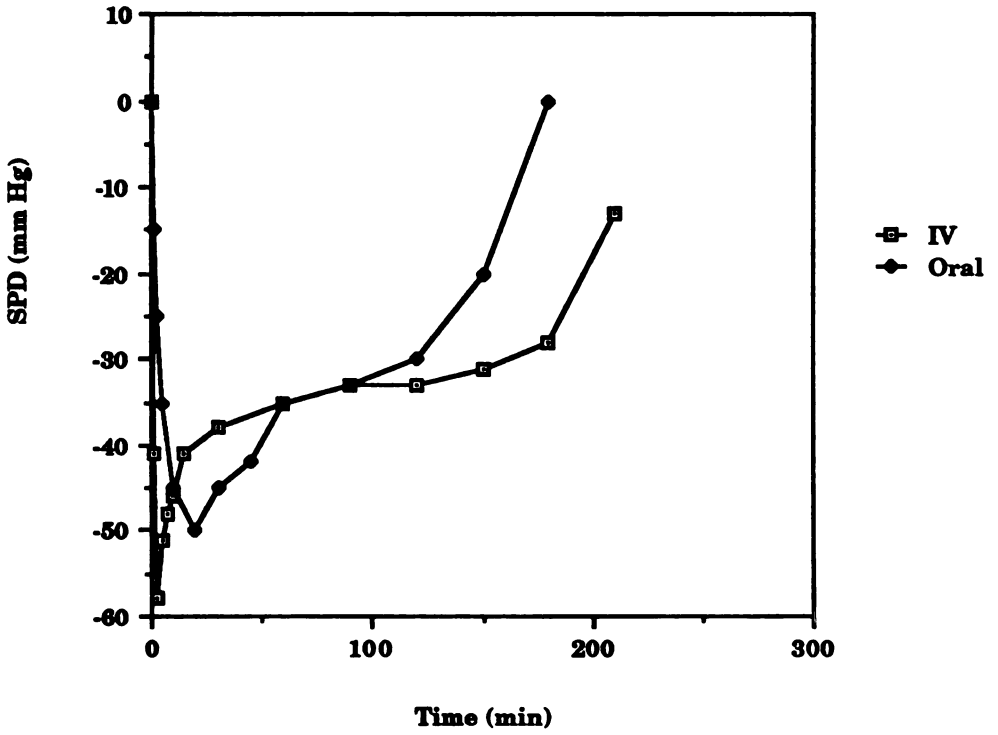

Figure III-15: Systolic blood pressure decrease vs time plots following iv and oral doses of 1,2-GDN (0.25 mg/kg) to dog #3.

Table III-12: Systolic blood pressure decrease following bolus intravenous doses of 1,2-GDN (250 μg/kg) to conscious dogs

Dog #	Dmax (mm Hg)	Tmax (min)	AUCspd (mmHg*min)	AUCpl (ng*min/ml)	AUCspd/ AUCpl	AUCspd/ Dose
3	58	3	6844	16724	0.409	27.4
4	37	10	4704	19577	0.240	18.8
5	40	3	1768	14612	0.121	7.07
6	35	15	2993	10057	0.298	12.0
7	37	4	2020	18086	0.112	8.08
8	38	5	3663	16662	0.220	14.7
Mean	41	7	3665	15953	0.233	14.7
S.D.	9	5	1894	3327	0.112	7.6

Table III-13: Systolic blood pressure decrease following oral doses of 1,2-GDN (250 μg/kg) to conscious dogs

Dog #	Dmax (mm Hg)	Tmax (min)	AUCspd (mmHg*min)	AUCpl (ng*min/ml)	AUCspd/ AUCpl	AUCspd/ Dose
3	50	20	5503	8863	0.621	22.0
4	33	30	3418	9450	0.362	13.7
5	25	20	2085	15030	0.139	8.34
6	60	10	3308	7375	0.449	13.2
7	36	20	3065	8957	0.342	12.3
8	37	10	1319	8037	0.164	5.28
Mean	40	18	3116	9619	0.346	12.5
S.D.	13	8	1423	2752	0.180	5.7

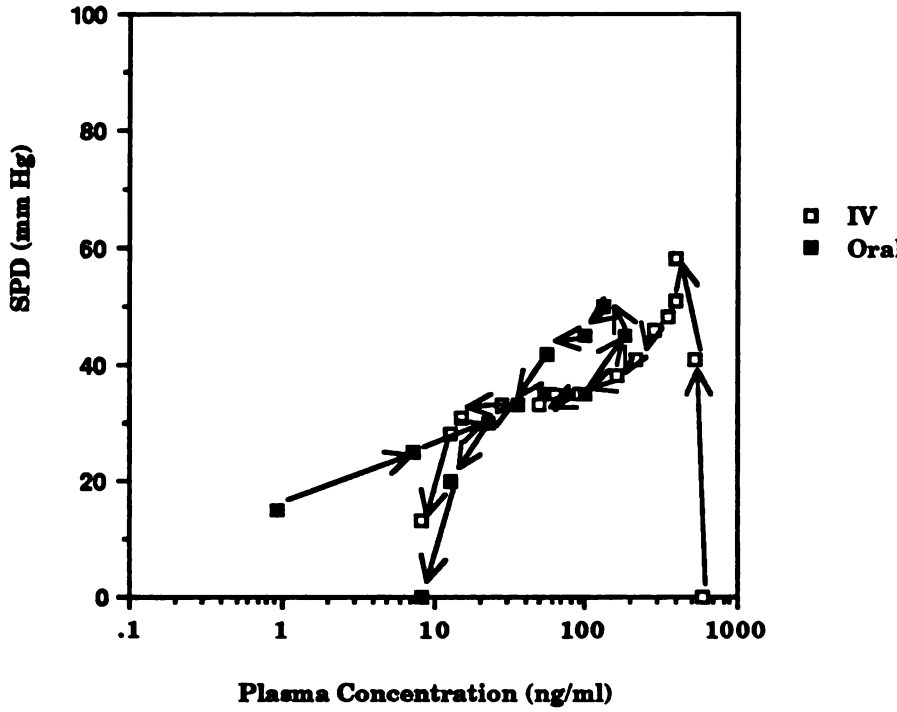

Figure III-16: Systolic blood pressure decrease vs 1,2-GDN plasma concentration plots following iv and oral doses of 1,2-GDN (0.25 mg/kg) to dog #3.

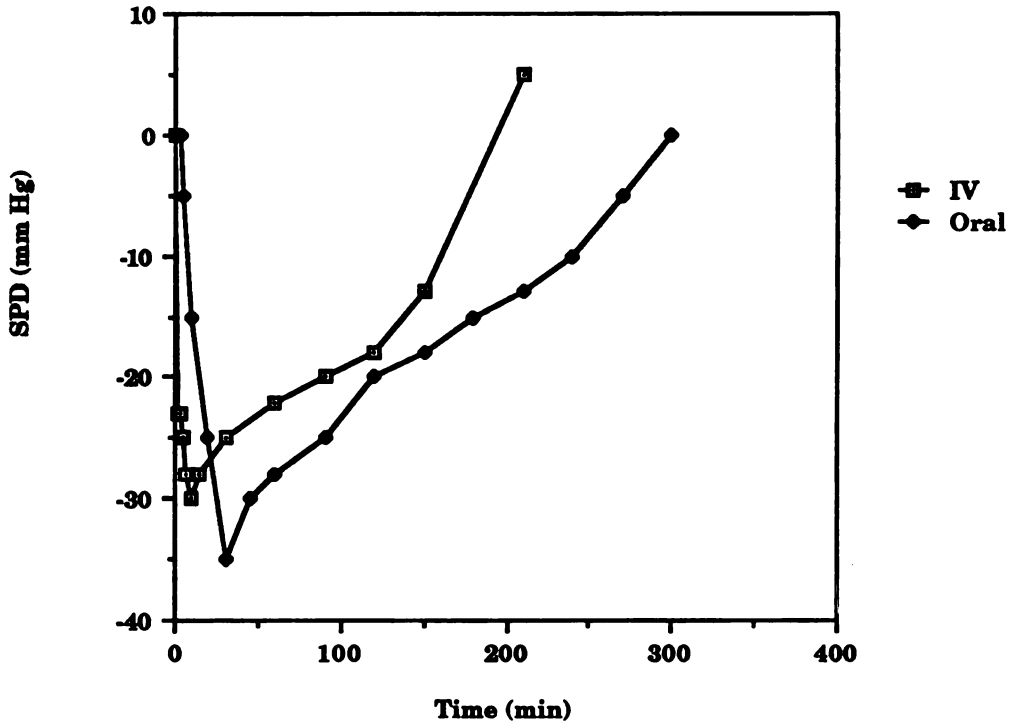

Figure III-17: Systolic blood pressure decrease vs time plots following iv and oral doses of 1,3-GDN (0.25 mg/kg) to dog #4

Table III-14: Systolic blood pressure decrease following bolus intravenous doses of 1,3-GDN (250 μg/kg) to conscious dogs

Dog #	Dmax (mm Hg)	Tmax (min)	AUCspd (mmHg*min)	AUCpl (ng*min/ml)	AUCspd/ AUCpl	AUCspd/ Dose
3	50	3	3856	11376	0.339	15.4
4	30	10	3578	18779	0.191	14.3
5	25	4	185	19341	0.010	0.740
6	20	5	808	15550	0.052	3.23
7	62	4	1190	20535	0.058	4.76
8	29	10	2364	18353	0.129	9.46
Mean	36	6	1997	17322	0.130	7.98
S.D.	16	3	1513	3349	0.121	6.04

Table III-15: Systolic blood pressure decrease following oral doses of 1,3-GDN (250 μg/kg) to conscious dogs

Dog #	Dmax (mm Hg)	Tmax (min)	AUCspd (mmHg*min)	AUCpl (ng*min/ml)	AUCspd/ AUCpl	AUCspd/ Dose
3	33	45	4438	8750	0.507	17.8
4	35	30	5078	10701	0.475	20.3
5	35	10	3843	15698	0.245	15.4
6	30	3	3115	11842	0.263	12.5
7	21	45	2740	11152	0.246	11.0
8	31	30	2369	11494	0.206	9.48
Mean	31	27	3597	11606	0.324	14.4
S.D.	5	17	1043	2280	0.131	4.2

after iv doses and 0.324 (S.D. = 0.131) after oral doses. Representative SPD versus 1,3-GDN concentration plots for dog #4 are shown in Figure III-18 after iv and oral doses.

4) Comparison of Potency

The parameters Dmax, AUCspd, AUCspd/AUCpl and AUCspd/Dose were used as the indices to compare the potency between drug treatments (i.e., GTN, 1,2-GDN and 1,3-GDN) via the same route (i.e., iv or oral) at the 0.25 mg/kg dose. The results are listed in Table III-16. Potency was also compared between routes of administration under the same drug treatment at 0.25 mg/kg (Table III-16). Intravenous GTN is more potent than 1,2-GDN, 1,3-GDN and oral GTN in comparing the maximum systolic blood pressure decrease (Dmax). When given intravenously 1,2-GDN produced the greatest AUCspd and AUCspd/Dose among these three drugs whereas given orally GTN, 1,2-GDN and 1,3-GDN showed no significant difference. Comparing AUCspd/Dose, orally dosed nitrates are equipotent with or more potent than intravenously dosed nitrates. Based on AUCspd/AUCpl ratios GTN is significantly more potent than 1,2-GDN and 1,3-GDN and oral administration is more efficient than iv administration.

DISCUSSION

Nitroglycerin is a difficult drug to study both in terms of pharmacokinetics and pharmacodynamics. Remarkable variability in pharmacokinetics and pharmacodynamics was exhibited both between dogs and between days in the same dogs. Large standard deviations and wide ranges were observed for all pharmacokinetic and pharmacodynamic parameters. Apparent dose dependent pharmacokinetics of GTN was shown as the CLapp decreased for the 0.25 mg/kg iv dose as compared to the 0.025 mg/kg iv dose of GTN, but this change is probably not due to metabolite (end-product) inhibition (to be discussed in detail in

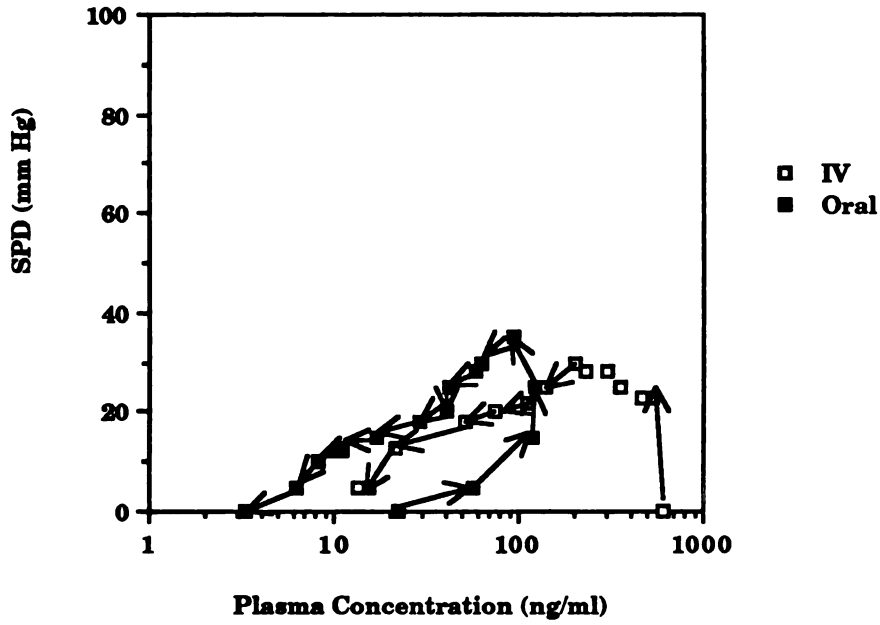

Figure III-18: Systolic blood pressure decrease vs 1,3-GDN plasma concentration plots following iv and oral doses (0.25 mg/kg) of 1,3-GDN to dog #4.

Table III-16: Comparison of potency between GTN, 1,2-GDN and 1,3-GDN using Dmax, AUCspd, AUCspd/Dose and AUCspd/AUCpl as indices in six dogs[a,b]

Route	GTN	1,2-GDN	1,3-GDN
		(Mean ± S.D.)	
Dmax (n=6)			
IV	63 ± 19[c]	41 ± 9	36 ± 16
PO	34 ± 17	40 ± 13	31 ± 15
	($p<0.05$)[d]	(N.S.)	(N.S.)
AUCspd (n=6)			
IV	1782 ± 833	3665 ± 1894	1997 ± 1513
PO	3447 ± 3670	3116 ± 1423	3597 ± 1043
	(N.S.)	(N.S.)	($p<0.05$)
AUCspd/Dose (n=6)			
IV	7.13 ± 3.33	14.7 ± 7.6	7.98 ± 6.04
PO	13.8 ± 14.7	12.5 ± 5.7	14.4 ± 4.2
	(N.S.)	(N.S.)	($p<0.05$)
AUCspd/AUCpl (n=6)			
IV	4.34 ± 2.43	0.233 ± 0.112	0.130 ± 0.121
PO	867 ± 849	0.346 ± 0.180	0.324 ± 0.131
	($p<0.05$)	($p<0.05$)	($p<0.05$)

a One-way ANOVA and the Newmann-Keuls test were used to compare the three drugs via the same dosing route.

b Each dog received 0.25 mg/kg doses of GTN, 1,2GDN and 1,3-GDN.

c A line connection between groups indicates that the two groups are significantly different ($p<0.05$).

d Paired Student's t test was used to compare the difference between iv and oral dosing routes.

Chapter V) as Cossum et al. (1986) suggested. It is most likely due to concentration dependent tissue uptake, saturable blood metabolism and/or a blood flow decrease. Blood vessel uptake of nitrates after intravessel administration (upper inferior vena cava) is rapid and extensive as Fung et al. (1984b) demonstrated in rats that the blood vessel concentration was 19.1 times higher than the plasma concentration at the peak time, 2 min. This first-pass blood vessel uptake of GTN may contribute to the large apparent sampling site dependent systemic clearance for GTN. If blood vessel uptake of GTN is concentration dependent then a change in blood vessel uptake will yield a change in the apparent total body clearance. In our dog studies Vapp,ss after high dose GTN was not different than that determined after low dose GTN. That is, only a limited space for tissue uptake of GTN is available in the body, and this remains constant from dose to dose.

The concentration dependency of GTN metabolism in human blood has been demonstrated in vitro by Cossum and Roberts (1985a). The half-life of GTN in blood increased from 2.7 to 16.6 min when the GTN concentration was increased from 0.8 to 600 ng/ml. In the in vivo situation when the metabolism of GTN in blood is saturated at higher GTN concentrations, other organs may still have full capacity to metabolize GTN. However, the metabolism pattern, as reflected in the ratio of metabolites formed, may be changed. This ratio difference of 1,2-GDN/1,3-GDN after various routes of GTN administration has been reported by Noonan and coworkers (1985, 1987), suggesting the possibility of different enzyme specificity for GTN metabolism to the GDNs in different tissues, i.e., liver (Needleman and coworkers, 1971, 1973; Lee, 1973), lung (Heinzow and Ziegler, 1981; Fung et al.,1984b; Cossum and Roberts, 1985b), small intestine (DeCarlo et al., 1968), blood (Noonan and Benet, 1982; Cossum and Roberts, 1985), blood vessels (Fung et al., 1984b), muscle (Fung et al., 1984b) and skin (Santus et al., 1986). The ratios of 1,2-GDN/1,3-GDN after sublingual, intravenous, oral and topical GTN administration in man were 3.22, 7.5, 1.99 and 5.25, respectively. In our dog studies, the ratio of $AUC_{1,2\text{-}GDN}/AUC_{1,3\text{-}GDN}$ after high

dose (0.25 mg/kg) iv GTN was significantly lower (3.41) as compared to the 8.44 ratio after low dose (0.025 mg/kg)iv GTN (Table III-4). It is interesting to note that the change in the ratio results from an apparent relative increase in 1,3-GDN concentrations with higher doses. Note in Tables III-1 and 2 that the AUC for 1,2-GDN increases 10 fold when the GTN dose increases 10 fold. In contrast, the AUC for 1,3-GDN increases approximately 25 fold for the 10 fold iv dose increase. This may also be seen in Table III-8 where the fraction of the GTN dose accounted for by 1,3-GDN increases significantly from low to high doses while the 1,2-GDN fraction remains constant. However, even though the AUC for 1,3-GDN increased disproportionately with the higher GTN dose while the AUC for 1,2-GDN showed a proportional increase, the apparent fractional clearances of GTN to 1,2-GDN (CLapp,m1) and 1,3-GDN (CLapp,m2) exhibited an opposite response. That is, CLapp,m1 decreased ($p<0.05$) while CLapp,m2 remained constant (Table III-7). In addition, the residual clearance of GTN decreased in going from the low to high doses both in terms of percent of clearance not accounted for by measurable dinitrate metabolites (24.3% of low dose vs 13.8% of high dose) and in apparent clearance values (350 ml/min/kg for low dose calculated as 0.243 * 1440 ml/min/kg, the latter term being the average GTN apparent clearance, vs 94.7 ml/min/kg for the high dose calculated as 0.138 * 686 ml/min/kg). These residual fractions of the GTN clearance are most likely the result of sequential metabolism whereby the GDNs are further metabolized to glucuronides, GMNs and glycerol in the various eliminating organs before reentering the systemic circulation. Thus, it appears from this limited study that a great deal of the nonlinear change in GTN clearance may result from the nonlinear change in the clearance of GTN to 1,2-GDN. GTN is a potent vasodilator which can decrease venous return to the heart (decreased preload) and decrease cardiac output (Williams et al., 1975). Especially at high doses, GTN can dilate both veins and arteries which can result in a drastic decrease in blood pressure, cardiac output and blood flow. These physiological changes can in turn affect nitroglycerin metabolism in different organs and tissues. It is

probably only fortuitous that the AUC for 1,2-GDN increased proportionally with the GTN dose while the apparent clearance of GTN and its partial clearance to 1,2-GDN decreased.

The terminal half-life of GTN varied between all three studies (high dose oral and iv, low dose iv). The high iv dose of GTN exhibited the longest terminal half-life (70 min), the low iv dose exhibited a median half-life (18 min) and the oral GTN dose exhibited a very short half-life (4 min). It is obvious that the measurement of the slow terminal phase is dependent on the detection limit of the assay method used. Nevertheless, this long half-life corresponding to the slow decline phase may not be significant for the low iv dose since it only contributed a small portion (<20%) to the total AUCpl. For the high iv GTN dose, a substantial portion (40%) of total AUCpl corresponded to this slow decline phase and contributed a significant portion to the apparent total body clearance. This long half-life corresponding to the slow decline phase may also be pharmacodynamically significant. However, GTN (>0.1 ng/ml) and GDN (>10 ng/ml) plasma levels were still measurable even when the SPDs returned to control values, although compensatory sympathetic reflex effects make any simple attempt to correlate the plasma concentrations of GTN and GDNs to hemodynamic responses difficult. The long half-life is most likely due to rate limited redistribution of GTN from tissue back to plasma.

The formation of 1,2-GDN and 1,3-GDN was rapid and extensive following iv and oral doses of GTN. After the low iv GTN dose the Cmax of total 1,2-GDN and 1,3-GDN averaged 5.1 times higher than that for GTN. Total AUC of 1,2-GDN and 1,3-GDN averaged about 43 times higher than the AUC of GTN (Table III-1). In contrast, for the high iv GTN dose study, the Cmax of total 1,2-GDN and 1,3-GDN averaged only 2 times higher than the Cmax for GTN, while the total AUC of 1,2-GDN and 1,3-GDN averaged about 24 times higher than that of GTN (Table III-2). After the oral GTN dose, the GTN plasma levels declined very rapidly and could not be detected after 15 min. The Cmax of total 1,2-GDN and 1,3-GDN

was about 94 times higher than the Cmax of GTN and the total AUC for GDNs was almost 1600 times higher than that of GTN (Table III-3). Measured 1,2-GDN and 1,3-GDN accounted for 76±19% of the low iv GTN dose, 86±21% of the high iv GTN dose and 70±29% of the oral GTN dose (Table III-8). These percentages are not significantly different from each other. Thus, independent of route of administration, approximately 75% of the dose may be accounted for in terms of measurable dinitrate metabolites in the systemic circulation. What changes from dose to dose and route of administration is the ratio of the two metabolites. After the oral GTN dose, the residual 30% of the dose which was not measurable in the systemic circulation can be accounted for as the result of sequential metabolism of GTN in the GI tract and liver.

The pharmacokinetic parameters for 1,2-GDN and 1,3-GDN were consistent and similar to each other. Their apparent clearances (averaged 15.5 ml/min/kg) and Vss (averaged 0.80 L/kg) were much lower than those for GTN. Their oral bioavailabilities (averaged 0.65) were also greater than that for GTN (i.e., 0.015). 1,2-GDN and 1,3-GDN exhibited considerably longer half-lives (45 min) than that of GTN. The GDN half-lives are in agreement with the values reported by Miyazaki et al. (1982) in dogs.

The pharmacokinetic/pharmacodynamic equilibration time for GTN in the body was extremely short since the Tmax of plasma levels was about the same as the Tmax for the systolic blood pressure decrease (Tables III-9 and III-10). The equilibration time for 1,2-GDN and 1,3-GDN, Tmax for SPD about 6 min after iv dosing, was slower than that for GTN (Tables III-12 and III-14). This is probably because the dinitrate metabolites are more polar than GTN and require a longer time to diffuse through the blood vessel smooth muscle cell membranes.

The mean values of Dmax, AUCspd, AUCspd/AUCpl and AUCspd/Dose are summarized in Table III-17. Comparing maximum net systolic blood pressure decreases (Dmax) for the low (0.025 mg/kg) iv bolus dose of GTN with the 0.25 mg/kg iv doses of the GDNs, nitroglycerin is about 10 times more potent than 1,2-GDN and 12 times more potent than 1,3-GDN, which is similar to the results reported by Needleman et al. (1969) and determined in approximately the same manner. When comparisons are made with the high iv dose of GTN (0.25 mg/kg), then nitroglycerin was only 1.54 times more potent than 1,2-GDN and 1.75 times more potent than 1,3-GDN. This probably reflects the fact that a maximum Dmax change is observed at a much lower GTN dose and this would not be an appropriate potency comparison. Comparisons of pharmacodynamic potency based on single point efficacy measurements (such as Dmax) can be misleading and it is often better to base the potency comparison on integrated measures of effect. That is, the duration of the effect induced by the drug should also be considered. The durations of the systolic blood pressure decrease caused by 1,2-GDN and 1,3-GDN were more prolonged than that of GTN and gave a larger AUCspd. Comparing the ratio of AUCspd/Dose, 1,2-GDN was about twice as potent as GTN while 1,3-GDN was about equipotent with GTN. This prolonged duration correlated well with the larger GDN plasma AUCs. When efficiency is compared using the ratio of AUC_{spd}/AUC_{pl}, GTN was more efficient than its dinitrate metabolites. Yet, as shown in Tables III-1 and 2, significant concentrations of the GDNs result from GTN dosing. However, any simple attempt to correct AUC_{spd} measurements for GDN concentrations does not yield reasonable correlations of efficacy with concentration. Since the formation of 1,2-GDN and 1,3-GDN is so rapid and extensive, it is obvious that the contribution of the GDNs to the pharmacological effect can not be ignored. Following oral GTN dosing, GTN plasma levels fall below the detectable limit 15 min post-dose. However, the SPD did not return to baseline until 90 min or longer which correlates well with the time course of 1,2-GDN and 1,3-GDN plasma levels. This finding is contrary to what Needleman and Johnson (1980) have suggested, i.e., high dose oral GTN saturates the hepatic first pass metabolism allowing

Table III-17: Average (±S.D.) Dmax, AUCspd, AUCspd/Dose and AUCspd/AUCpl following intravenous and oral doses of GTN, 1,2-GDN and 1,3-GDN to dogs

Drug	Route	Dose (μg/kg)	Dmax (mm Hg)	AUCspd	AUCspd/ Dose	AUCspd/ AUCpl
GTN	iv	25	42±13	202±181	8.08±7.22	9.12±8.67
GTN	iv	250	63±19	1782±833	7.13±3.33	4.34±2.43
GTN	oral	250	34±17	3447±3670	13.8±14.7	867±849
1,2-GDN	iv	250	41±9	3665±1894	14.7±7.6	0.233±0.112
1,2-GDN	oral	250	40±13	3116±1423	12.5±5.7	0.346±0.180
1,3-GDN	iv	250	36±16	1997±1513	7.98±6.04	0.130±0.121
1,3-GDN	oral	250	31±5	3597±1043	14.4±4.2	0.324±0.131

sufficient GTN to be available to cause the pharmacological effect. Rather, we suggest that the high levels of GDNs play a major role in the systolic blood pressure decrease. Therefore, it is necessary to measure the plasma levels of GTN and the GDNs in order to understand fully the pharmacokinetics and pharmacodynamics of GTN. Our 1,2-GDN and 1,3-GDN studies also demonstrate that for the same degree of systolic blood pressure decrease, plasma levels of the GDNs after oral GDN dosing were lower than that after intravenous dosing. Based on the ratio AUCspd/AUCpl (Table III-17) the oral 1,2-GDN dose is about 1.5 times more potent than the iv 1,2-GDN dose and the oral 1,3-GDN dose is about 2.7 times more potent than the iv 1,3-GDN dose. Based on the ratio AUCspd/Dose (Table III-17) the orally dosed nitrates are equipotent with or more potent than intravenously dosed nitrates. This could be explained by the findings reported by Chen et al. (1979,1981) in the anesthetized cat that the major site of nitroglycerin-induced venous pooling is in the splanchnic circulation. Thus, orally dosed organic nitrates are first absorbed into the splanchnic circulation, where they induce venous pooling prior to passing through the liver and reaching the systemic circulation. Even though orally dosed organic nitrates may produce a low systemic drug level resulting from gastro-intestinal and hepatic first pass metabolism, the hemodynamic effects may be similar to that observed following iv dosing. Obviously hemodynamic measures are not useful as a comparison of antianginal efficacy for orally vs. intravenously dosed drug.

In conclusion, nitroglycerin exhibits a very short half life, high clearance, high volume of distribution, dose and administration site dependent elimination, and a short equilibration time between plasma concentrations and hemodynamic effects. GTN is about 10 to 12 times more potent than 1,2-GDN and 1,3-GDN in depressing the systolic blood pressure when a comparison is made in terms of maximal effects produced by a therapeutic dose. 1,2-GDN and 1,3-GDN exhibit much longer half-lives, lower clearances and lower volumes of distribution then GTN. Oral administration of nitroglycerin is pharmacologically effective,

when measuring hemodynamic effects, despite the extremely low GTN bioavailability. The effectiveness of oral doses of organic nitrates in reducing systolic blood pressure can be attributed to the formation of high levels of pharmacologically active dinitrate metabolites and to the finding that the splanchnic circulatory bed is the major venous pooling site for organic nitrates.

CHAPTER IV

INTRAVENOUS INFUSIONS OF GTN, 1,2-GDN AND 1,3-GDN TO CONSCIOUS DOGS

INTRODUCTION

Dose dependent pharmacokinetics of nitroglycerin after multiple intravenous infusions in healthy volunteers have been reported by Noonan et al. (1985). Although Cossum and Roberts (1985a) demonstrated concentration dependent metabolism of GTN and its dinitrate metabolites in human blood, they found that the dose dependency of GTN was not conclusive after GTN was infused into anesthetized sheep at three different infusion rates (Cossum et al., 1986). The purpose of this study in conscious dogs was: 1) to determine the dose dependency of nitroglycerin and the glyceryl dinitrates, 2) to determine the pharmacokinetic parameters for these compounds, and 3) to determine the hemodynamic effects after graded intravenous infusions of nitroglycerin and the glyceryl dinitrates.

MATERIALS AND METHODS

Organic Nitrates

Nitroglycerin (Tridil IV) was purchased from Du Pont Critical Care (McGaw Park, IL). 1,2-GDN and 1,3-GDN were provided by Marion Laboratories, Inc. (Kansas City, MO) as pure chemicals (>99%) and used without further purification. Suitable aliquots of stock solutions were diluted with normal saline to make the following dose solutions: 52.4 μg/ml, 157.1 μg/ml, 261.8 μg/ml, and 366.5 μg/ml for GTN; 104.7 μg/ml and 523.6 μg/ml for 1,2-GDN and for 1,3-GDN. All dose solutions were prepared under sterile conditions.

Animals

Four female mongrel dogs were used in each infusion study. These dogs had previously been studied and prepared with chronic indwelling catheters as described in Chapter III. Body weight, body temperature and complete blood counts (CBC) were checked weekly in each dog to insure that each dog was maintained in a healthy condition. Dog #3 was not available for the 1,2-GDN infusion studies and was replaced by another female dog (#9).

Experimental Protocol

Food was withheld from the dogs overnight and throughout each study period. Water was available ad libitum but not during the studies. Four dogs were dosed randomly with GTN at infusion rates of 10 μg/min, 30 μg/min, 50 μg/min and 70 μg/min and with 1,2-GDN and 1,3-GDN, each at 20 μg/min and 100 μg/min . The dose solution was transferred into a 50 cc syringe and slowly infused into a front leg vein using a Harvard Infusion/Withdrawal pump (Millis, MA) with the pump speed at 0.191 ml/min (50 cc syringe). High density PE tubing (Tridil infusion set) was used to prevent the loss of dose due to adsorption (Baaske et al., 1980). All glassware and PE tubing were sterilized before use and the infusions were performed under aseptic conditions. At least 5 days elapsed between studies for each dog. Plasma samples taken prior to the second dose administration showed that no nitrate residues were carried over from the previous studies. This was also demonstrated by Moffat et al. (1984) in dogs. Blood samples (5 ml) were collected through an indwelling femoral venous catheter at 0, 2, 5, 8, 15, 30, 60, 90, 120, 150, 180, 210, 212, 215, 218, 225, 240, 270, 300, and 330 min after the infusion was begun. The blood volume loss due to blood sampling was replaced with normal saline immediately following each blood sample withdrawal. The infusion was continued for 210 min to insure that steady-state had been reached for all three glyceryl nitrates. Arterial blood pressure was monitored with a Gould Statham p23db

transducer (Oxnard, CA) connected to a Grass model 7 polygraph (Quincy, MA). All experiments were conducted in a quiet room with the dog in a sling which provided support but minimal restraint. Prior to these studies, the dogs were trained for one week to acclimatize them to the experimental environment. Before each study the arterial blood pressure was monitored for one hour as the control period. Blood samples were centrifuged using an Eppendorf centrifuge (Brinkmann Instruments, Westbury, NY) immediately after collection. The plasma was separated and frozen in dry ice. Plasma samples were assayed within a month of sample collection.

Assay Method

A capillary GC-ECD method was used to determine the plasma concentrations of GTN, 1,2-GDN and 1,3-GDN. The detailed procedure was described in Chapter II.

RESULTS

A. Pharmacokinetics

1) Intravenous Infusion of GTN

Steady-state concentrations (Css) of GTN were reached at about 60 min post-dose (Figure IV-1). Average Css measurements for GTN using concentrations from 60 to 210 min and average Css for the dinitrate metabolites using concentrations from 150 to 210 min following intravenous infusions of GTN are listed in Table IV-1. The plasma levels of GTN decreased rapidly post-infusion (T1/2 = 4 min) followed by a slow decay phase (Fig IV-1) which was observed in previous iv bolus dose studies of GTN (Chapter III). The conversion of GTN into dinitrate metabolites was rapid (Figure IV-2). Steady-state concentrations of 1,2-GDN and

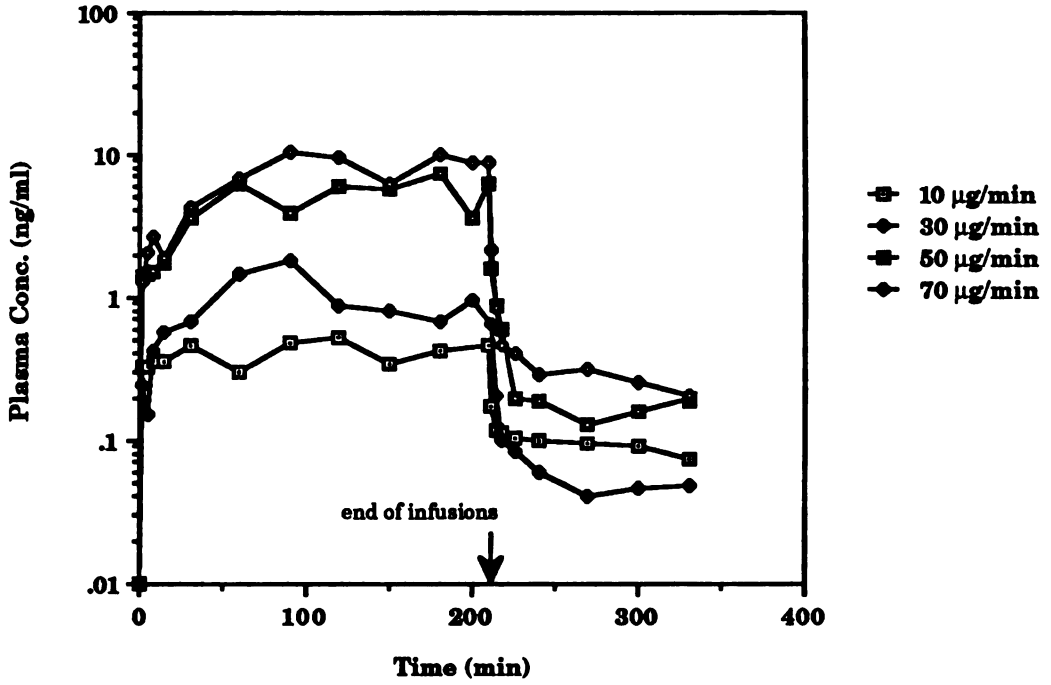

Figure IV-1: Plasma levels of GTN vs time plots following various iv infusion rates of GTN to dog #4

Table IV-1: Average (S.D.) steady-state concentrations of GTN, 1,2-GDN and 1,3-GDN following intravenous infusions of GTN to conscious dogs

Infusion Rate (μg/min)	Css (ng/ml)							
	Dog #3		#4		#5		#8	
				GTN				
10	1.11	(0.30)	0.422	(0.085)	0.219	(0.057)	0.184	(0.038)
30	4.62	(1.18)	1.04	(0.43)	0.683	(0.221)	1.33	(0.21)
50	1.53	(0.08)	5.60	(1.34)	1.37	(0.28)	1.36	(0.24)
70	13.8	(1.8)	8.71	(1.57)	1.51	(0.25)	2.31	(0.35)
Y-int	-1.73		-1.91		0.031		0.014	
Slope	0.175		0.147		0.023		0.032	
r	0.765		0.969		0.983		0.951	
				1,2-GDN				
10	13.0	(0.77)	12.0	(0.3)	9.40	(0.33)	9.36	(0.44)
30	55.6	(5.6)	49.7	(0.76)	39.1	(1.1)	36.8	(2.76)
50	59.0	(4.4)	66.2	(2.3)	78.4	(7.6)	53.0	(4.8)
70	75.2	(3.5)	83.7	(11.5)	47.0	(4.9)	84.1	(20.2)
Y-int	12.7		6.58		13.1		-2.27	
Slope	0.950		1.16		0.761		1.20	
r	0.924		0.977		0.692		0.994	
				1,3-GDN				
10	2.16	(0.15)	2.04	(0.14)	1.26	(0.11)	1.45	(0.10)
30	7.66	(0.60)	5.82	(0.21)	9.49	(0.54)	6.64	(0.38)
50	10.9	(0.51)	16.4	(1.5)	12.2	(1.2)	8.77	(0.56)
70	20.2	(0.8)	17.0	(2.5)	9.94	(0.16)	13.1	(1.2)
Y-int	-1.24		-0.777		2.47		0.074	
Slope	0.287		0.277		2.47		0.185	
r	0.979		0.950		0.775		0.989	

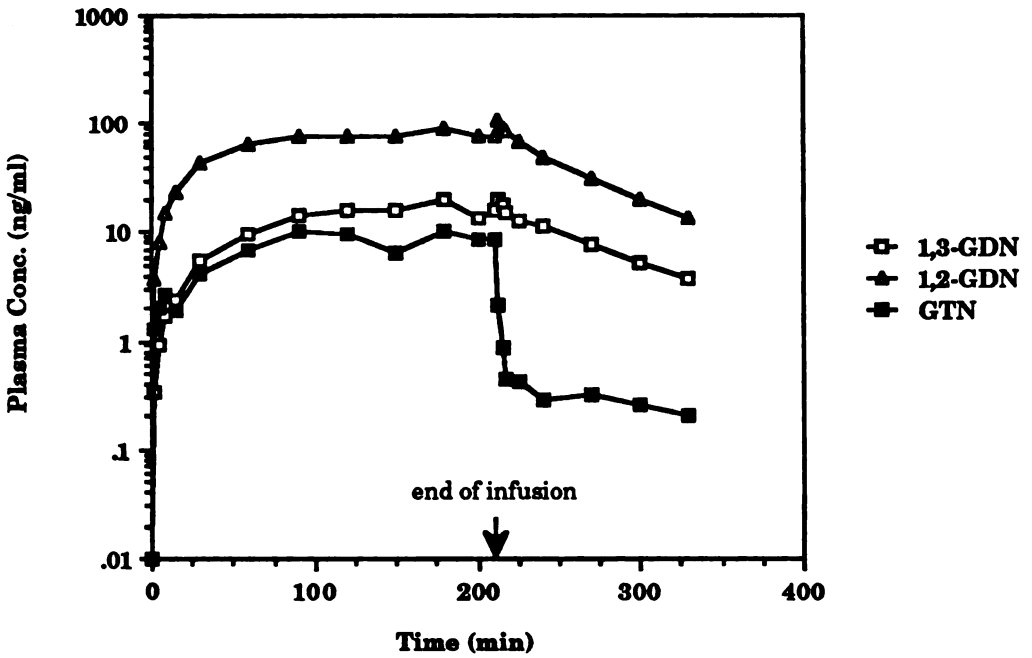

Figure IV-2: Plasma levels of GTN, 1,2-GDN and 1,3-GDN vs time plots following iv infusion (70 μg/min) of GTN to dog #4.

1,3-GDN were reached at about 150 min following intravenous infusion of GTN at various infusion rates. In order to assess the proportionality between Css values and infusion rates, the ratio of Css of GTN at infusion rates (R_0) of 30, 50 and 70 μg/min relative to Css of GTN at 10 μg/min were calculated (Table IV-2) and plotted in Figure IV-3a. In Figure IV-3b the average Css of GTN depicted are normalized to the 10 μg/min infusion rate. That is, the Css for the 30 μg/min infusions are divided by three, the Css for the 50 μg/min infusions divided by 5 and the Css for 70 μg/min infusions divided by 7. As can be seen in Figure IV-3b the normalized concentrations varied extensively from dog to dog and except in dog #5 from infusion rate to infusion rate. Except for dog #5 the Css of GTN were not proportional to infusion rate, however, all dogs together showed a good linear relationship between Css of GTN and infusion rates with an average correlation coefficient of 0.917 ± 0.102. Average Css of 1,2-GDN and 1,3-GDN following GTN infusions were also normalized to 10 μg/min and plotted in Figures IV-4a and IV-4b , respectively. It is obvious that the range of Css from dog to dog for the dinitrate metabolites is not as extensive as that for GTN. The apparent average clearances of GTN were determined as CL = Ro/Css and are listed in Table IV-3 and plotted in Figure IV-5. Large variability in GTN clearance after various infusion rates was observed in all dogs. It is difficult to make any conclusion concerning the effect of dose on clearance for these highly variable results.

The Css ratios of 1,2-GDN/GTN and 1,3-GDN/GTN are listed in Table IV-4 and yield overall averages of 31.5 ± 17.2. and 5.47 ± 3.19, respectively. The Css ratios of metabolites 1,2-GDN/1,3-GDN are also listed in Table IV-4, yielding an overall average of 5.78 ± 1.23. It seems that the ratio 1,2-GDN/1,3-GDN declined as the GTN infusion rate was increased although it is not statistically significant (one-way ANOVA with repeated-measures, $p>0.3$). The Css ratios of GDN/GTN were much more variable than the ratios of metabolites.

Table IV-2: Ratio of GTN Css/Css,10

Infusion Rate Ratio	Css Ratio			
	Dog #3	#4	#5	#8
10/10	1	1	1	1
30/10	4.16	2.46	3.19	7.23
50/10	1.38	13.3	6.40	7.23
70/10	12.4	20.6	7.06	12.6

Table IV-3: Clearance of GTN following intravenous infusions of GTN to conscious dogs

Infusion Rate (μg/min)	CL (ml/min/kg)			
	Dog #3	#4	#5	#8
10	322	878	1870	1940
30	232	1070	1760	806
50	1170	331	1460	1340
70	181	298	1850	1080

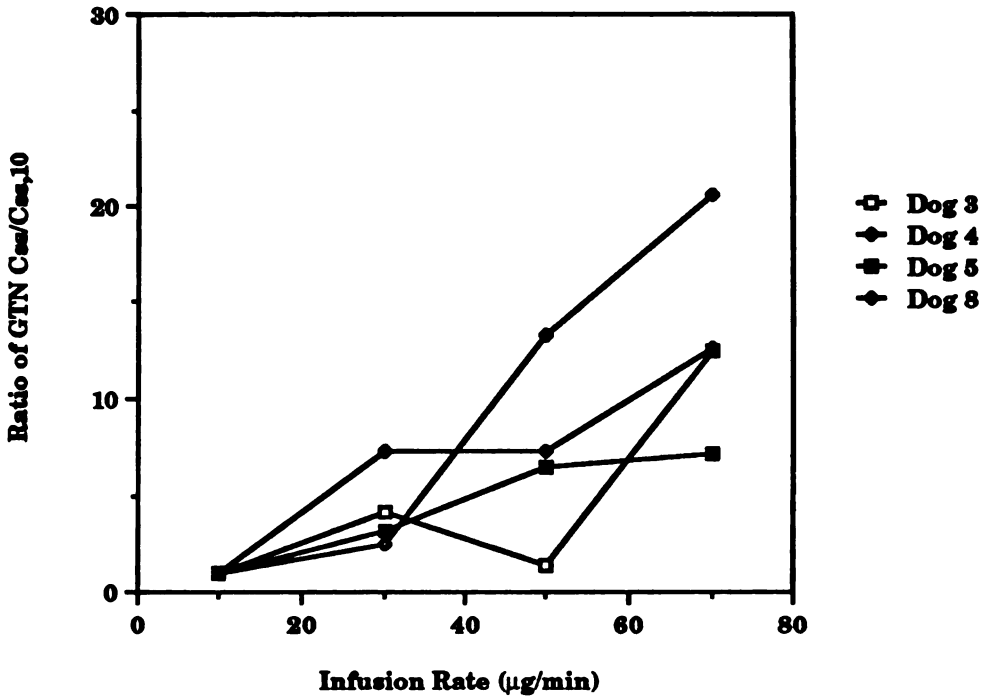

Figure IV-3a: Ratio of GTN Css/Css,10 vs infusion rates plots.

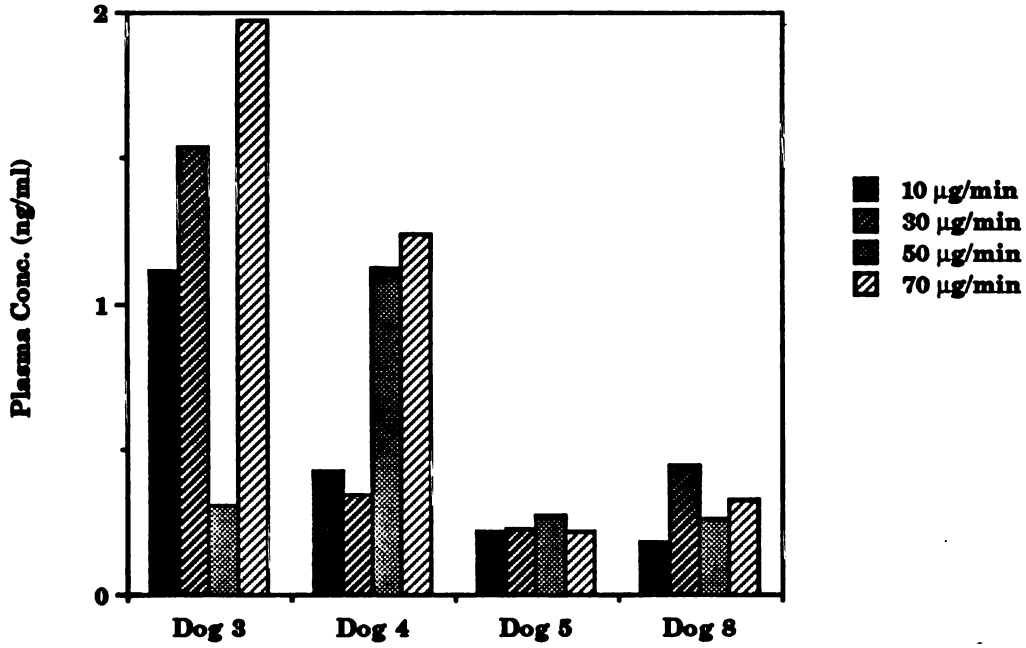

Figure IV-3b: Css of GTN normalized to 10 μg/min.

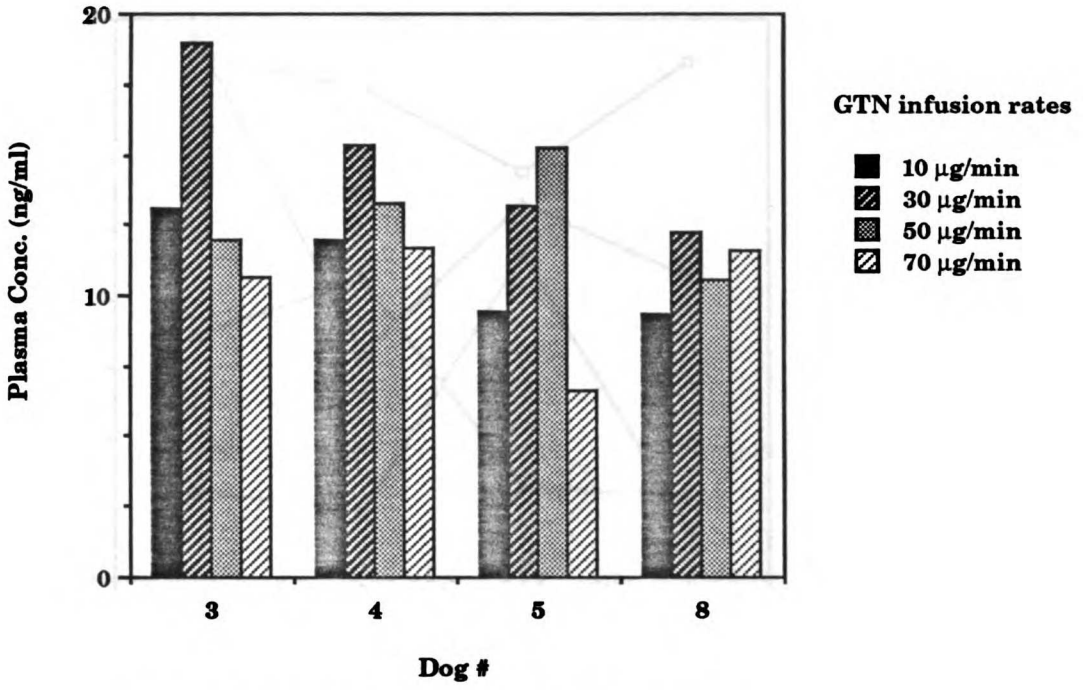

Figure IV-4a: Css of 1,2-GDN normalized to 10µg/min.

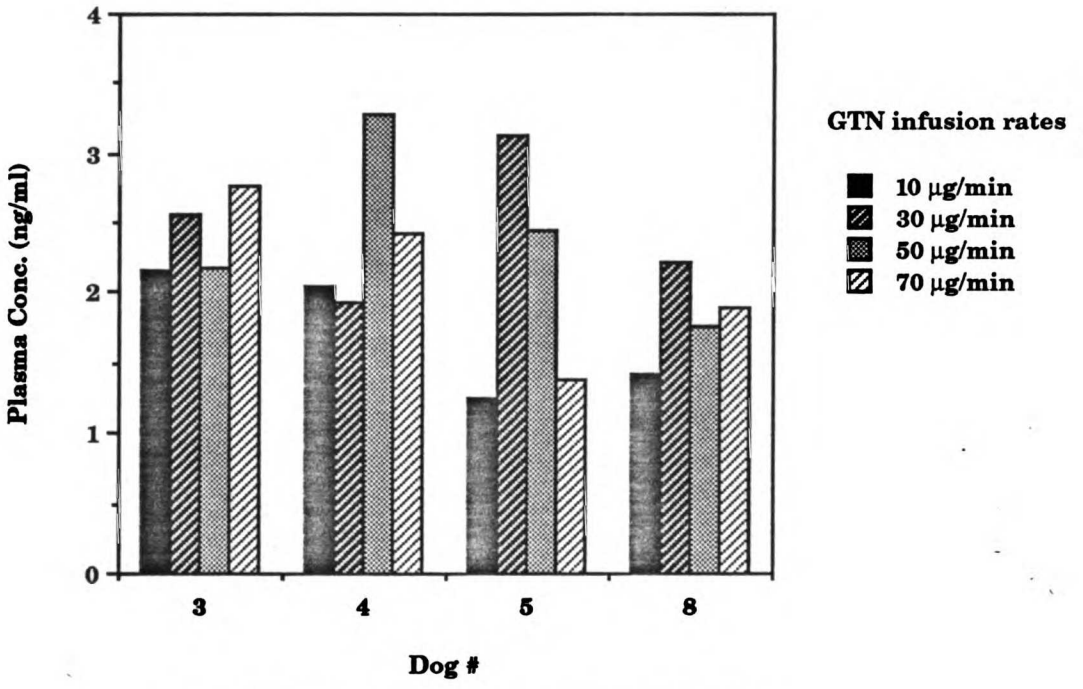

Figure IV-4b: Css of 1,3-GDN normalized to 10 µg/min.

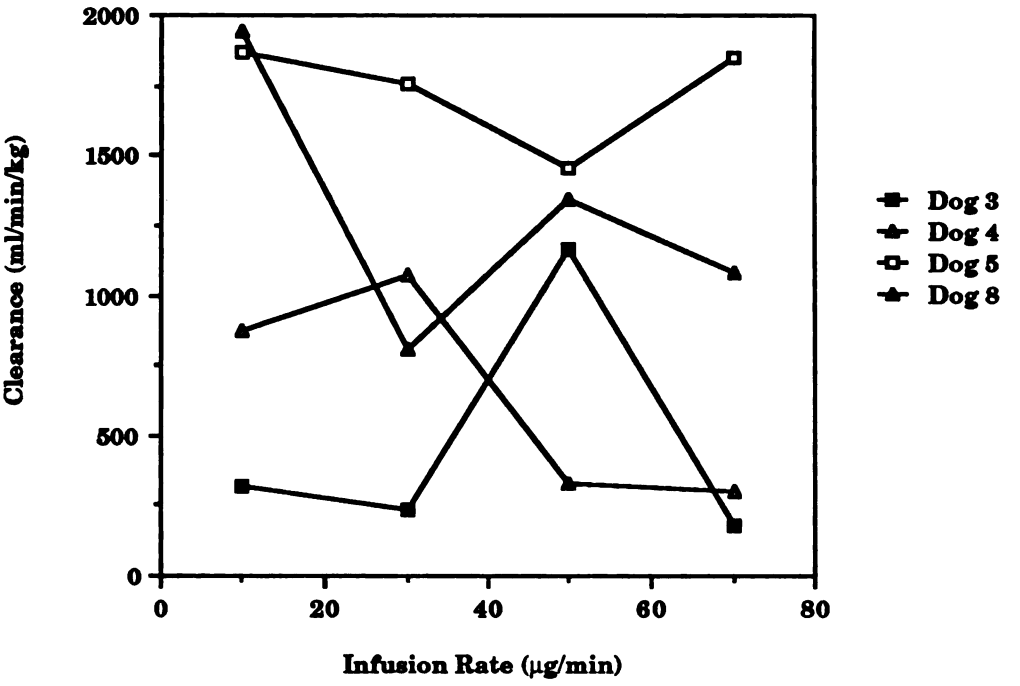

Figure IV-5: GTN Clearance in dogs after various GTN infusions.

Table IV-4: Ratio of Css

Infusion Rate (μg/min)		Dog #3	#4	#5	#8
			$C_{ss,1,2\text{-GDN}}/C_{ss,GTN}$		
10		11.8	28.4	44.0	50.9
30		12.3	44.3	58.0	27.7
50		39.0	11.8	55.5	39.8
70		5.38	9.41	30.8	35.2
Overall					
Mean	31.5				
S.D.	17.2				
			$C_{ss,1,3\text{-GDN}}/C_{ss,GTN}$		
10		1.95	4.83	5.89	7.77
30		1.65	5.60	13.7	4.99
50		7.12	2.93	8.91	6.59
70		1.41	1.95	6.45	5.76
Overall					
Mean	5.47				
S.D.	3.19				
			$C_{ss,1,2\text{-GDN}}/C_{ss,1,3\text{-GDN}}$		
10		6.06	5.88	7.47	6.55
30		7.45	7.92	4.23	5.54
50		5.48	4.04	6.24	6.04
70		3.83	4.82	4.77	6.11
Oveall					
Mean	5.78				
S.D.	1.23				

2) Intravenous Infusions of 1,2-GDN and 1,3-GDN

Plasma concentrations of 1,2-GDN versus time plots for the 20 μg/min and 100 μg/min intravenous infusions to four conscious dogs are illustrated in Figures IV-6a and IV-6b, respectively, while similar plots for 1,3-GDN at 20 μg/min and 100 μg/min are illustrated in Figures IV-7a and IV-7b, respectively. Steady-state plasma concentrations (Css) were reached at about 150 min for both 1,2-GDN and 1,3-GDN at both infusion rates. Average Css values, calculated from 150 to 210 min for each dog, are listed in Table IV-5. The Css ratio (100 μg/min infusion rate to 20 μg/min) was 5.00 $\pm$ 1.05 for 1,2-GDN and 4.96 $\pm$ 1.02 for 1,3-GDN. Therefore, clearances of 1,2-GDN and 1,3-GDN, as tabulated in Table IV-6, were apparently unchanged over this dose range. The clearance of 1,2-GDN averaged 24.8 ml/min/kg (S.D. = 7.8) after the 20 μg/min infusions and 24.8 ml/min/kg (S.D. = 5.3) after the 100 μg/min infusions. The clearance of 1,3-GDN averaged 19.2 ml/min/kg (S.D. = 7.1) after the 20 μg/min infusions and 19.1 ml/min/kg (S.D. = 4.5) after 100 μg/min infusions. The terminal half-lives of 1,2-GDN and 1,3-GDN post infusion were similar to those values obtained after a single bolus dose, i.e., 45 min for both metabolites. The variations of pharmacokinetic parameters for the glyceryl dinitrates were much lower than those for GTN.

3) Fractional GTN Clearance

As in Chapter III, it is possible to calculate the fractional GTN clearance to the GDN metabolites, that is, the formation clearances of 1,2-GDN (CLm1) and 1,3-GDN (CLm2) from GTN. The equation listed below to calculate the metabolite formation clearance is similar to that given in Chapter III for iv bolus studies except that AUC measurements are replaced by steady-state concentrations and clearance terms need no longer be identified as apparent clearance values.

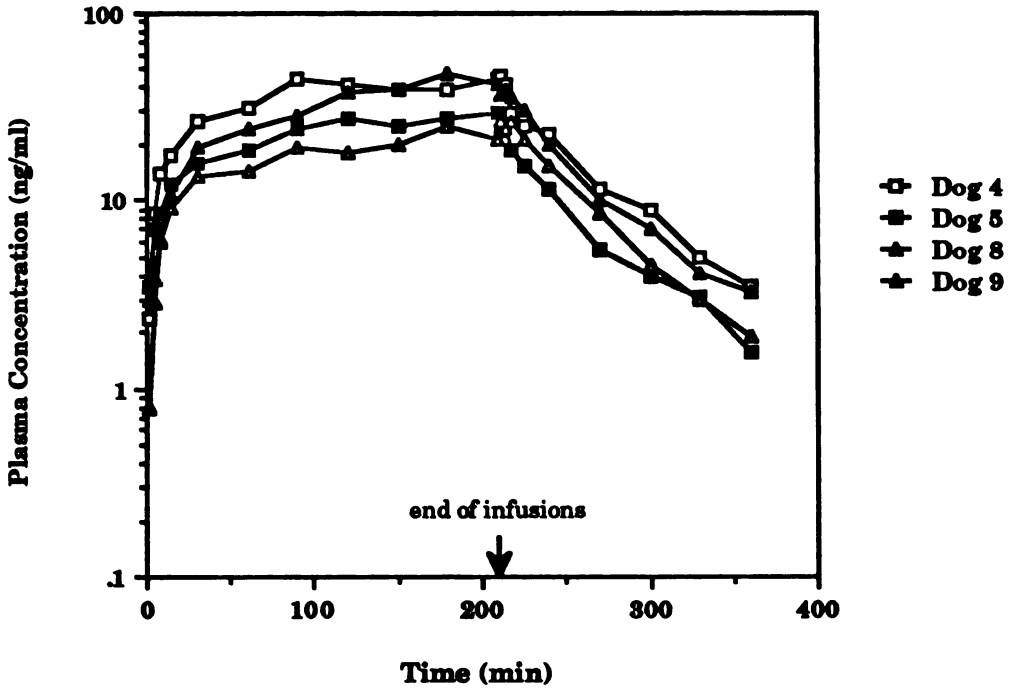

Figure IV-6a: 1,2-GDN plasma levels vs time plots following iv infusions of 1,2-GDN (20μg/min) to conscious dogs.

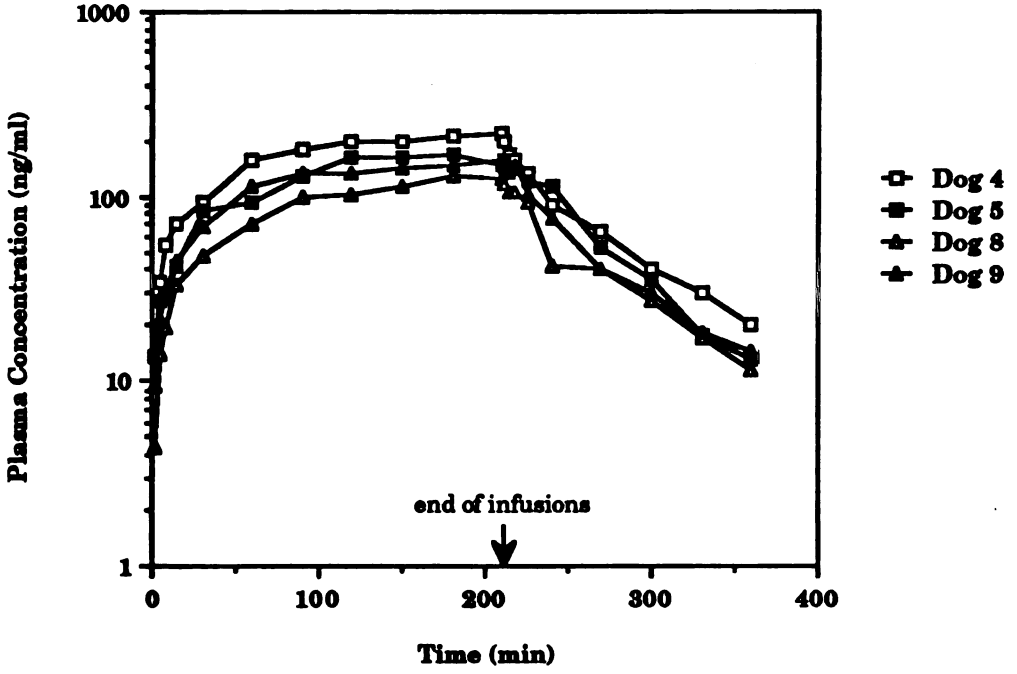

Figure IV-6b: 1,2-GDN plasma levels vs time plots following iv infusions of 1,2-GDN (100 μg/min) to conscious dogs.

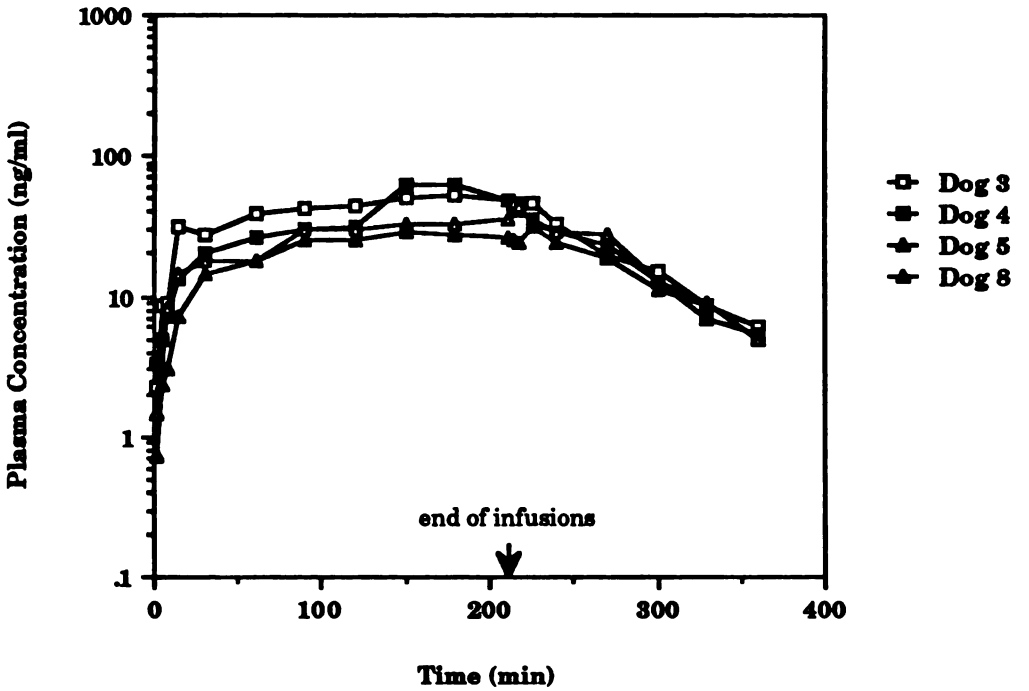

Figure IV-7a: 1,3-GDN plasma levels vs time plots following iv infusions of 1,3-GDN (20 μg/min) to conscious dogs.

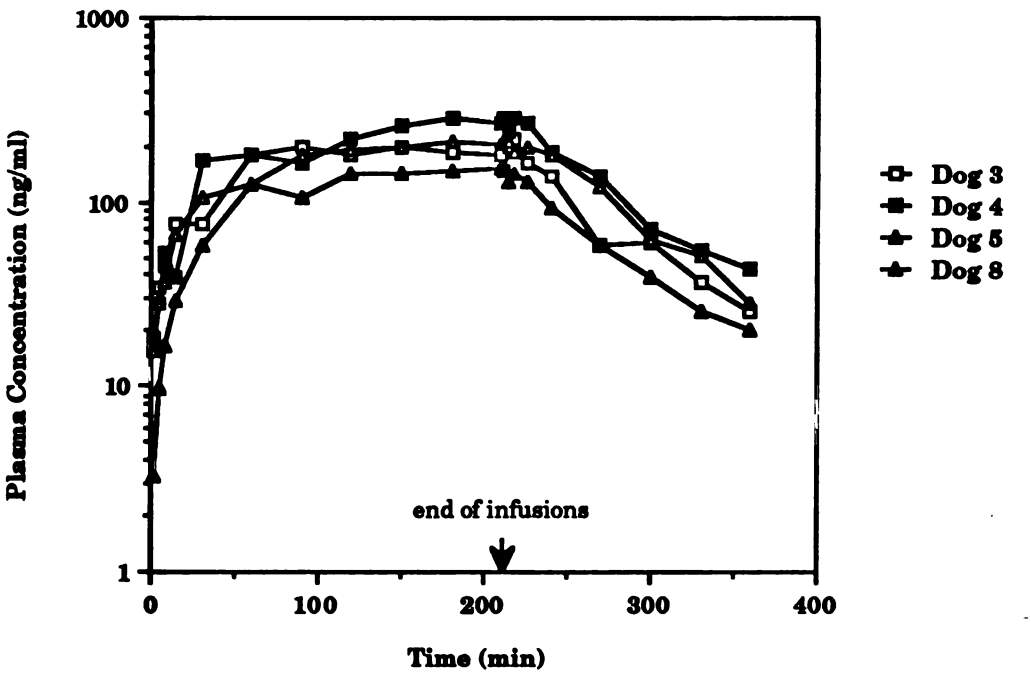

Figure IV-7b: 1,3-GDN plasma levels vs time plots following iv infusions of 1,3-GDN (100 μg/min) to conscious dogs.

Table IV-5: Average (±S.D.) steady-state concentrations of GDNs following iv infusions of GDNs to conscious dogs

1,2-GDN						
Infusion Rate (μg/min)	Dog #4	#5	#8	#9	Mean	S.D.
20	41.0 ±3.0	27.3 ±2.5	22.0 ±2.5	42.6 ±4.3		
100	208 ±11	160 ±10	122 ±8	149 ±9		
Css,100/Css,20	5.07	5.86	5.55	3.50	5.00	±1.05
1,3-GDN	Dog #3	#4	#5	#8		
20	50.6 ±1.6	58.5 ±9.5	34.0 ±1.4	27.5 ±1.0		
100	188 ±10	271 ±12	207 ±7	148 ±6		
Css,100/Css,20	3.72	4.63	6.09	5.38	4.96	±1.02

Table IV-6: Clearances of GDNs following iv infusions of GDNs to conscious dogs

Infusion Rate (μg/min)	CL (ml/min/kg)					
	Dog #4	#5	#8	#9	Mean	S.D.
1,2-GDN						
20	17.4	30.5	32.5	18.8	24.8	7.8
100	17.2	26.0	29.2	26.8	24.8	5.3
1,3-GDN	Dog #3	#4	#5	#8		
20	14.1	12.2	24.5	26.0	19.2	7.1
100	19.0	13.2	20.1	24.1	19.1	4.5

$$CLm = (Css,m/182) * CL(m) / (Css,GTN/227)$$

where Css,m is the metabolite concentration in plasma at steady-state; CL(m) is the plasma clearance of the metabolite; Css,GTN is the GTN concentration in plasma at steady-state; and the numbers 227 and 182 are the molecular weights of GTN and GDN, respectively. The fraction of the GTN dose which was converted to 1,2-GDN (fm1) or 1,3-GDN (fm2) at steady-state is calculated as follows:

$$fm = (Css,m / 182) * CL(m) * B.W. / (Ro / 227)$$

where B.W. is the body weight since clearance terms are given as ml/min/kg and Ro is the infusion rate.

It appears from the GDN clearance results (Table IV-6) that GDN clearance is linear over the concentration range studied. Therefore, the mean clearance in each dog (average of clearances for 20 and 100 μg/min infusions) for 1,2-GDN and 1,3-GDN was substituted in the above equations for CL(m). Only three dogs (#4, 5 and 8) received all eight infusions (10, 30, 50 and 70 μg/min GTN and 20 and 100 μg/min of both 1,2-GDN and 1,3-GDN), therefore, the appropriate parameters for these dogs only are listed in Table IV-7. The fraction of GTN clearance (or of the GTN steady-state dose) which was accounted for (i.e., fm1+ fm2) is also listed. There are no significant differences over the four infusion rates for these parameters (one-way ANOVA with repeated-measures). It appears that all of the GTN dose at steady-state can be accounted for by measured 1,2-GDN and 1,3-GDN since the mean value 1.12 in the last column of Table IV-7 is not significantly different than 1.0.

Table IV-7: Clearances (ml/min/kg) of GTN in forming 1,2-GDN (CLm1) and 1,3-GDN (CLm2) and fractions of GTN converted to 1,2-GDN (fm1) and 1,3-GDN (fm2) following GTN infusions in dogs

Dog #	Ro (μg/min)	CL_{GTN}	CLm1	CLm2	fm1	fm2	Fraction CL_{GTN} Accounted for
4	10	878	613	76.6	0.725	0.090	0.815
(28 kg)	30	1070	1030	88.6	1.00	0.086	1.09
	50	331	255	46.4	0.800	0.143	0.945
	70	298	207	30.9	0.722	0.108	0.830
mean		644	526	60.6	0.812	0.107	0.919
S.D.		389	382	26.6	0.131	0.026	0.126
5	10	1870	1520	160	0.796	0.084	0.880
(24 kg)	30	1760	2020	386	1.10	0.211	1.31
	50	1460	2020	248	1.33	0.163	1.49
	70	1850	1100	183	0.569	0.095	0.664
mean		1740	1670	244	0.949	0.138	1.09
S.D.		190	440	102	0.335	0.060	0.38
8	10	1940	1960	247	1.01	0.127	1.14
(28 kg)	30	806	1070	156	1.32	0.194	1.51
	50	1340	1500	202	1.14	0.154	1.29
	70	1080	1400	178	1.30	0.164	1.46
mean		1290	1480	196	1.19	0.160	1.35
S.D.		480	368	39	0.15	0.028	0.17
Overall Mean		1220	1220	167	0.984	0.135	1.12
S.D. (Dogs #4, 5 and 8)		580	630	100	0.261	0.043	0.29

B. Pharmacodynamics

1) Intravenous Infusion of GTN

A decrease in systolic blood pressure was the most pronounced hemodynamic response observed after organic nitrate dosing. Hence, we used the net systolic blood pressure decrease (SPD) as the index to evaluate the pharmacodynamics of GTN, 1,2-GDN, and 1,3-GDN in our studies.

Systolic blood pressure reached steady-state at about 60 min following intravenous infusion of GTN (Figure IV-8) at the time corresponding to when plasma concentrations reached steady-state. The average net decreases of systolic blood pressure at steady-state (SPDss) for each dog (60 to 210 min) are listed in Table IV-8. Large intra and interdog variation in SPDss was also observed. Only dog #4 appeared to show a dose related systolic blood pressure decrease following graded infusions. However, all dogs showed a clear systolic blood pressure decrease when the highest infusion rate (70 μg/min) was given. Mean SPDss vs infusion rate or log infusion rate plots for all dogs are presented in Figures IV-9a and IV-9b. At the cessation of the GTN infusions an initial rapid increase in systolic blood pressure was observed followed by a slowly increasing phase until the pressure returned to control values. This is similar to the post infusion phase in plasma level versus time plots. When SPD values were plotted versus log GTN concentrations in all four dogs, a counter clockwise hysteresis was observed. A representative plot of SPD vs GTN plasma concentrations in dog #4 following the 70 μg/min infusion of GTN is illustrated in Figure IV-10.

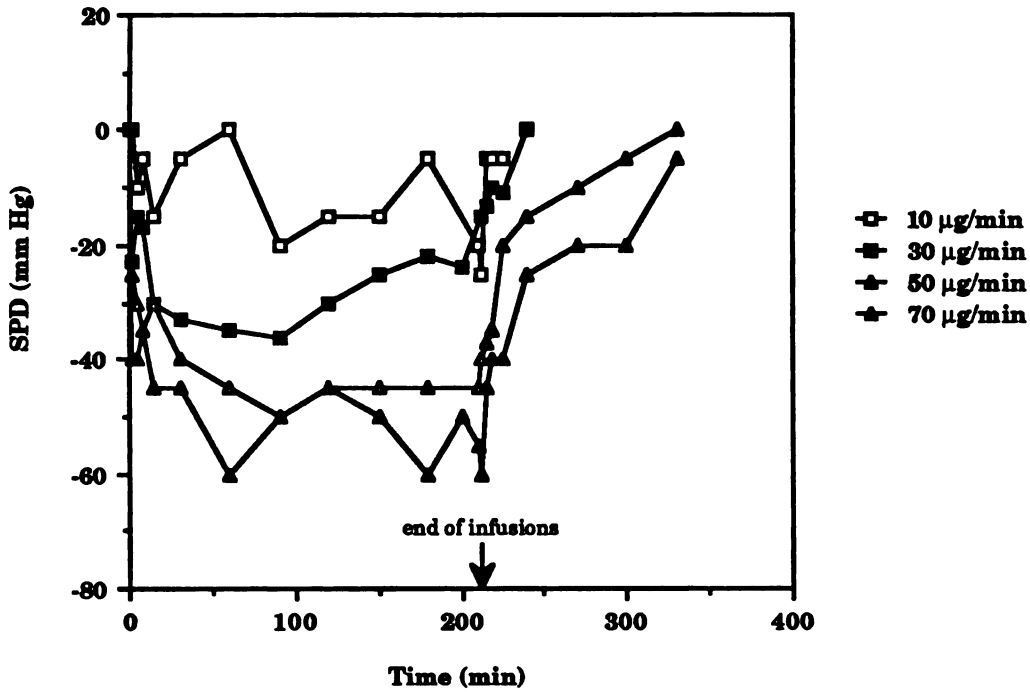

Figure IV-8: SPD vs time plots after various GTN infusions to dog #4.

Table IV-8: Mean SPDss after various iv infusions of GTN to conscious dogs

Infusion Rate (μg/min)	Dog #3	#4	#5	#8
10	34	15	10	13
30	43	29	16	26
50	47	46	10	25
70	83	53	26	32
Y-int (E_0)[a]	22	9.6	7.1	13
Slope	0.755	0.655	0.210	0.280
r	0.905	0.988	0.718	0.908

[a] Systolic blood pressure at control level.

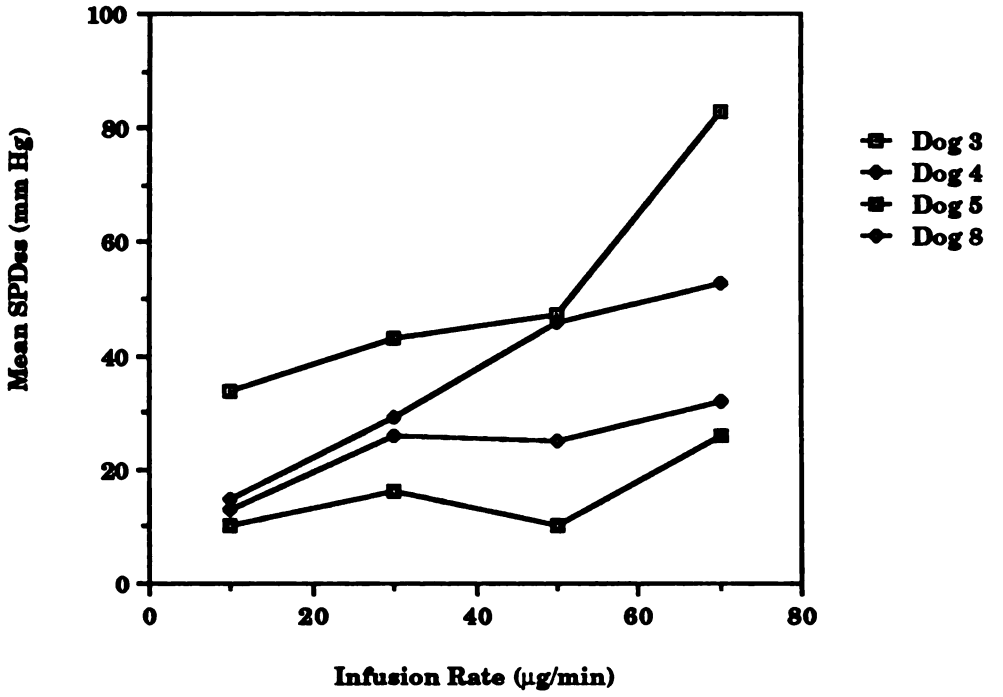

Figure IV-9a: Mean SPDss versus infusion rate plots following various iv infusions of GTN in dogs.

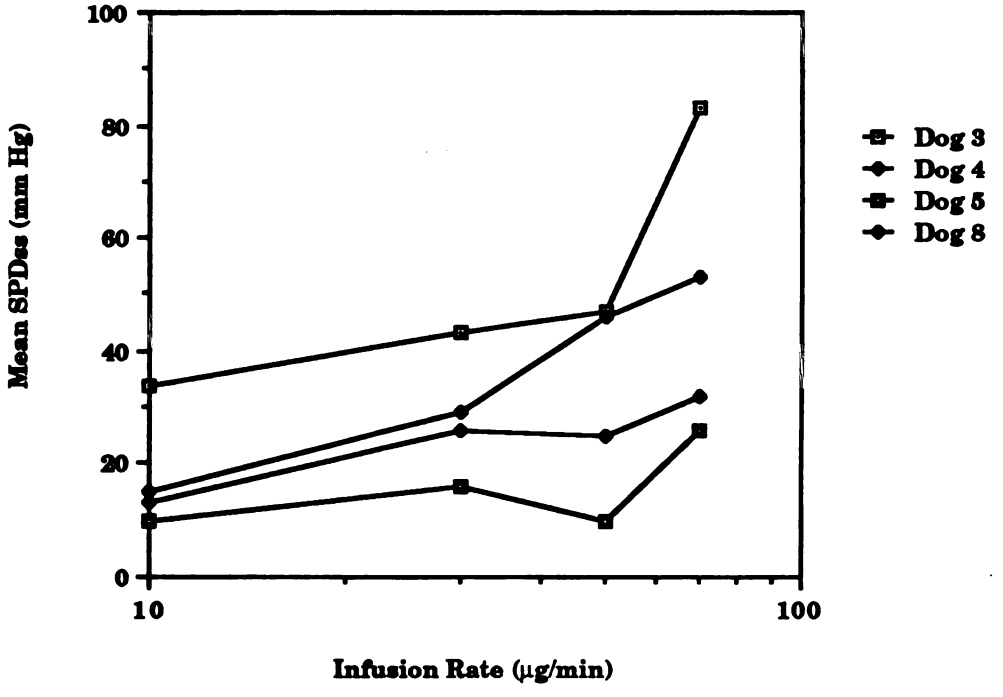

Figure IV-9b: Mean SPDss versus log infusion rate plots following various iv infusions of GTN in dogs.

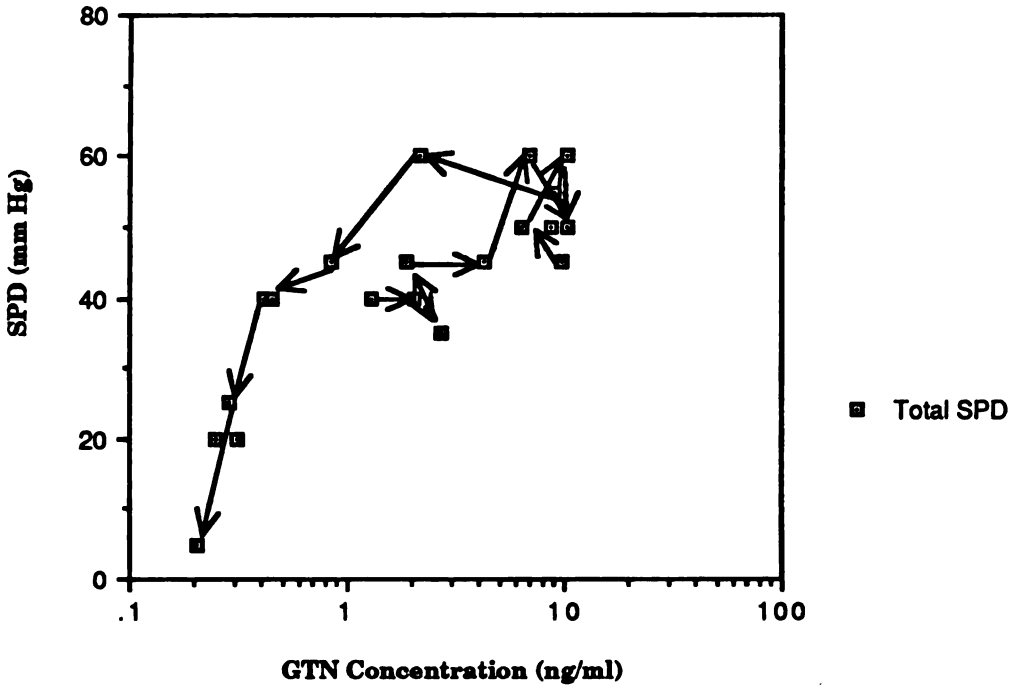

Figure IV-10: SPD vs GTN plasma levels following GTN infusion (70 μg/min) in dog #4.

2) Intravenous Infusions of 1,2-GDN and 1,3-GDN

Systolic blood pressure decrease versus time plots following 20 μg/min and 100 μg/min intravenous infusions of 1,2-GDN in all four dogs are illustrated in Figures IV-11a and IV-11b, respectively. 1,2-GDN at the dose of 20 μg/min with corresponding steady-state plasma levels (Css) which ranged from 20.0 to 42.6 ng/ml appeared to yield very little or no effect on systolic blood pressure. Dog #5 exhibited a slow systolic blood pressure decrease after the infusion was terminated (Figure IV-11a) but the pressure returned to the control at 360 min. The reason for this post-infusion systolic blood pressure decrease is unknown. It is clear that systolic blood pressure in all four dogs decreased following the 100 μg/min 1,2-GDN infusion (Figure IV-11b). Corresponding Css ranged from 122 to 208 ng/ml. After the cessation of the infusion the systolic blood pressure gradually returned to control values in all four dogs.

Systolic blood pressure decrease versus time plots following 20 μg/min and 100 μg/min intravenous infusions of 1,3-GDN in all four dogs are presented in Figures IV-12a and IV-12b, respectively. The systolic blood pressure in three dogs (dogs #3, 5 and 8) decreased following the 20 μg/min 1,3-GDN infusion with corresponding Css in the range of 27.5 to 58.5 ng/ml. Following the 100 μg/min infusion of 1,3-GDN, systolic blood pressure in all four dogs decreased with corresponding Css in the range of 148 to 271 ng/ml (Figure IV-12b) and gradually returned to control pressures at 360 min.

The systolic blood pressure decreases at steady-state (SPDss) averaged over 150 to 210 min following intravenous infusions of 1,2-GDN and 1,3-GDN to conscious dogs are listed in Table IV-9. The mean systolic blood pressure decreases following 20 μg/min and 100 μg/min of iv infusions of 1,2-GDN were 4 $\pm$ 1 mm Hg and 20 $\pm$ 9 mm Hg, respectively. The mean SPD decrease following 20 μg/min and 100 μg/min of iv infusions of 1,3-GDN were 11 $\pm$ 8 mm Hg and 21 $\pm$ 6 mm Hg, respectively. The corresponding 1,2-GDN and 1,3-GDN plasma

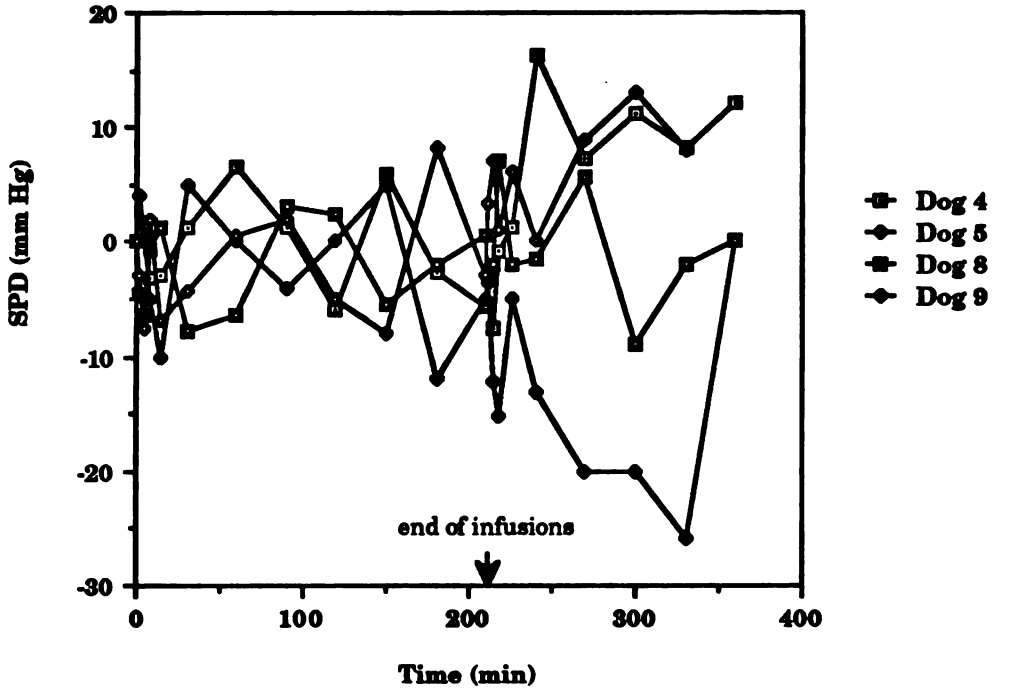

Figure IV-11a: SPD vs time plots following iv infusions of 1,2-GDN (20 μg/min) to conscious dogs.

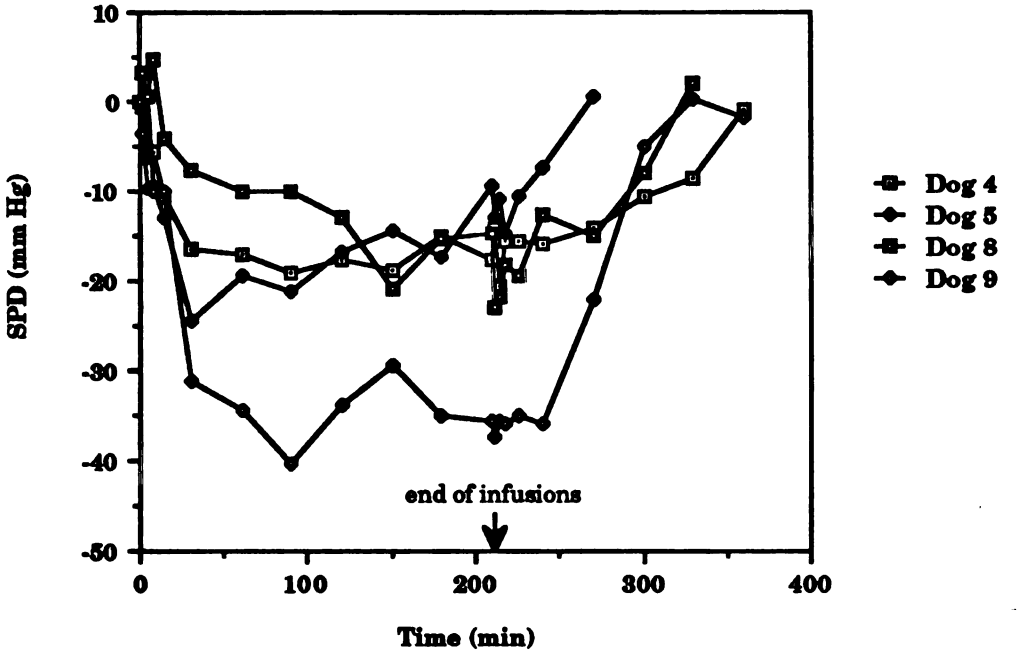

Figure IV-11b: SPD vs time plots following iv infusions of 1,2-GDN (100 μg/min) to conscious dogs.

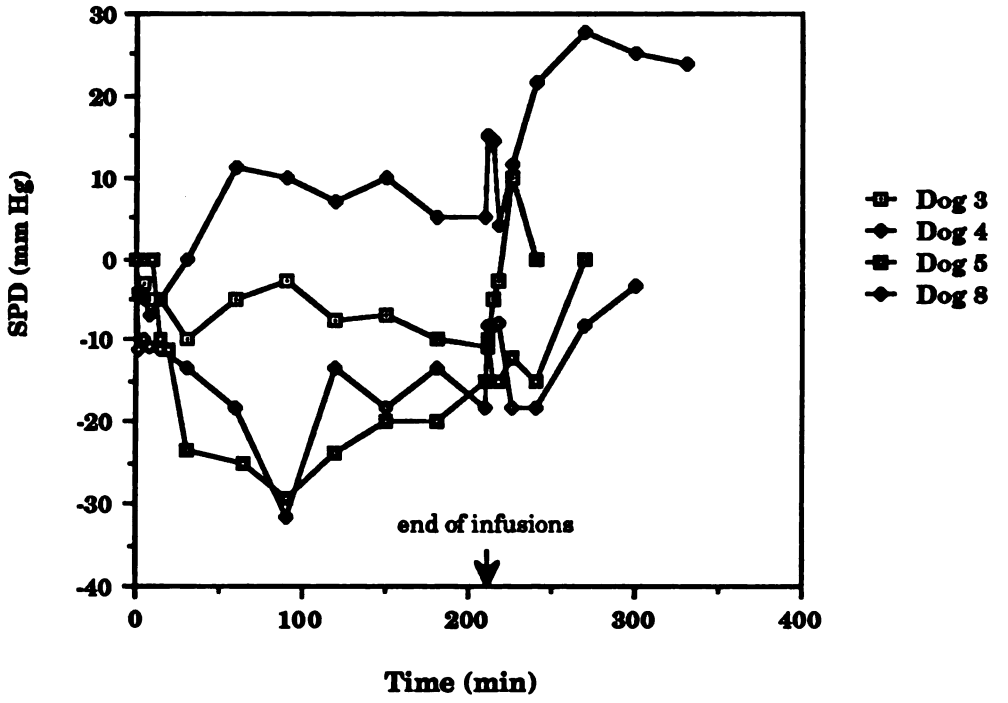

Figure IV-12a: SPD vs time plots following iv infusions of 1,3-GDN (20 μg/min) to conscious dogs.

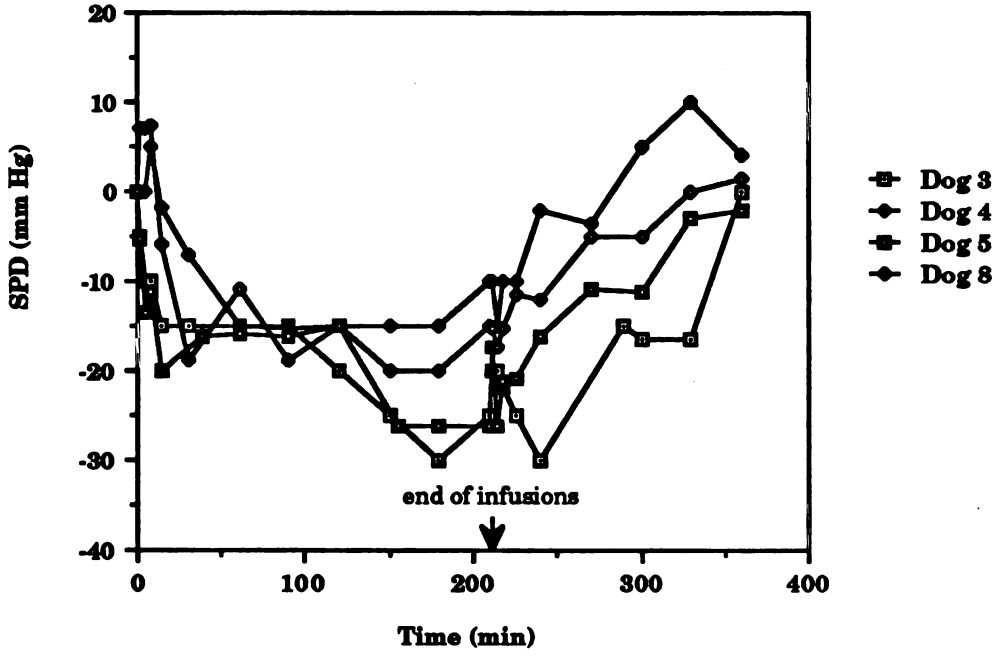

Figure IV-12b: SPD vs time plots following iv infusions of 1,3-GDN (100 μg/min) to conscious dogs.

Table IV-9: Mean SPDss following iv infusions of GDNs to conscious dogs

Infusion Rate (μg/min)	Mean SPDss (mm Hg)			
	Dog #4	#5	#8	#9
1,2-GDN				
20	3	6	3	4
100	17	14	17	33
1,3-GDN	Dog #3	#4	#5	#8
20	9	0	18	16
100	27	13	26	18

levels at each infusion rate were similar. It seems that 1,3-GDN depressed systolic blood pressure more than 1,2-GDN at least at the low dose, although the difference is not significant. The SPD vs log plasma concentration relationship for dog #9 following the 100 μg/min infusion of 1,2-GDN is illustrated in Figure IV-13a, while that for the 100 μg/min infusion of 1,3-GDN in dog #3 is illustrated in Figure IV-13b. A summary plot of SPDss versus Css for GTN, 1,2-GDN and 1,3-GDN in all studies is presented in Figure IV-14.

3) Contribution of GDN to GTN Pharmacodynamics

The post infusion SPD versus plasma levels of GTN, 1,2-GDN and 1,3-GDN following 70 μg/min GTN and 100 μg/min GDN infusions of the respective drugs to dogs #4, 5, and 8 were fitted to an Emax model as $E = Emax * C/(EC50 + C)$ and a sigmoid Emax model (Hill equation) as $E = Emax * C^n/[(EC50)^n + C^n]$ using the computer program MKMODEL from PROPHET public procedures (NIH, U.S. Department of Health and Human Services) The data fitted a sigmoid Emax model much better than the Emax model as demonstrated in the representative Figures IV-15a, IV-15b and IV-15c. The sigmoid Emax model exhibited a higher regression coefficient ($r^{**}2 > 0.9$) and less deviation for each data point. The parameters Emax, EC50 and n for the fitted sigmoid Emax model are listed in Table IV-10. Comparing the three parameters between different treatments (i.e., GTN, 1,2-GDN and 1,3-GDN), no significant difference was found between Emax whereas EC50 for GTN was much lower than that for the GDNs ($p<0.05$). The n-slope factor for GTN seems lower than that for the GDNs but they are not significantly different. Comparing the EC50 values, GTN is about 130 times more potent than 1,2-GDN and 480 times more potent than 1,3-GDN in terms of decreasing systolic blood pressure in dogs.

The following analysis was undertaken to assess the contribution of the GDNs to GTN pharmacodynamics. An example is given in Figure IV-16 of such an analysis for dog #4

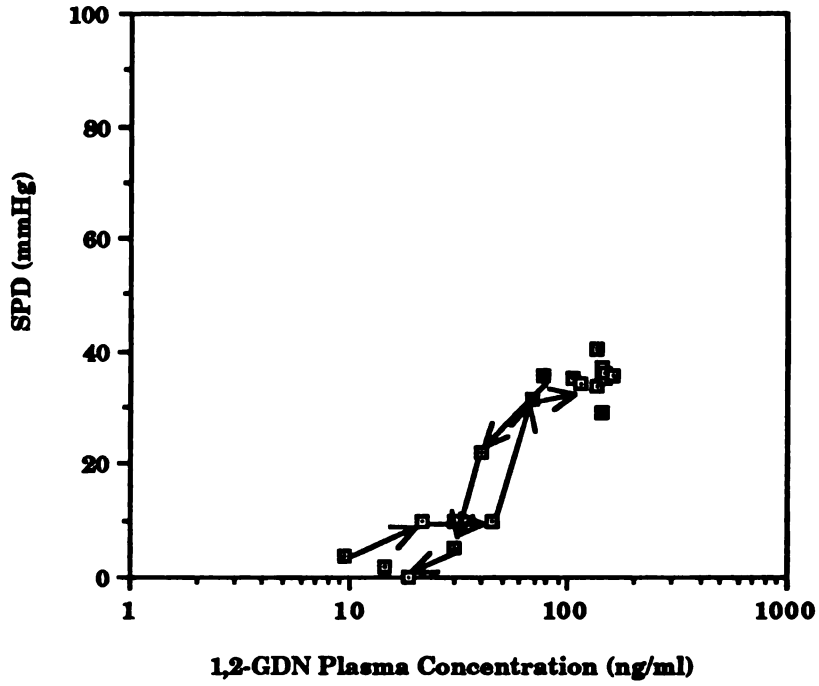

Figure IV-13a: SPD vs 1,2-GDN plasma levels following iv infusion of 1,2-GDN (100 μg/min) to dog #9.

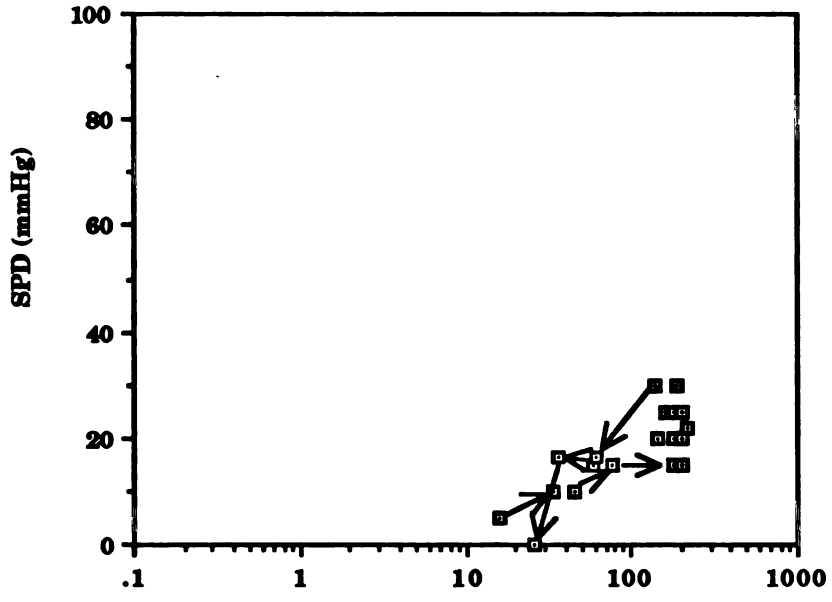

Figure IV-13b: SPD vs 1,3-GDN plasma levels following iv infusion of 1,3-GDN (100 μg/min) to dog #3.

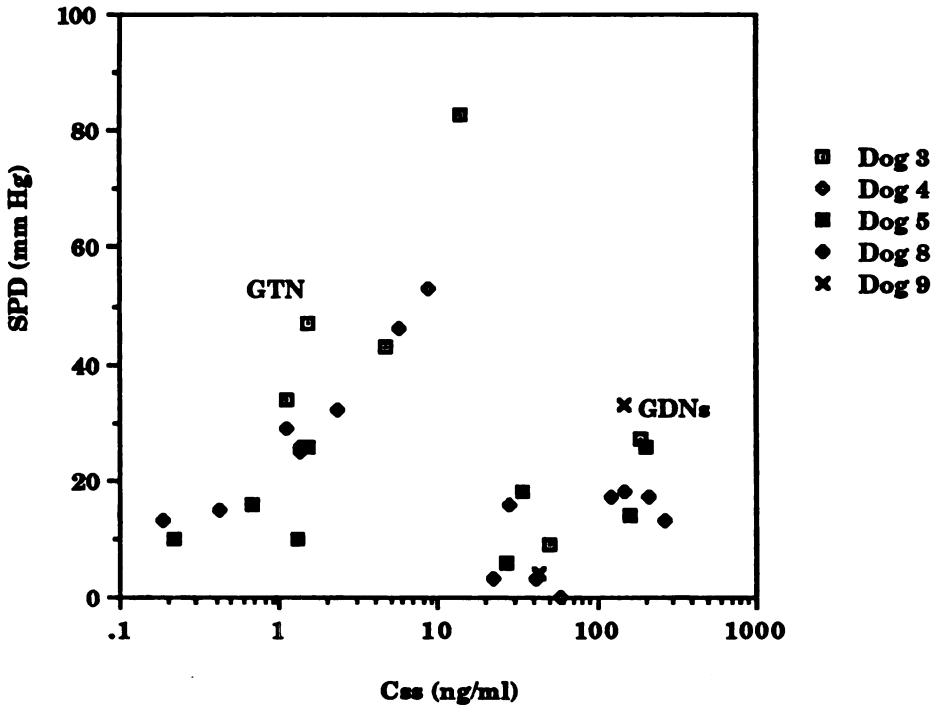

Figure IV-14: SPDss vs Css plot for GTN, 1,2-GDN and 1,3-GDN following infusions of the respective drugs in conscious dogs

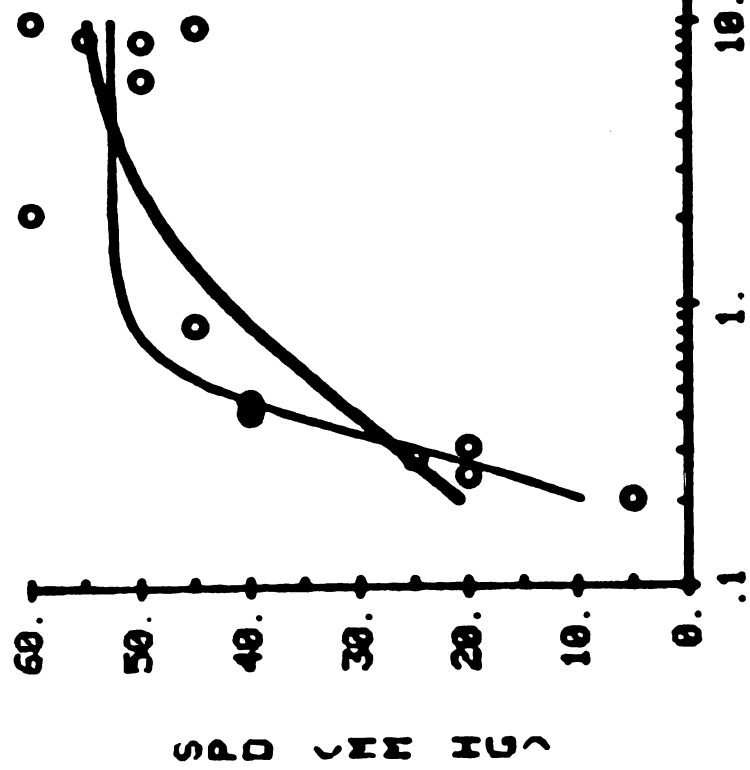

Figure IV-15a: Sigmoid Emax and Emax model fitting for the post infusion phase of SPD vs GTN plasma concentration following 70 µg/min GTN infusion to dog #4.

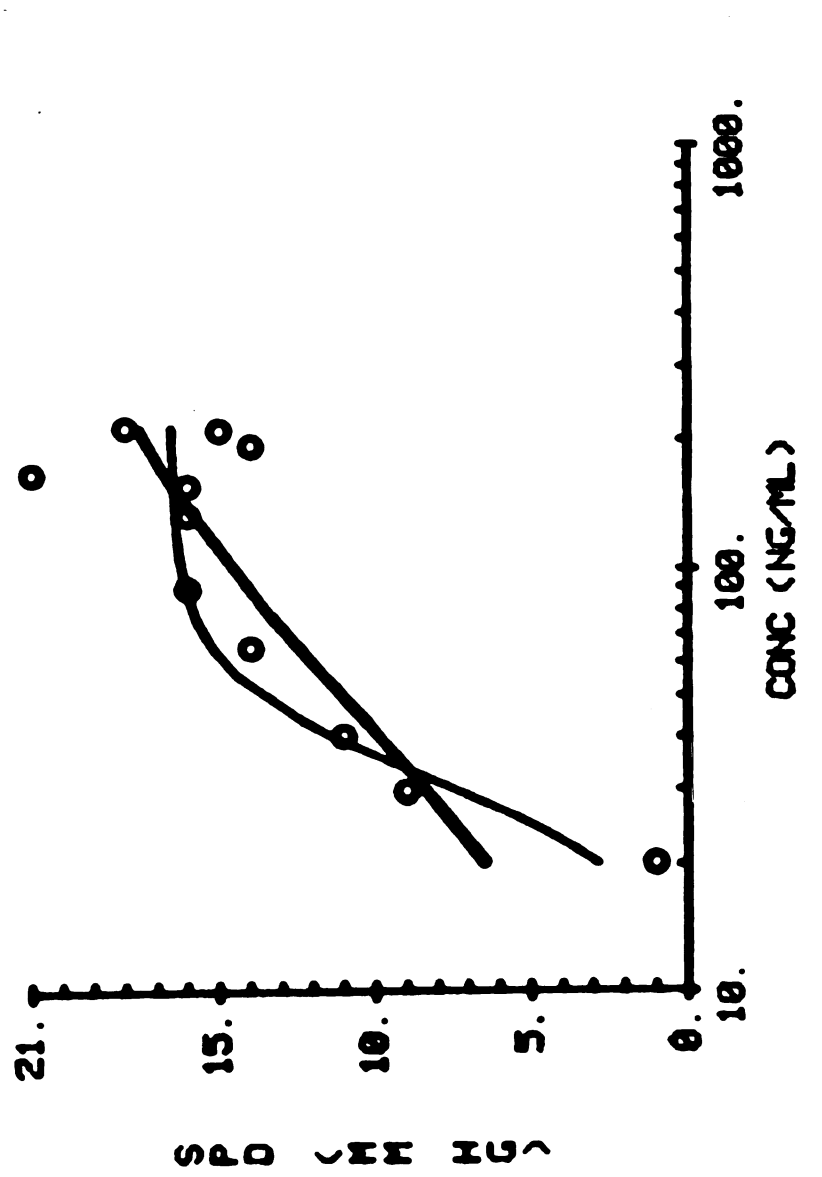

Figure IV-15b: Sigmoid Emax and Emax model fitting for the post infusion phase of SPD vs 1,2-GDN plasma concentration following 100 µg/min 1,2-GDN infusion to dog #4.

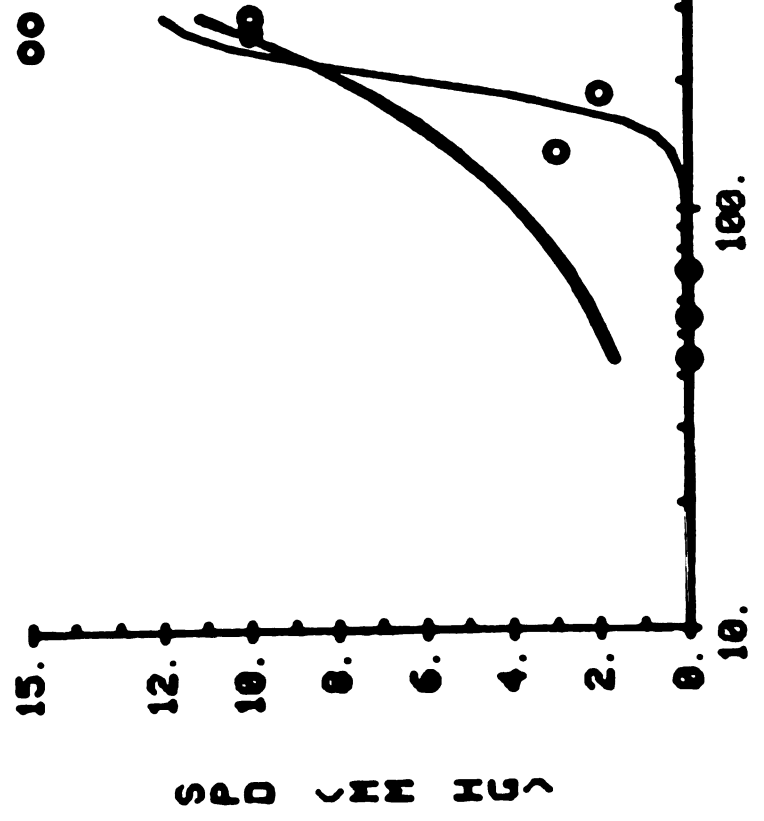

Figure IV-15c: Sigmoid Emax and Emax model fitting for the post infusion phase of SPD vs 1,3-GDN plasma concentration following 100 μg/min 1,3-GDN infusion to dog #4.

Table IV-10: Pharmacodynamic parameters (Emax, EC50 and n) obtained for GTN, 1,2-GDN and 1,3-GDN when the post infusion[a] phase plasma concentrations and SPD were fitted to a sigmoid Emax model

Dog #	Emax (mm Hg)	EC50 (ng/ml)	n	r**2
		GTN		
4	52.6	0.320	3.30	0.987
5	22.4	0.120	1.68	0.961
8	49.6	1.33	0.349	0.911
Mean	41.5	0.590	1.78	
S.D.	16.6	0.649	1.48	
		1,2-GDN		
4	16.5	31.9	3.39	0.983
5	30.6	165	2.89	0.973
8	18.8	30.8	4.29	0.975
Mean	22.0	75.9	3.52	
S.D.	7.6	77.2	0.71	
		1.3-GDN		
4	12.5	203	8.89	0.938
5	102	564	1.23	0.971
8	21.1	84.2	2.36	0.974
Mean	45.2	284	4.16	
S.D.	49.4	250	4.14	

[a] Post infusion phase of 100 μg/min GDN infusions and 70 μg/min GTN infusions.

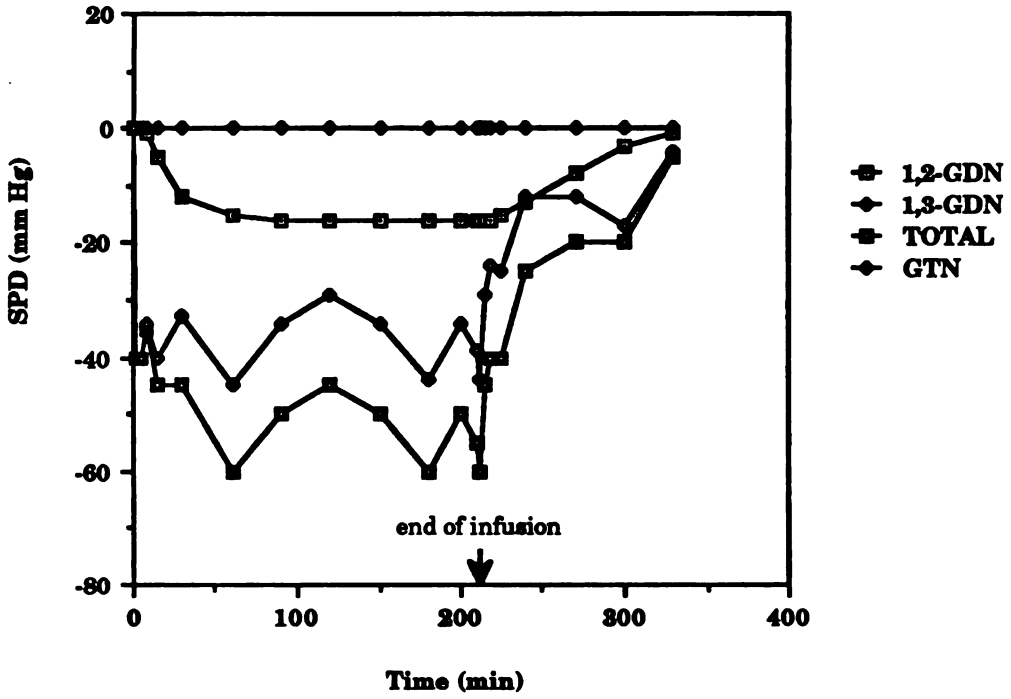

Figure IV-16: Systolic blood pressure decrease contributed by GTN, 1,2-GDN and 1,3-GDN following 70 μg/min GTN infusion to dog #4.

following a 70 μg/min GTN infusion. The lowest curve in this figure corresponds to the SPD measured in dog #4 as a function of time as previously shown in Figure IV-8. Using the measured GDN concentrations in this dog during the GTN infusion (Figure IV-2), the SPD attributed to 1,2-GDN and 1,3-GDN was calculated using the sigmoid Emax parameters given for this dog in Table IV-10. These calculated SPD values for the GDNs (the top two curves in Figure IV-16 were subtracted from the measured SPD to yield the SPD attributed to GTN alone as plotted in the third highest curve in Figure IV-16. The SPD attributed to GTN versus the measured GTN plasma concentration is plotted in Figure IV-17. (Figure IV-10 depicting measured SPD versus measured GTN concentrations is repeated here for comparison purposes.) Note that the hysteresis seen in Figure IV-10 disappears when the SPD attributed to the active metabolites is subtracted from the measured SPD as shown in Figure IV-17. However, if the combined action of 1,2-GDN and 1,3-GDN can potentiate the SPD response then the GDN contribution will be more significant than what we estimated. More detailed studies are needed to clarify this point.

DISCUSSION

Although the half-life of GTN is very short (~4 min), steady-state plasma concentrations were not reached until about 60 min instead of 12-16 min (3 to 4 half-lives) as might be expected. It seems that tissue uptake of GTN in addition to the rapid metabolism of GTN in the body is also involved in achieving the steady-state during a GTN infusion. Therefore, steady-state will not be reached until all three factors, infusion input, tissue uptake and metabolism reach equilibrium. Indeed, extensive vascular uptake of GTN has been demonstrated by Fung et al. (1984b). Previously reported studies (Armstrong et al., 1982), in which the infusion of GTN was continued for only 20 min, might not have achieved steady-state. Our studies show that the Css interdog and intradog variability for the glyceryl nitrates after each infusion was large, especially after the GTN infusions. Fluctuating Css

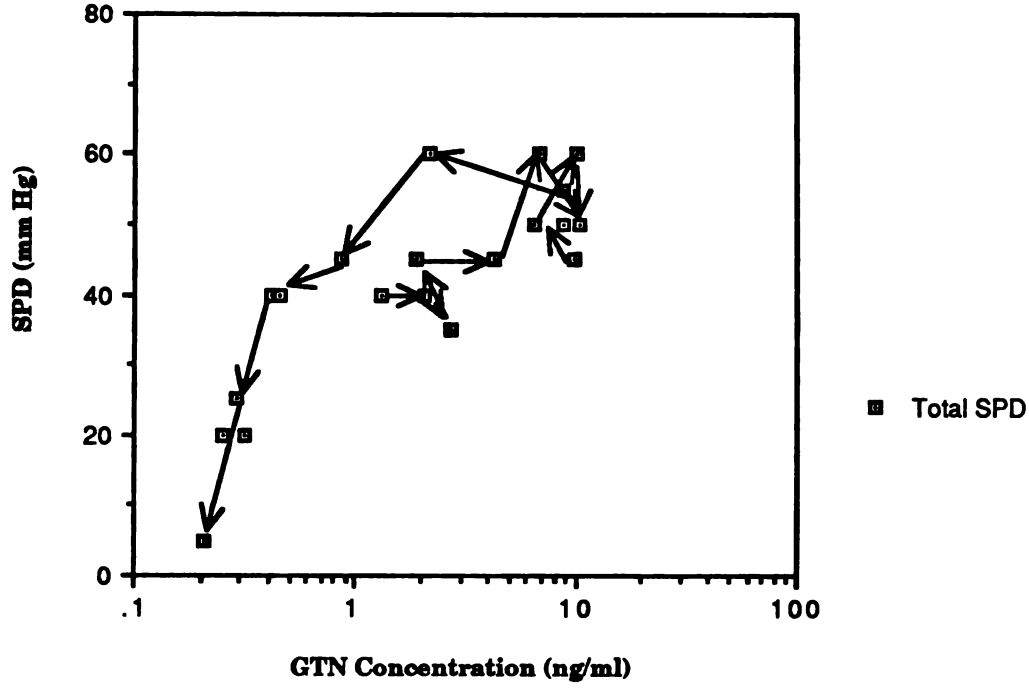

Figure IV-10: SPD vs GTN plasma levels following GTN infusion (70 μg/min) in dog #4.

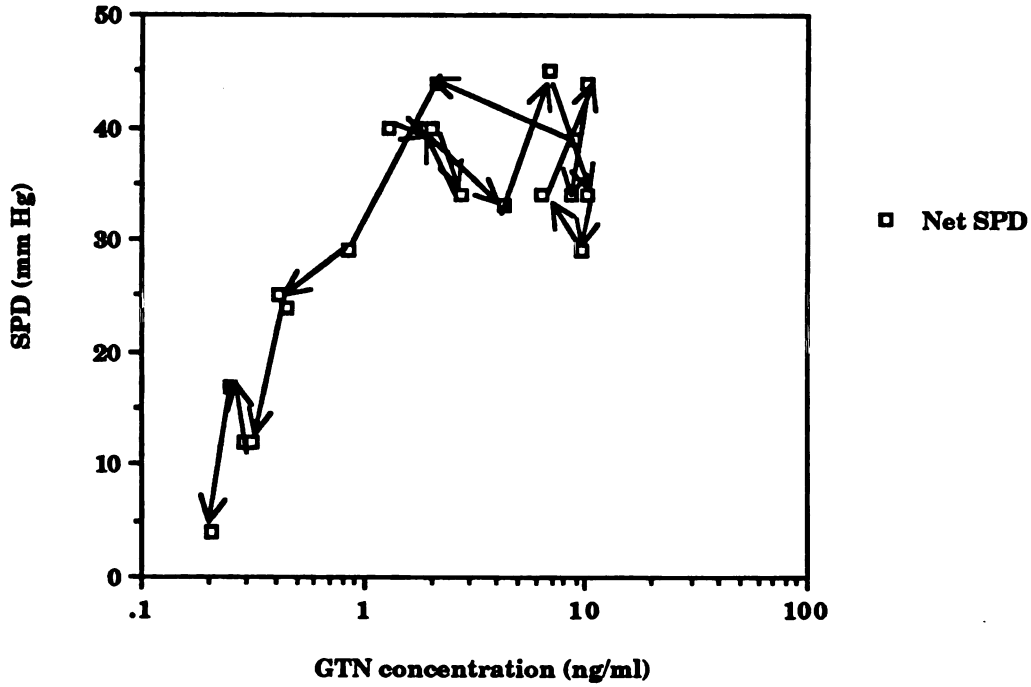

Figure IV-17: Net SPD vs GTN plasma levels following GTN infusion (70 μg/min) to dog #4.

under apparent steady-state conditions has also been observed previously in man (Armstrong et al., 1982; Karim, 1983; Parker and Fung, 1984; Noonan et al., 1985) and in dogs (Moffat et al., 1984). Armstrong et al. (1982) administered nitroglycerin infusion doses to 20 congestive heart failure patients. The end point of the infusion was either a reduction in pulmonary capillary wedge pressure by at least 25% of the control value or a 10-fold increment over the initial infusion rate. The infusion rate was increased every 10-15 min until the end point was achieved. These investigators found that a wide range of GTN infusion rates was required and a considerable variation in plasma GTN concentrations was observed. In our dog studies the variation in GTN Css in all four dogs at various infusion rates (Figure IV-3a) was very similar to that found by Moffat et al. (1984) in their dog studies (Figure IV-18). They infused GTN into six anesthetized dogs for 20 min at infusion rates of 4, 7.8, 15 and 21 μg/min. Css was determined from the plasma sample taken at the end of each infusion. They also discovered a wide variation in GTN concentrations between dogs and a lack of proportionality for Css between infusions. The reason for this variability in GTN concentrations at steady-state is unclear but the recent reports by Fung et al., (1984b, 1986) may give some explanations. These investigators demonstrated that organic nitrates are extensively taken up and metabolized by rat blood vessels and other organs and further demonstrated that the pharmacokinetics of GTN are dependent on cardiac output providing a hemodynamic explanation for this large variability in Css of GTN. Only in one of four dogs (dog #5), did GTN Css increase proportionately with dose (Table IV-2). The increase of GTN Css in the other three dogs was not proportional to the infusion rate, however, a linear relationship between Css of GTN and infusion rate was observed in all four dogs (y-intercept = -0.899 $\pm$ 1.066 ng/ml, slope = 0.094 $\pm$ 0.078) which is similar to the findings reported by Moffat et al. (1984) in dogs with a slope of 0.26 $\pm$ 0.12. Although clearances of GTN in dogs showed a large variation, as also observed by Cossum et al. (1986) in sheep, they were not significantly different at various infusion rates which indicates there was no obvious dose dependency of GTN in the dose range we studied. The formation of 1,2-GDN and 1,3-GDN

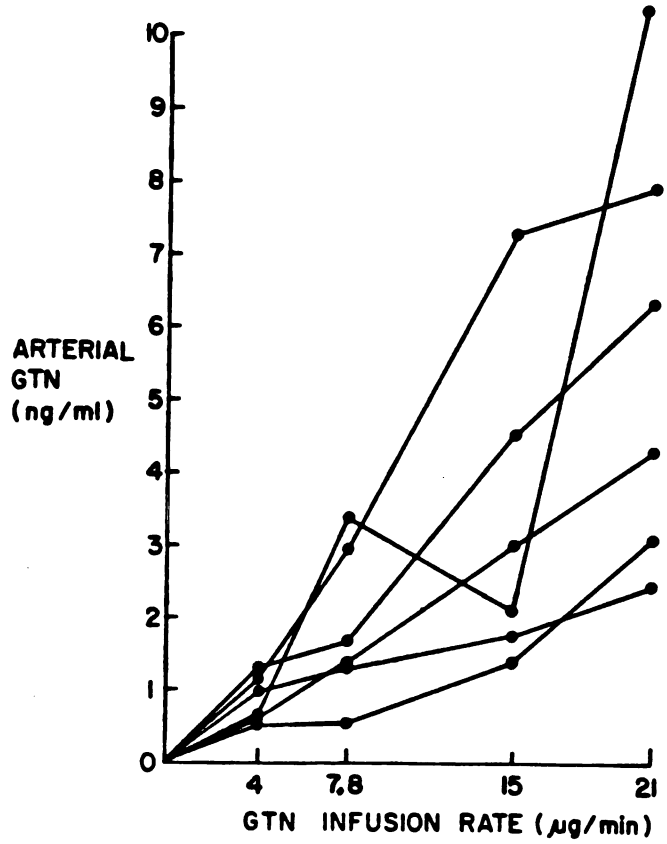

Figure IV-18: The relationship between infusion rate and GTN concentration in arterial blood. The infusion was maintained at each infusion rate for at least 20 min before a blood sample was withdrawn (Moffat et al., 1984)

after GTN infusions was rapid and the metabolites reached levels much higher than that of GTN. The Css of 1,2-GDN was 31.5 times (S.D. = 17.2) higher than that of GTN whereas 1,3-GDN was 5.47 times (S.D. = 3.19) higher than that of GTN. These ratios are much higher than the values reported by Noonan et al. (1985) in man following multiple infusions of GTN. This could result from either a higher GTN metabolic clearance in dog than that in man or the clearance of the dinitrate metabolites is markedly less in dogs than that in man. GDN pharmacokinetic data in man are required to clarify this point. The Css for the dinitrate metabolites following graded GTN infusions also failed to show a proportional increase with dose but a linear relationship between Css of dinitrate metabolites and GTN dose does exist. The ratio of 1,2-GDN/1,3-GDN (5.78 $\pm$ 1.23) is lower than the value (7.49 $\pm$ 0.38) observed in man (Noonan et al., 1985) and is intermediate of the ratios obtained in dogs (Chapter III) after low intravenous bolus doses (8.44 $\pm$ 1.94), high intravenous bolus doses (3.41 $\pm$ 0.90) and oral doses (1.61 $\pm$ 0.19). The clearance of GTN to form 1,2-GDN (CLm1) and 1,3-GDN (CLm2) was different following low and high intravenous GTN administration to dogs as discussed in Chapter III. Since the clearance and volume of distribution of 1,2-GDN and 1,3-GDN are similar to each other, the different ratio of 1,2-GDN/1,3-GDN following different GTN dosing routes may indicate that the biotransformation of GTN to 1,2-GDN and 1,3-GDN was different for each dosing route. Further investigation is needed to clarify whether there are enzymes or mechanisms specific for 1,2-GDN and 1,3-GDN metabolism.

The Css of 1,2-GDN and 1,3-GDN were reached at about 150 min following infusions of 1,2-GDN and 1,3-GDN at 20 μg/min and 100 μg/min. This length of time was the same as that required for concentrations of 1,2-GDN and 1,3-GDN to reach steady state after GTN infusions. This is consistent with the predicted time to reach steady-state in terms of GDN half-lives (~45 min), i.e., that 90% of Css will be reached after 3-1/2 half-lives infusion time. Good dose and Css proportionality was observed between 20 μg/min and 100 μg/min in the

1,2-GDN and 1,3-GDN infusion studies. The Css increased five fold when the infusion rate was increased 5 fold. Therefore, over this dose range clearances of 1,2-GDN and 1,3-GDN were constant. It is very clear that the pharmacokinetics for 1,2-GDN and 1,3-GDN are much more consistent and predictable than that observed for GTN. However, it is interesting to note that the GDN clearances were higher following infusion administration than that observed after bolus administration (Table IV-11), except for 1,3-GDN in dog #4, indicating dose dependent pharmacokinetics. The average Cmax for 1,2-GDN and 1,3-GDN after bolus GDN doses (0.25 mg/kg) were 438 and 518 ng/ml (Chapter III), respectively, whereas the mean Css for 1,2-GDN and 1,3-GDN were 33.2 and 42.7 ng/ml, respectively, following 20 μg/min infusion doses and 160 and 204 ng/ml, respectively, following 100 μg/min infusion doses. It is possible that the pattern for GDN metabolism might be changed at higher plasma concentrations, similar to that observed for GTN as discussed in Chapter III. The metabolic pathways for the GDNs are more complicated than that for GTN since the GDNs also can be eliminated through glucuronidation in addition to denitration to form glyceryl mononitrates.

Systolic blood pressure at steady-state decreased in a non-proportional way as the infusion rate of GTN increased but it exhibited a linear relationship with differing infusion rates (Table IV-8 and Figure IV-9a). Large interdog and intradog SPDss variability was also observed. Such variability is appreciable and as we have discussed previously the high variability in Css can affect SPDss. The SPDss can then affect the physiology of the dog which can in turn affect clearance and Css. In Figure IV-10 GTN concentrations of the ascending limb are higher than those of the descending limb, i.e., reverse hysteresis. This phenomenon could be attributed to either an equilibration time between the sampling site and the action site or to the formation of active metabolites. Glyceryl dinitrate metabolites of GTN have been shown to be pharmacologically active in our previous studies (Chapter III) and the equilibration time for GTN was very short (< 1 min) after a single bolus dose of GTN

Table IV-11: Clearances (ml/min/kg) of GTN, 1,2-GDN and 1,3-GDN after intravenous bolus (low 0.025 and high 0.25 mg/kg) and infusion (10, 20, 30, 50, 70 and 100 μg/min) doses to conscious dogs

GTN

Dog #	CLlow	CLhigh	CL10	CL30	CL50	CL70
3	635	482	322	232	1170	181
4	720	249	878	1070	331	298
5	1190	611	1870	1760	1460	1850
8	2060	940	1940	806	1080	1700

1,2-GDN

	CLhigh	CL20	CL100
4	12.8	17.4	17.2
5	17.1	30.5	26.0
8	15.0	32.5	29.2

1,3-GDN

	CLhigh	CL20	CL100
4	13.3	12.2	13.2
5	12.9	24.5	20.1
8	13.6	26.0	24.1

(Chapter III). Therefore, this counter clockwise hysteresis is most likely due to the formation of active metabolites - 1,2-GDN and 1,3-GDN. Indeed, after the subtraction of the SPD corresponding to 1,2-GDN and 1,3-GDN from total SPD (Figure IV-17) the counter clockwise hysteresis disappeared demonstrating the significance of the active dinitrate metabolites on nitroglycerin pharmacodynamics.

The systolic blood pressure decreases at steady-state following 20 μg/min infusions of 1,2-GDN and 1,3-GDN were very small. A significant systolic blood pressure decrease was observed after the infusion rate was increased to 100 μg/min for both 1,2-GDN and 1,3-GDN. Comparing Css values between GTN and GDNs based on comparable SPDss, GTN was about 100 times more potent than 1,2-GDN and 1,3-GDN (Figure IV-14). However, the dinitrate metabolites formed after GTN infusion may have partially contributed to the nitroglycerin potency. The SPD vs plasma concentration plot after 1,2-GDN and 1,3-GDN infusions did not show a counter clockwise hysteresis as observed in GTN infusions (Figures IV-10, IV-13a and IV-13b). Thus, it seems that the mononitrate metabolites formed following 1,2-GDN and 1,3-GDN infusions do not affect the SPD induced by 1,2-GDN and 1,3-GDN. We are not able to assess the effects of mononitrates on the pharmacokinetics of the dinitrates since the plasma levels of mononitrates were not measured. The descending limb of SPD versus 1,2-GDN and 1,3-GDN curves fitted a sigmoid Emax model (Hill equation) much better than an Emax model. However, a wide range for Emax, EC50 and slope factor (n) in dogs was observed. Because the doses administered to dogs were not the maximal, it was difficult to obtain good estimates for Emax, EC50 and n.

CHAPTER V

DO NITROGLYCERIN DINITRATE METABOLITES AFFECT NITROGLYCERIN PHARMACOKINETICS?

INTRODUCTION

Nitroglycerin (GTN) and its active dinitrate metabolites, 1,2-GDN and 1,3-GDN, are metabolized via the same enzyme, organic nitrate reductase (Needleman and Hunter, 1965). After chronic dosing of GTN, the high levels of dinitrates in plasma may affect the metabolism and/or distribution of GTN. Glyceryl dinitrate inhibition of nitroglycerin metabolism in vitro in sheep tissue homogenates (i.e., liver, lung, muscle, arterial and venous tissue) has been demonstrated by Cossum and Roberts (1985b). Cossum and Roberts (1985a) also reported that the in vitro metabolism of GTN in human blood can be inhibited by 1,2-GDN and 1,3-GDN. Sutton and Fung (1984) and Morrison and Fung (1984) demonstrated that metabolites of isosorbide dinitrate (ISDN), isosorbide-2-mononitrate (2-ISMN) and isosorbide-5-mononitrate (5-ISMN), decrease the in vivo plasma clearance of ISDN in rats. Cossum et al. (1986) showed that administration of glyceryl dinitrates to anesthetized sheep following GTN infusion at 5.7 μg/kg/min significantly inhibited GTN metabolism across the hind leg. Noonan et al. (1985) reported dose dependent pharmacokinetics of GTN after multiple intravenous infusions in healthy volunteers. They infused GTN for a total of 160 min. The infusion rates were adjusted to 10, 20, 40, 10 μg/min at 0, 40, 80, 120 min. They found that the steady-state plasma concentration during the last 10 μg/min infusion was always higher than that obtained during the initial 10 μg/min infusion and that the Css versus infusion rate curve exhibited a hysteretic type

response. They further suggested that this hysteretic response may be explained by either end-product inhibition or saturable binding of GTN to blood vessels.

The purpose of this study was to examine the metabolite inhibition effects of 1,2-GDN and 1,3-GDN on GTN pharmacokinetics and pharmacodynamics in conscious dogs, and to possibly elucidate the mechanism of the previously observed hysteretic response.

MATERIALS AND METHODS

Organic Nitrates

Nitroglycerin (Tridil IV) was purchased from Du Pont Critical Care (McGaw Park, IL). 1,2-GDN and 1,3-GDN were provided by Marion Laboratories, Inc. (Kansas City, MO) as pure chemicals (>99%) and used without further purification. All dose solutions were prepared using normal saline under sterile conditions.

Animals

Five conditioned mongrel dogs, 2 male (#7 and #11) and 3 female (#4, #9 and #12), weighing 17-28 kg were used. Chronic catheters were surgically implanted into the femoral artery and vein of the dogs. The conditioning schedule and surgical procedures were as described in detail in Chapter III. Dogs were given a ration of dry chow (Purina) daily at 1400 hr and had water available ad libitum.

Experimental Protocol

Experiments were normally conducted in the morning. Food was withheld from the dogs overnight before study and throughout the study period. Water was not available during the study period. Generally, 5 days separated studies in each dog. All experiments were conducted in a quiet room with the dog in a sling (Alice King Chatham Medical Arts, Los Angels, CA) which provided support but minimal restraint. The dogs were trained to familiarize them to the environment before the actual studies were conducted. Two types of experiment were carried out:

A. Intravenous Bolus Dose of GTN and Infusion of GDNs:

Three mongrel dogs were given a single iv bolus dose of GTN (0.025 mg/kg) as the control. In the interaction studies, steady state concentrations (Css) of either 1,2-GDN or 1,3-GDN were rapidly achieved by giving a single iv bolus (77 μg/kg), followed immediately by an infusion (50 μg/min, Harvard Infusion/Withdrawal pump) of GDN . Steady-state of GDN plasma concentrations was achieved within 50 min of starting the infusion, at which time a single iv bolus dose of GTN (0.025 mg/kg) was given. Thus three studies: control, GTN/1,2-GDN interaction and GTN/1,3-GDN interaction studies were performed in all three dogs. The GDN infusion was continued for 200 min, that is, for 150 min after the GTN bolus dose. Venous blood samples (5 ml/sample) were drawn into a heparinized syringe at 0, 1, 2, 3, 4, 5, 7, 10, 15, 30, 60, 90, 120 and 150 min post GTN dose.

B. Infusions of GTN and GDN:

An infusion of nitroglycerin was begun in each of 4 dogs and continued for 160 min at an infusion rate of 100 μg/min. Steady-state concentrations of GTN were achieved within 100

min, at which time the dog received simultaneously, an iv bolus dose (5.14 mg) of one of the GDNs and an infusion dose (100 μg/min) of the same GDN. Both GTN/1,2-GDN and GTN/1,3-GDN interaction studies were conducted in each dog. Both arterial and venous blood samples (5 ml/sample) were collected at 0, 2, 10, 15, 30, 60, 90, 100, 120, 140, 150, 160, 162, 165, 170, 175, 190, 220 and 250 min post GTN dose.

GTN and GDN doses were administered into a front leg vein using an Angiocath (20g, 1-1/4 in, Deseret Medical, Inc. Sandy, UT). Blood samples were collected from the femoral artery and vein and centrifuged immediately using a Eppendorf centrifuge (Brinkmann Instruments, Westbury, NY). The plasma was separated and frozen with dry ice and stored in a freezer (-20°C) pending assay. Arterial blood pressure was monitored with a Gould Statham p23db transducer (Oxnard, CA) connected to a Grass model 7 polygraph (Quincy, MA). Blood volume loss due to sampling was replaced with normal saline following each sample collection.

Assay Method

A capillary GC-ECD method was used to determine simultaneously the plasma concentrations of GTN, 1,2-GDN and 1,3-GDN. The detailed procedure was described in Chapter II.

RESULTS

A. Intravenous Bolus Dose of GTN and Infusion of GDNs

Steady-state plasma concentrations (~ 100 ng/ml) of 1,2-GDN and 1,3-GDN were reached rapidly (<50 min) after co-administration of a single iv bolus dose and an infusion dose of the

respective GDN. Plasma concentrations of GTN during the control, GTN/1,2-GDN and GTN/1,3-GDN interaction studies versus time plots for all three dogs are presented in Figures V-1, V-2 and V-3, respectively. The GTN data were fitted to a two-compartment model (i.e., $C = A_1 * e^{-k1t} + A_2 * e^{-k2t}$) using the PCNONLIN program (Statistical Consultants, Inc., 1986). Initial estimates for PCNONLIN were obtained from CSTRIP (Sedman and Wagner, 1976). Weights of 1/C were used to obtain the best fit. Areas under the GTN plasma concentration versus time curves were calculated using the method reported by Benet and Galeazzi (1979) as $AUC = A_1/k_1 + A_2/k_2$ and are listed in Table V-1. Apparent clearance (CLapp) of GTN as listed in Table V-1 was determined as CLapp = Dose/AUC. One-way ANOVA for repeated-measures was used to test the difference between control studies and interaction studies. It was shown that there was no significant difference between the interaction results and the control results in comparing AUC_{total} ($p>0.9$) and CLapp ($p>0.7$). Because GTN plasma levels dropped rapidly below the assay limit, there were insufficient data to accurately estimate area under the moment curve(AUMC). Therefore, the apparent volume of distribution at steady-state (Vapp,ss) of GTN was not determined. However, from Figures V-1 through V-3 it appears that the GDNs had no significant effect on the GTN time course, and therefore no changes would be expected in terms of clearance or volume.

After the simultaneous iv bolus and infusion administration of 1,2-GDN or 1,3-GDN to dogs the systolic pressure decreased and reached a steady-state rapidly (Figure V-4). When the iv bolus dose of GTN (0.025 mg/kg) was given to the dog at 50 min following the onset of GDN dosing, it caused a transient (~ 1 min) further systolic blood pressure decrease (net mean maximum decrease = 40 mm Hg) but the pressure returned rapidly to the previous steady-state pressure induced by the GDN infusion, which was maintained until the termination of the GDN infusion. Heart rate did not change throughout the GDN infusion period except for

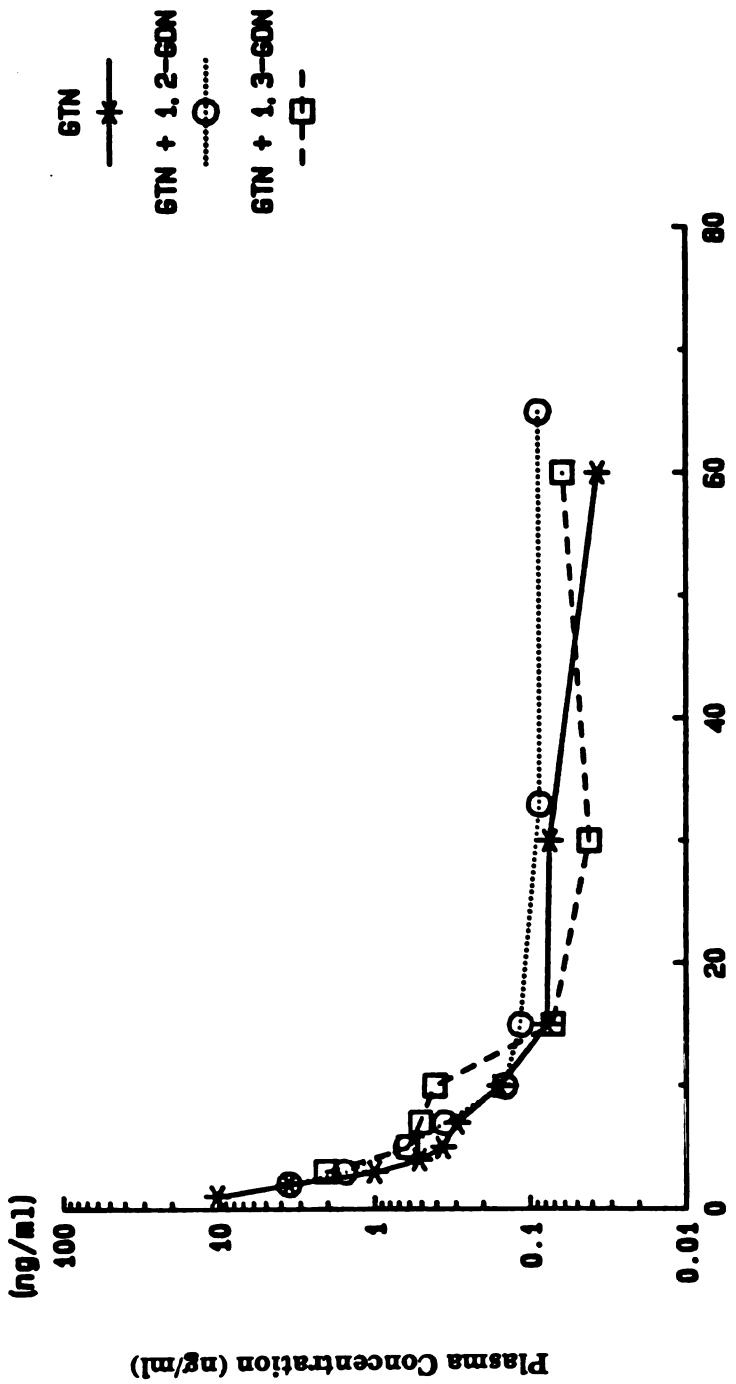

Figure V-1: Plasma levels of GTN versus time plots following GTN, 1,2-GDN and 1,3-GDN doses to dog #4. For the GTN/GDN interaction studies, a GTN bolus dose (0.025 mg/kg) was given at 50 min after the 1,2-GDN or 1,3-GDN infusions (50 µg/min) were begun.

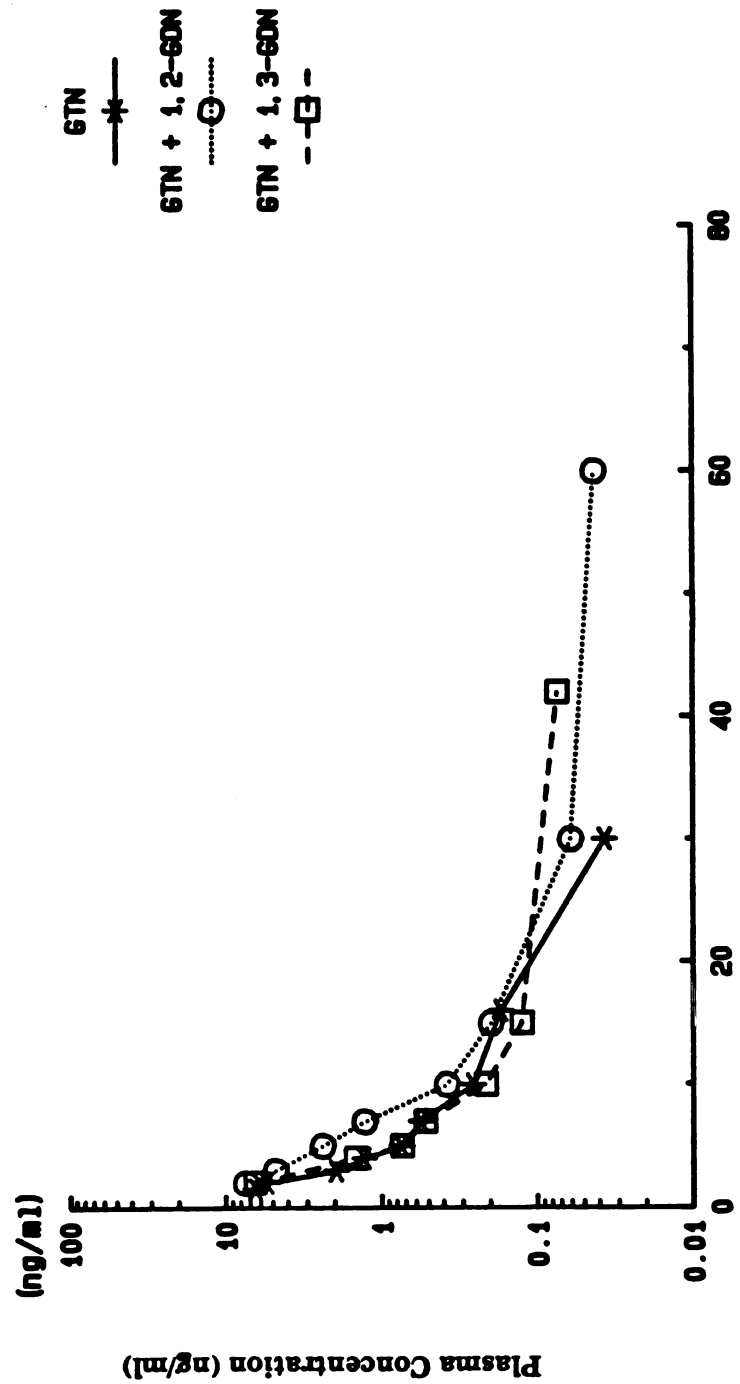

Figure V-2: Plasma levels of GTN versus time plots following GTN, 1,2-GDN and 1,3-GDN doses to dog #7. For the GTN/GDN interaction studies, a GTN bolus dose (0.025 mg/kg) was given at 50 min after the 1,2-GDN or 1,3-GDN infusions (50 µg/min) were begun.

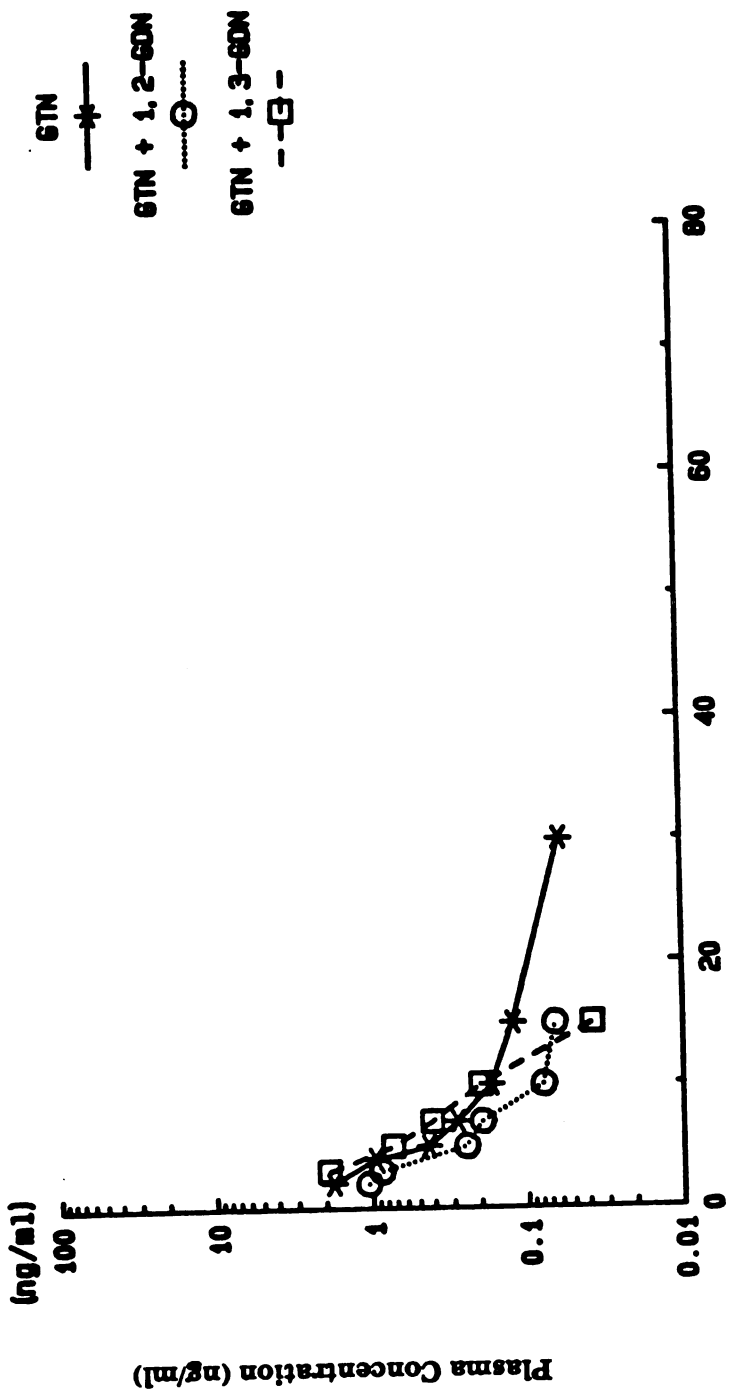

Figure V-3: Plasma levels of GTN versus time plots following GTN, 1,2-GDN and 1,3-GDN doses to dog #9. For the GTN/GDN interaction studies, a GTN bolus dose (0.025 mg/kg) was given at 50 min after the 1,2-GDN or 1,3-GDN infusions (50 µg/min) were begun.

Table V-1: AUC of GTN plasma levels versus time and clearance following nitroglycerin single iv bolus doses (0.025 mg/kg) to conscious dogs

	AUC (ng*min/ml)			
	Dog #4	Dog #7	Dog #9	one-way ANOVA
GTN(Control)	32.7	50.6	14.5	
GTN/1,2-GDN[a,c]	25.3	46.7	24.8	p>0.9
GTN/1,3-GDN[b,c]	22.7	46.3	32.2	
	CLapp (ml/min/kg)			
GTN (Control)	765	494	1720	
GTN/1,2-GDN	988	535	1010	p>0.7
GTN/1,2-GDN	1100	540	774	

a GTN dose was given at 50 min after the 1,2-GDN administration.
b GTN dose was given at 50 min after the 1,3-GDN administration.
c 1,2-GDN or 1,3-GDN doses were given as a combination of iv bolus dose (77 μg/kg) and infusion dose (50 μg/min).

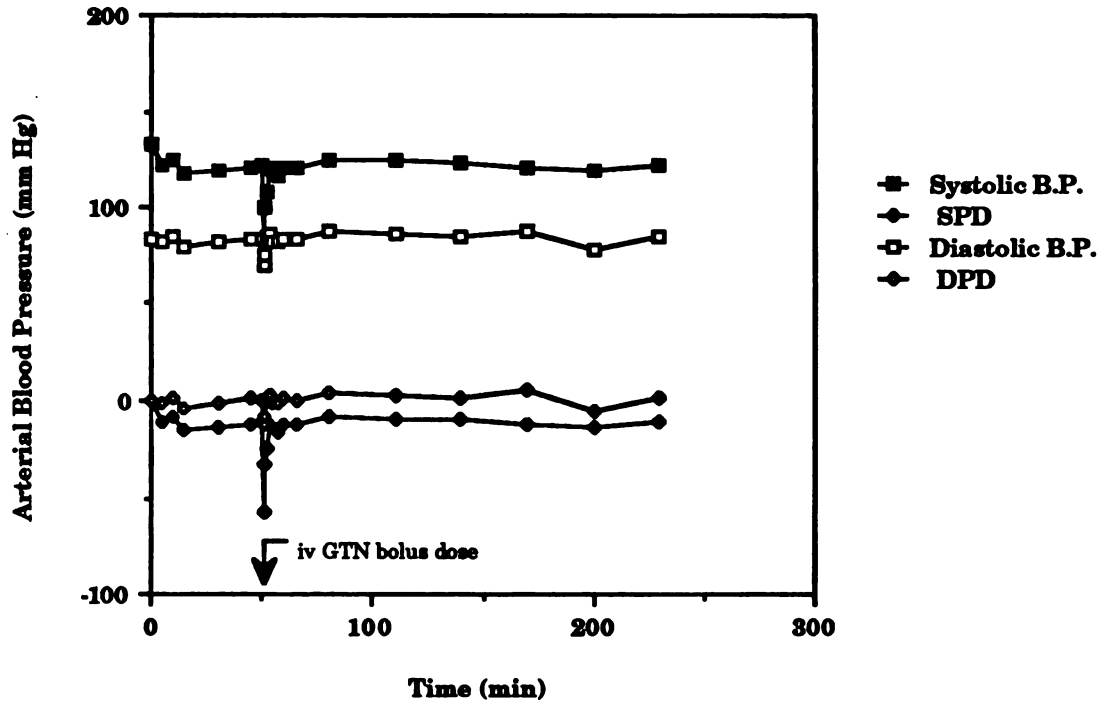

Figure V-4: Arterial blood pressure and blood pressure change following 1,2-GDN infusion and GTN bolus doses to dog #4. The GTN dose (0.025 mg/kg) was given at 50 min after the 1,2-GDN infusion (50 μg/min) was begun.

a transient increase at the moment when the GTN dose was given. There was no clear diastolic blood pressure change except at the time GTN was injected (Figure V-4).

B. Infusions of GTN and GDN

Steady-state arterial and venous GTN plasma concentrations were reached about 60 min following the beginning of the GTN infusion. The averages of plasma levels of GTN at 60, 90 and 100 min in each dog were used as control concentrations, whereas the averages of plasma levels of GTN at 120, 150 and 160 min were used as the interaction plasma concentrations. The control and interaction plasma levels of GTN in each dog were compared using Student's paired t test (two-tail). For both the GTN/1,2-GDN and GTN/1,3-GDN interaction studies, no significant differences were observed between control and interaction plasma levels of GTN when either arterial or venous levels were compared except for venous GTN levels after co-administration of GTN and 1,3-GDN ($p < 0.04$). The marked rise of venous GTN plasma levels as reported by Cossum et al. (1986) was not observed in any of the four dogs after the GDN doses were given. Mean steady-state arterial and venous plasma GTN concentrations are listed in Table V-2. The arterial and venous GTN plasma levels versus time plots for all dogs following simultaneous GTN and GDN dosing are presented in Figures V-5 to V-12. Clearances (CL) before and after GDN dosing were also determined as the ratio of the GTN infusion rate divided by the average steady-state GTN plasma concentration. Clearances based on both arterial and venous levels were calculated (Table V-3). Arterial clearances were much lower than venous clearances and fluctuated less as demonstrated by a lower %CV (Table V-3). Since nitroglycerin can be extensively taken up and metabolized by blood vessels and muscles, especially venous vessels, it is expected that venous GTN plasma levels will be lower and venous clearances will be higher than arterial measurements and show higher intra and inter-dog variation as was discussed in Chapter IV. For both GTN/1,2-GDN and GTN/1,3-GDN interaction studies there were no

Table V-2: Mean steady-state GTN plasma levels (Css) following intravenous infusions of GTN in conscious dogs with and without concomitant GDN infusions

		GTN Control			GTN with 1,2-GDN		
Dog #	Body Weight (kg)	Ca,ss[a]	Cv,ss[b]	A/V[c]	Ca,ss	Cv,ss	A/V
4	29	30.4	13.3	2.29	34.5	13.0	2.79
7	27	29.5	14.4	2.04	27.4	14.9	1.96
11	25	33.3	7.65	4.35	29.5	12.3	2.40
12	17	63.8	15.8	4.06	64.8	21.8	2.81
Mean		39.3	12.8	3.19	39.0	15.0	2.49
S.D.		16.4	3.6	1.19	17.4	4.9	0.40
		GTN Control			GTN with 1,3-GDN		
4	29	32.1	7.57	4.24	25.5	8.20	3.31
7	27	37.4	11.6	3.23	32.5	14.7	2.34
11	25	31.2	3.91	7.98	26.9	5.61	4.80
12	17	39.0	9.68	4.02	45.2	11.7	4.02
Mean		34.9	8.19	4.87	32.2	10.2	3.62
S.D.		3.9	3.29	2.12	9.2	3.8	1.05

[a] Arterial plasma level
[b] Venous plasma level
[c] Arterial/venous ratio

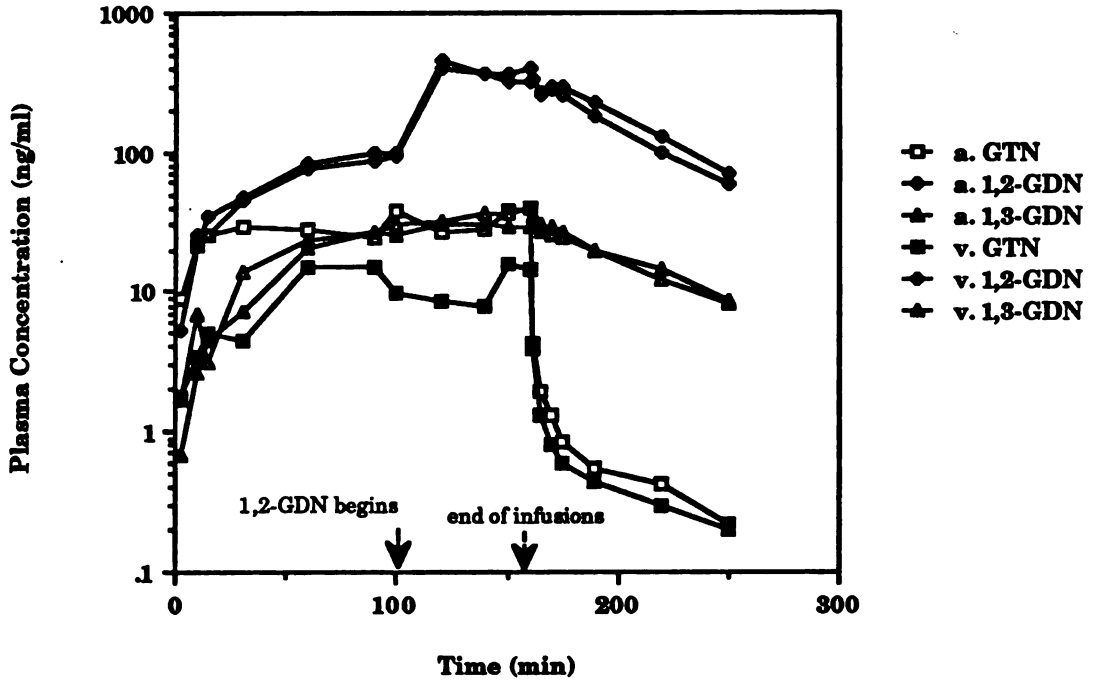

Figure V-5: Arterial and venous plasma levels of GTN, 1,2-GDN and 1,3-GDN following GTN and 1,2-GDN infusions in dog #4.

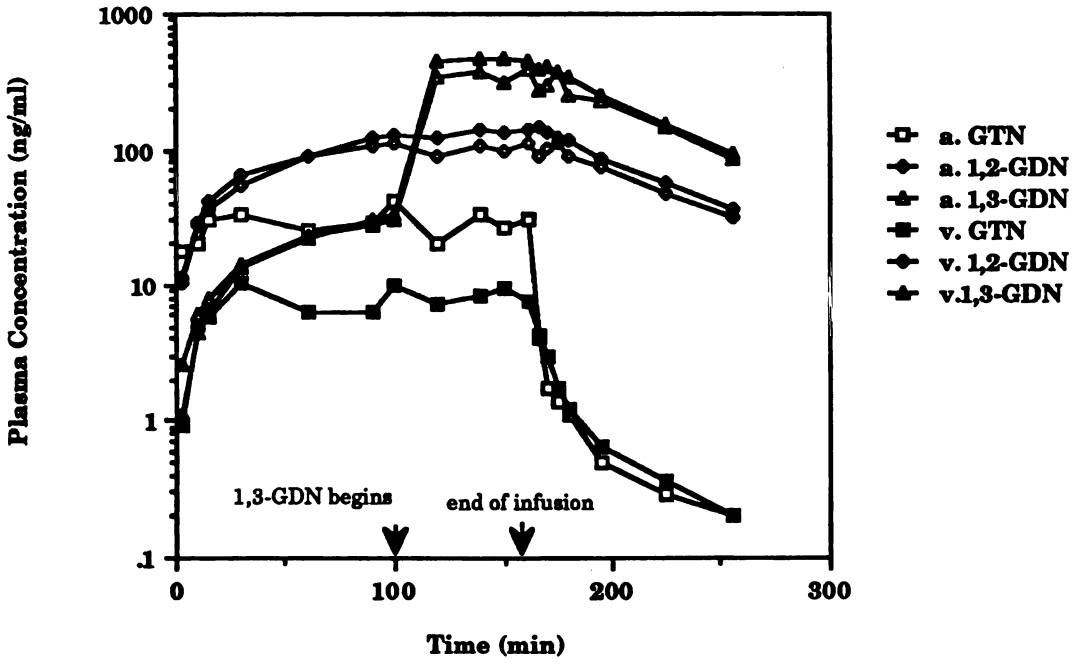

Figure V-6: Arterial and venous plasma levels of GTN, 1,2-GDN and 1,3-GDN following GTN and 1,3-GDN infusions in dog #4.

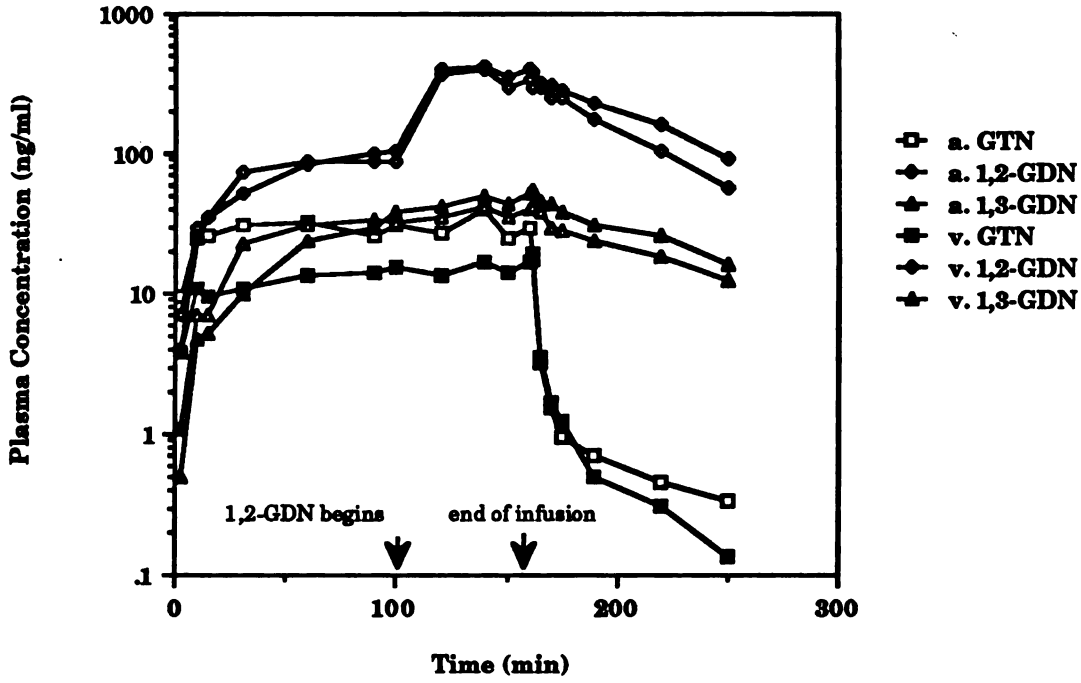

Figure V-7: Arterial and venous plasma levels of GTN, 1,2-GDN and 1,3-GDN following GTN and 1,2-GDN infusions in dog #7.

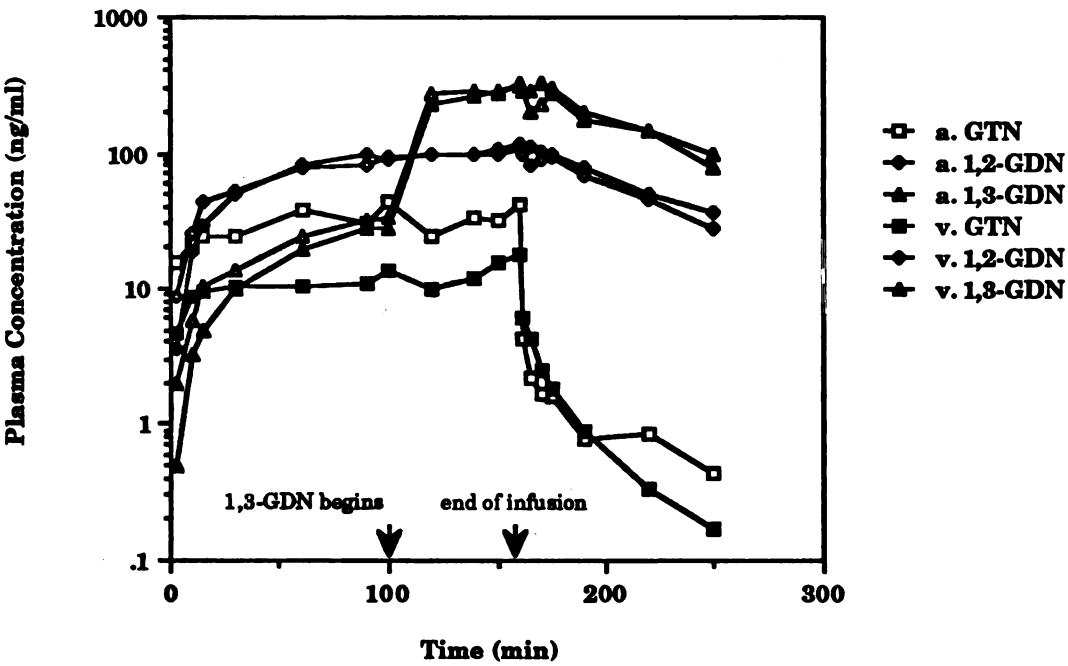

Figure V-8: Arterial and venous plasma levels of GTN, 1,2-GDN and 1,3-GDN following GTN and 1,3-GDN infusions in dog #7.

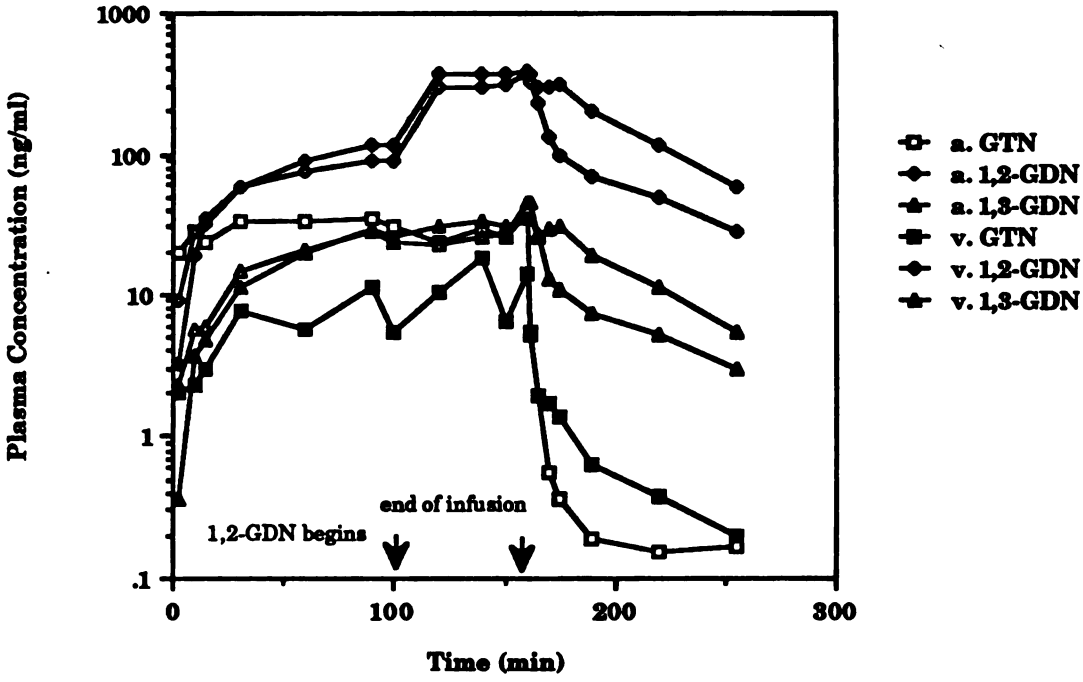

Figure V-9: Arterial and venous plasma levels of GTN, 1,2-GDN and 1,3-GDN following GTN and 1,2-GDN infusions in dog #11.

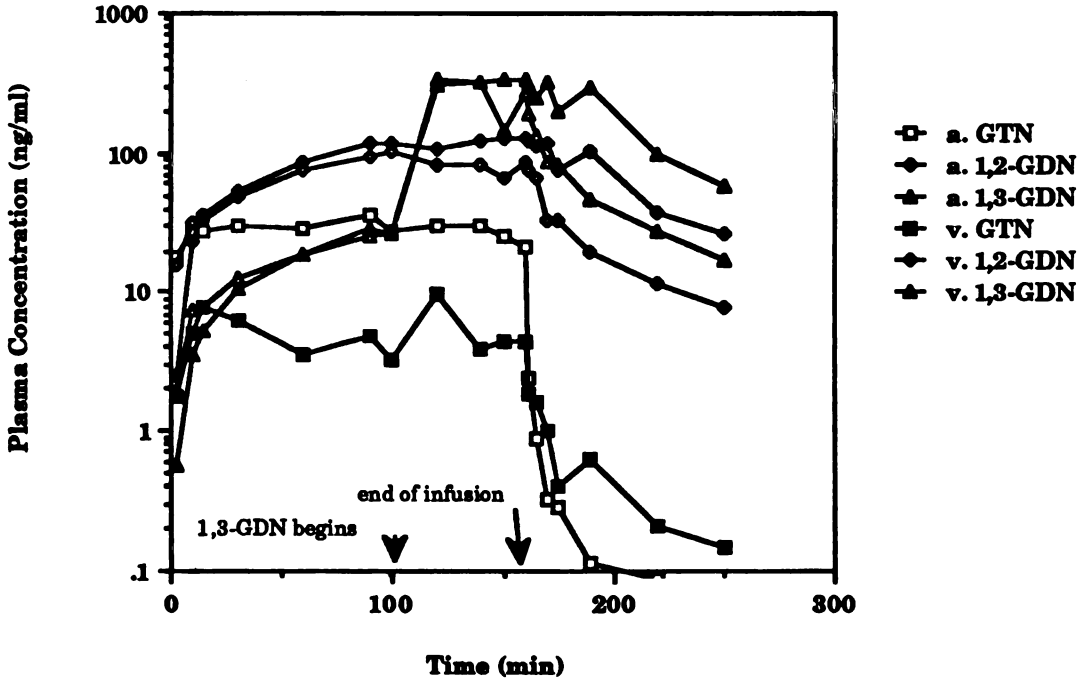

Figure V-10: Arterial and venous plasma levels of GTN, 1,2-GDN and 1,3-GDN following GTN and 1,3-GDN infusions in dog #11.

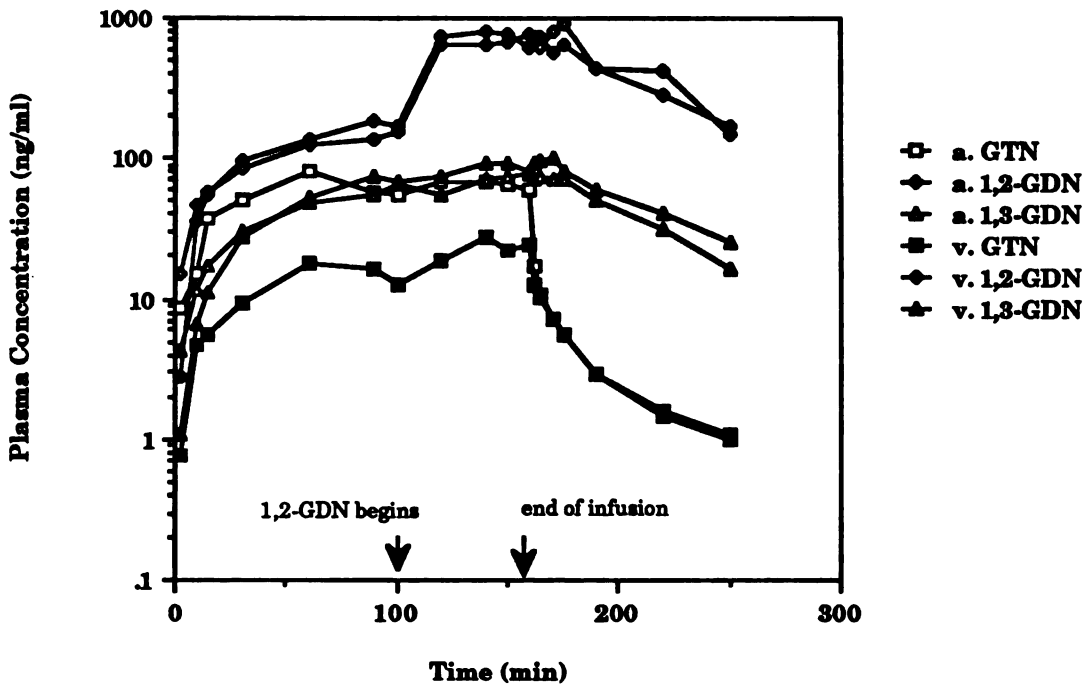

Figure V-11: Arterial and venous plasma levels of GTN, 1,2-GDN and 1,3-GDN following GTN and 1,2-GDN infusions in dog #12.

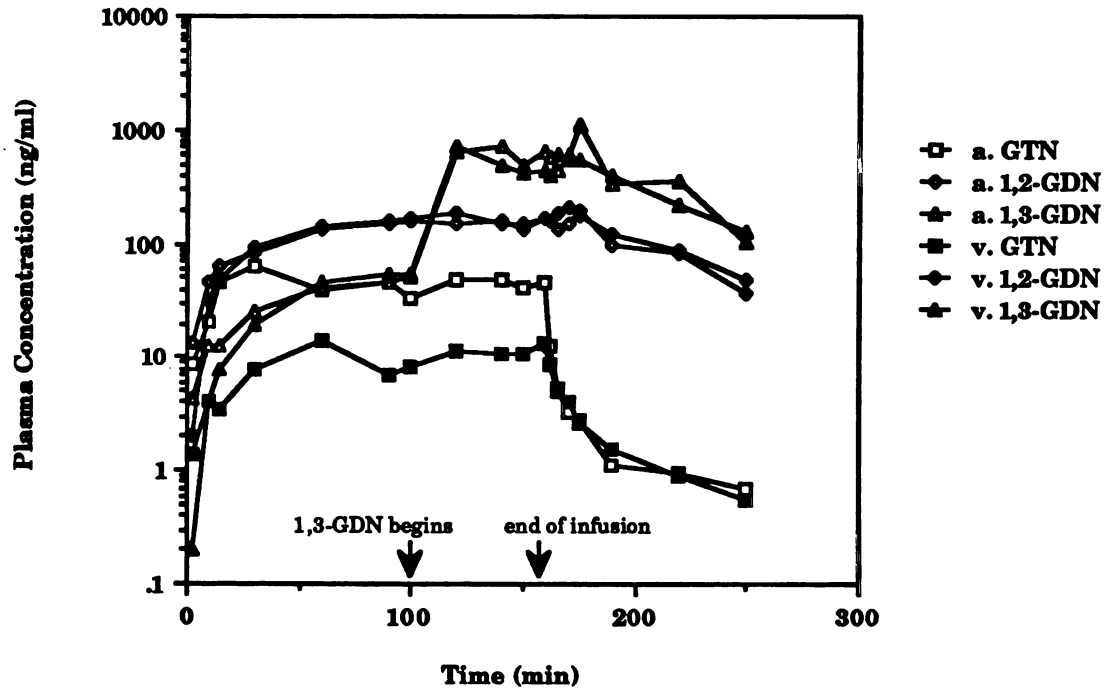

Figure V-12: Arterial and venous plasma levels of GTN, 1,2-GDN and 1,3-GDN following GTN and 1,3-GDN infusions in dog #12.

Table V-3: Clearance (L/min/kg) of GTN following intravenous infusions of GTN in conscious dogs with and without concomitant GDN infusions

		GTN Control		GTN with 1,2-GDN	
Dog#	B.W. (kg)	Arterial	Venous	Arterial	Venous
4	29	0.113	0.259	0.105	0.295
7	27	0.126	0.257	0.122	0.239
11	25	0.120	0.524	0.136	0.325
12	17	0.092	0.372	0.090	0.252
Mean		0.113	0.353	0.113	0.278
S.D.		0.015	0.126	0.020	0.040
%CV		13.3	35.7	17.7	14.4
		GTN Control		GTN with 1,3-GDN	
4	29	0.107	0.455	0.126	0.417
7	27	0.099	0.319	0.113	0.264
11	25	0.128	1.02	0.149	0.712
12	17	0.151	0.606	0.128	0.511
Mean		0.121	0.600	0.129	0.476
S.D.		0.023	0.304	0.015	0.187
%CV		19.0	50.7	11.6	39.3

significant difference ($p>0.5$) between control and interaction arterial clearances, whereas venous clearance showed a general trend to decrease (7 out of 8 dogs) after the addition of 1,2-GDN or 1,3-GDN. However, the difference is not significant ($p>0.1$, Student's paired t test). The mean arterial/venous (A/V) GTN level ratios for control and GTN/1,2-GDN (Table V-2) were 3.19 (S.D. = 1.19) and 2.49 (S.D. = 0.40), respectively. The mean A/V ratios for control and GTN/1,3-GDN (Table V-2) were 4.87 (S.D. = 2.12) and 3.62 (S.D. = 1.05), respectively. The mean A/V ratios also exhibited a trend to decrease when control groups were compared with interaction groups, however, the difference is not significant ($p>0.1$, Student's paired t test).

During the GTN infusion, following the simultaneous iv bolus and infusion dose administration of 1,2-GDN to dogs, the arterial plasma levels of 1,2-GDN at 120, 140 150 and 160 min ranged from 294 to 779 ng/ml, whereas the venous levels ranged from 318 to 789 ng/ml. When iv bolus and infusion doses of 1,3-GDN were given simultaneously during GTN infusion to dogs, the arterial plasma levels of 1,3-GDN ranged from 145 to 703 ng/mg, whereas the venous levels ranged from 226 to 723 ng/ml. The addition of the 1,2-GDN bolus dose and infusion during the GTN infusion did not affect the 1,3-GDN plasma levels and vice versa (Figures V-5 to V-12). In contrast to GTN, the GDNs did not show a clear A/V difference at steady-state (Table V-4). This is probably due to the fact that little GDN metabolism takes place in the tissues. Therefore, little difference would be seen for a GDN infusion alone. When GTN is given concomitantly, the metabolism of GTN in the tissues (i.e., A/V difference) would only result in a 7% increase in GDN concentrations. This is probably too small a difference to detect. However, in 12 of 16 1,2-GDN measurements (Table V-4) venous levels did exceed arterial concentrations. This occurred for only 9 of the 16 1,3-GDN measurements. The A/V differences observed for GDNs at the beginning of the infusions and post infusions are most likely the result of blood vessel cell membrane adsorption and release of GDNs.

Table V-4: Arterial (A) and venous (V) plasma concentrations (ng/ml) of the dosed GDN following an iv bolus dose (5.14 mg) and infusion dose (100 μg/min) of the GDN[a] to conscious dogs during the course of a GTN infusion

Time	Dog #4		Dog #7		Dog #11		Dog #12	
(min)	A	V	A	V	A	V	A	V
1,2-GDN								
120	394	450	375	404	302	374	642	734
140	367	367	408	421	294	368	656	789
150	364	328	300	348	313	372	676	765
160	408	318	343	402	367	387	779	626
Mean	383	366	356	394	319	375	688	729
S.D.	21	60	46	32	33	8	62	72
1,3-GDN								
120	341	453	278	226	308	335	627	723
140	378	471	281	259	327	318	803	483
150	314	459	276	290	145	342	500	416
160	388	443	333	313	266	335	659	443
Mean	355	456	292	272	262	333	622	516
S.D.	34	12	27	38	82	10	87	141

[a] GDN doses were given at 100 min after GTN infusion begun.

During the GTN infusion (100 μg/min), a steady-state systolic blood pressure was reached at about 60 min with an average net decrease of 40 mm Hg. No significant effect on diastolic blood pressure was observed. Addition of 1,2-GDN or 1,3-GDN bolus and infusion doses at 100 min during the course of the GTN infusion caused only minor or no changes in systolic blood pressure (Figures V-13 and V-14).

DISCUSSION

The results from the design A experiments (GTN iv bolus with and without concomitant GDN infusions) indicate no significant difference in GTN AUC and clearance as a result of GDN infusions (Table V-1). However, large intra- and inter-dog variations in AUC were observed. This variation could be due to missing a number of early and later blood samples. Since GTN exhibits a rapid distribution, extensive metabolism, and a large volume of distribution (DiCarlo et al., 1968; Fung et al., 1984a; Noonan et al., 1985; Cossum et al., 1986; and Chapter III in this thesis) the GTN plasma levels at early time points are essential in obtaining an accurate total AUC and the levels at later time points are important in estimating the area under the moment curve (AUMC) which in turn can be used to calculate the apparent volume of distribution at steady-state (Vapp,ss). Nitroglycerin clearance between dogs was quite different, as has also been reported in humans (Armstrong et al., 1982). The results from the design A experiments demonstrate that there was no dramatic inhibition of nitroglycerin clearance by the dinitrate metabolites; however, the limited number of dogs studied and the many variables involved make it difficult to conclude that metabolite inhibitory effects on nitroglycerin pharmacokinetics and pharmacodynamics were not present to some degree.

Design B experiments (GTN infusions before and after concomitant GDN infusions) were performed consecutively on the same day, in the same dog. Therefore, within dog (between

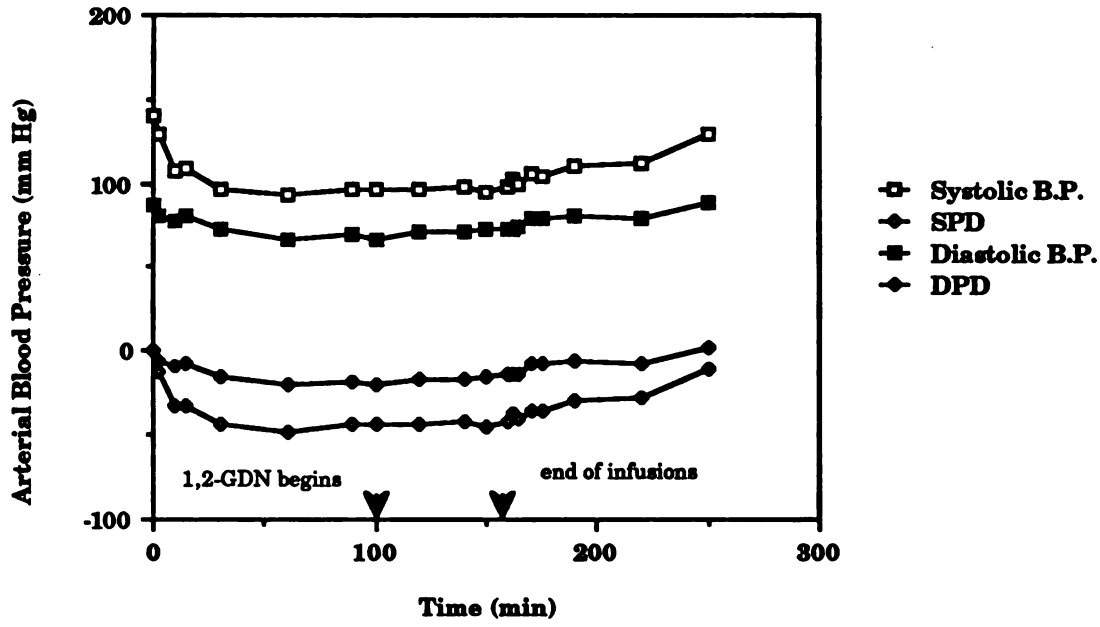

Figure V-13: Arterial blood pressure and blood pressure changes following GTN and 1,2-GDN doses to dog #4. 1,2-GDN infusion (100 μg/min) and bolus (5.14 mg) doses were given simultaneously at 100 min concomitantly with a GTN infusion (100 μg/min) begun at time zero.

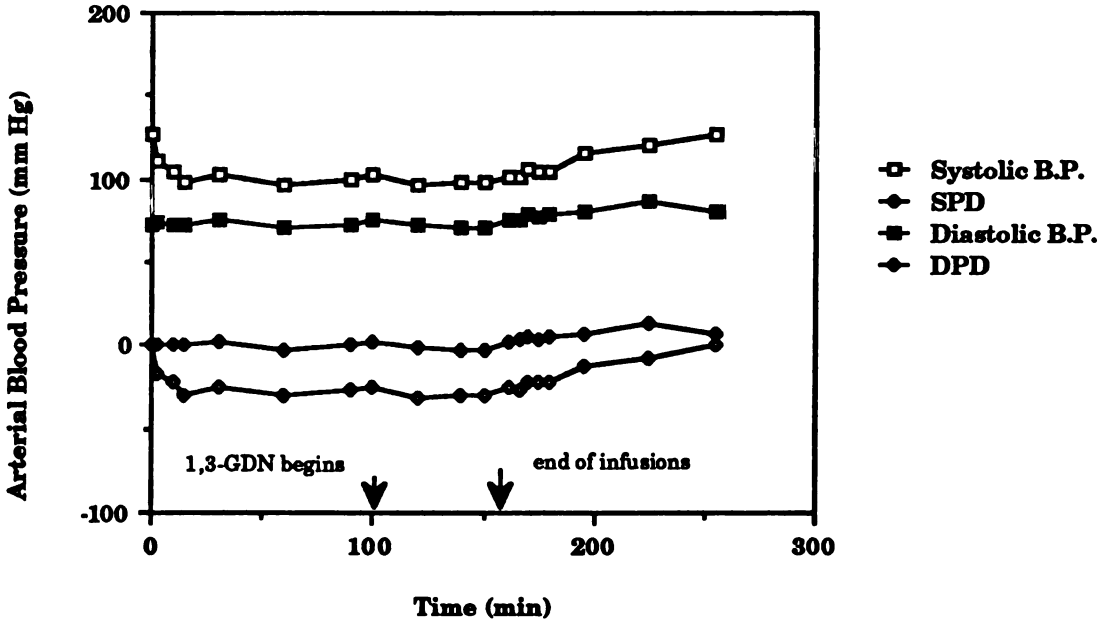

Figure V-14: Arterial blood pressure and blood pressure changes following GTN and 1,3-GDN doses to dog #4. 1,3-GDN infusion (100 μg/min) and bolus (5.14 mg) doses were given simultaneously at 100 min concomitantly with a GTN infusion (100 μg/min) begun at time zero.

days) variation was eliminated. The unchanged GTN arterial plasma levels and clearances after co-administration of GTN/1,2-GDN and GTN/1,3-GDN, which was also observed by Cossum et al. (1986), indicate that the systemic clearance, an integrated clearance of different organs (i.e., lung, liver, blood and muscle, etc.), was not affected . The GTN venous plasma levels, A/V ratios and clearances after co-administration of GTN/1,2-GDN and GTN/1,3-GDN showed a trend to decrease, however, the decrease is not statistically significant. It is possible that the dose level we studied was approaching the range of metabolite inhibition in the dog hind leg. However, these steady-state GDN levels in dogs are much higher than that observed when a therapeutic dose of GTN was given (Chapters III and IV). Therefore, during chronic therapeutic GTN dosing, dinitrate metabolite plasma levels may not be high enough to inhibit the metabolism of GTN, if the results in dogs can be extrapolated to man.

The results of these dog studies are contrary to those reported by Sutton and Fung (1984) and Morrison and Fung (1984) where isosorbide mononitrate (ISMN) inhibited the metabolism of isosorbide dinitrate (ISDN) in rats, and the report of Cossum et al. (1986) where the dinitrates significantly impaired GTN metabolism across the hind leg in anesthetized sheep. It is interesting to note that ISMN inhibition of ISDN metabolism in rats was injection site specific as reported by Morrison and Fung(1984). When 5-ISMN was injected at the same site as ISDN there was an increased AUC of ISDN, whereas the AUC of ISDN was unchanged when 5-ISMN was given at different site. It is also interesting to note that the Vapp,ss of ISDN decreased after co-administration of ISMNs. It is quite possible that the ISMNs perturb the uptake of ISDN and replace ISDN from blood vessels since blood vessel uptake of organic nitrates has been well demonstrated in rats by Fung et al. (1984b). In our design A experiments the experimental procedures were similar to those of Morrison and Fung (1984) except that the GTN and GDN doses were co-administered into the front leg vein and venous blood samples were collected from a femoral vein, whereas in the ISDN and

ISMN interaction studies carried out by Morrison and Fung (1984) the ISDN and ISMNs were co-administered into and then collected from the jugular vein. Sheep studies reported by Cossum et al. (1986) demonstrated a substantial blood vessel uptake of GDNs after GTN infusion in sheep for 40 min, where they observed higher GDN levels in artery than in vein during the GTN infusions and higher venous levels of GDN during the post infusion phase. In our dog studies GDN arterial plasma levels were initially higher than venous concentrations at the beginning of the GTN infusions, but venous and arterial levels rapidly became equal. Arterial GDN plasma levels were lower than venous concentrations during the post-infusion phase (Figures V-5 to V-12). If GDNs can perturb the blood vessel uptake of GTN it would be expected that the femoral venous GTN levels would increase during the co-administration of GTN and GDNs and the GTN levels in vein would be higher than that in artery during the post infusion phase as was observed in the sheep studies. These different results between our dog studies and other animal studies could be attributed to either species differences, the effect of anesthesia or the site of dose injection. In the sheep studies the GDN doses were administered into the aorta whereas in our dog studies the GDN doses were infused into a front leg vein. Nevertheless, based on the discussion above the hysteretic response observed in healthy volunteers for the Css versus infusion rate curve after multiple intravenous infusions as reported by Noonan et al. (1985) is most likely due to saturable binding (adsorption) of nitroglycerin to blood vessels, not to an end-product inhibitory effect on nitroglycerin metabolism.

The hemodynamic response results show that GTN is considerably more potent than 1,2-GDN and 1,3-GDN. The steady-state systolic blood pressure during glyceryl dinitrate infusions could be further reduced by dosing GTN; however, the steady-state systolic blood pressure decrease caused by GTN could not be further reduced by the GDN infusions. We conclude that there was no glyceryl dinitrate inhibition of nitroglycerin metabolism or hemodynamics at the dose levels studied here.

CHAPTER VI

EFFECTS OF AGE ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF NITROGLYCERIN IN DOGS

INTRODUCTION

Nitroglycerin is often used to treat patients with angina pectoris and congestive heart failure. Most patients having these diseases are aged and the physiological condition of their bodies are different from those of young individuals. Hence, elderly patients may respond differently to GTN as compared to young people. Age-dependent alterations in the volume of distribution and total body clearance for several drugs in humans (Ludwig et al., 1983; Owen et al., 1983; Roux et al., 1983) and animals (Kapetanovic et al., 1982a and 1982b; Schmucker, 1985; Tsang and Wilkinson, 1982) have been reported. In the current study we compared the pharmacokinetic and hemodynamic responses to GTN in old and young dogs.

EXPERIMENTAL

Nitroglycerin

Nitroglycerin (Tridil, IV, 5mg/ml) was purchased from Du Pont Critical Care (McGaw Park, IL) and diluted to a lower concentration with normal saline before administration to dogs.

Animals

Four young (< 3 years) and four old (> 8 years) mongrel dogs were prepared with chronic indwelling catheters in the femoral artery and vein. The detailed surgical procedures were described in Chapter III.

Experimental Protocol

Food was withheld from the dogs overnight and throughout each study. Water was available ad libitum but not during the studies. Dogs were given a single 0.1 mg/kg intravenous bolus dose of GTN through a front leg vein. Blood samples (5ml per sample) were drawn from the femoral vein catheter at 0, 1, 2, 3, 4, 5, 7, 10, 15, 20, 60, 90, 120, 150 and 180 min following dose administration. The blood volume loss due to blood sampling was replaced with normal saline following each blood sample withdrawal. Plasma was separated from red blood cells immediately after blood collection. Centrifugation, transfer of plasma and freezing of the plasma with dry ice were completed within two minutes to prevent blood mediated GTN decomposition. The capillary GC-ECD method described in Chapter II was used to assay the plasma samples.

Hemodynamic Measurements

Arterial blood pressure was measured using a Gould Statham model p23db pressure transducer (Oxnard, CA) connected to a Grass model 7D polygraph (Quincy, MA). A Buxco model CVA-1 cardiovascular analyzer module was used to derive heart rate and systolic, diastolic, mean and pulse pressures from the arterial pressure signal. Monitoring of cardiovascular pressures was accomplished using an on-line, microcomputer based data acquisition system. Typically, data from the arterial pressure channel on the Grass

polygraph and the Buxco cardiovascular analyzer were collected every second for the duration of the experiment and reduced to one minute averages. The dogs were allowed 30 min to become accustomed to the surroundings. Arterial pressure was then monitored for another 30 min as the control period.

RESULTS AND DISCUSSION

A. Pharmacokinetics

1. Area Under Curve (AUC)

Plasma concentrations of GTN, 1,2-GDN and 1,3-GDN versus time plots are presented in Figures VI-1, VI-2 and VI-3, respectively. The AUCs for GTN, 1,2-GDN and 1,3-GDN after GTN administration were calculated using the log-trapezoidal method. Mean values and standard deviations for the old and young dog groups are listed in Table VI-1. There was no marked difference between old and young dog groups when the mean AUCs of GTN, 1,2-GDN and 1,3-GDN were compared.

2. Apparent Clearance (CLapp)

The apparent clearance of GTN was calculated as dose divided by area under curve (AUC). Apparent is used to describe clearance since the calculation is made using venous plasma concentrations. Therefore, the first pass metabolism of GTN in the lung and blood flowing through the lung is included in the clearance term. Furthermore, data in man and our work in dogs (Chapter III) indicate that GTN clearance may contain saturable components, thus apparent clearance is used even though identical doses were administered to all animals.

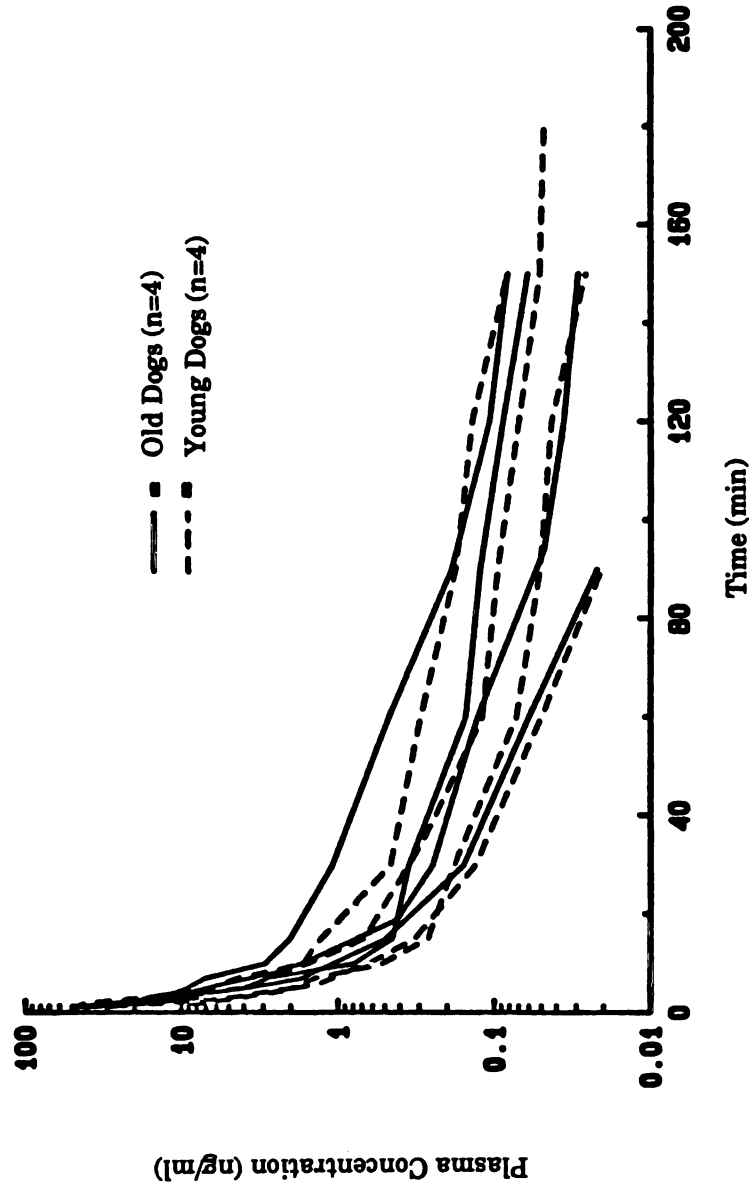

Figure VI-1: Plasma concentrations of GTN in dogs following 0.1 mg/kg GTN iv bolus doses

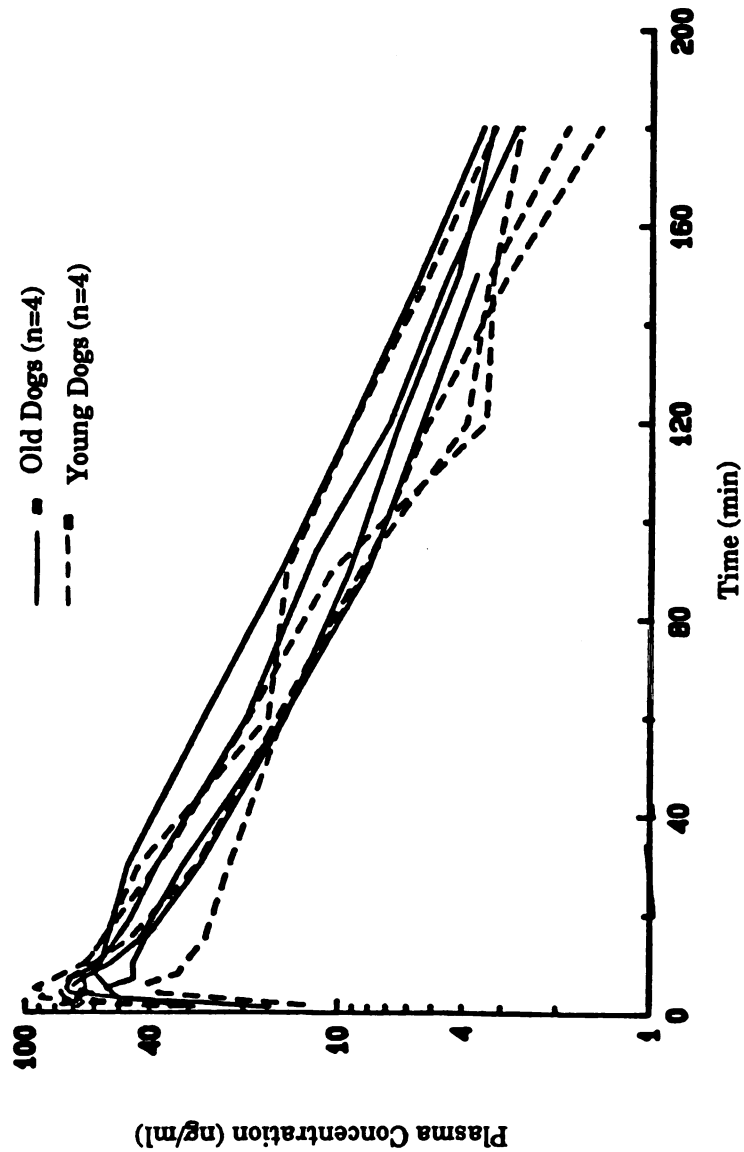

Figure VI-2: Plasma concentrations of 1,2-GDN in dogs following 0.1 mg/kg GTN iv bolus doses.

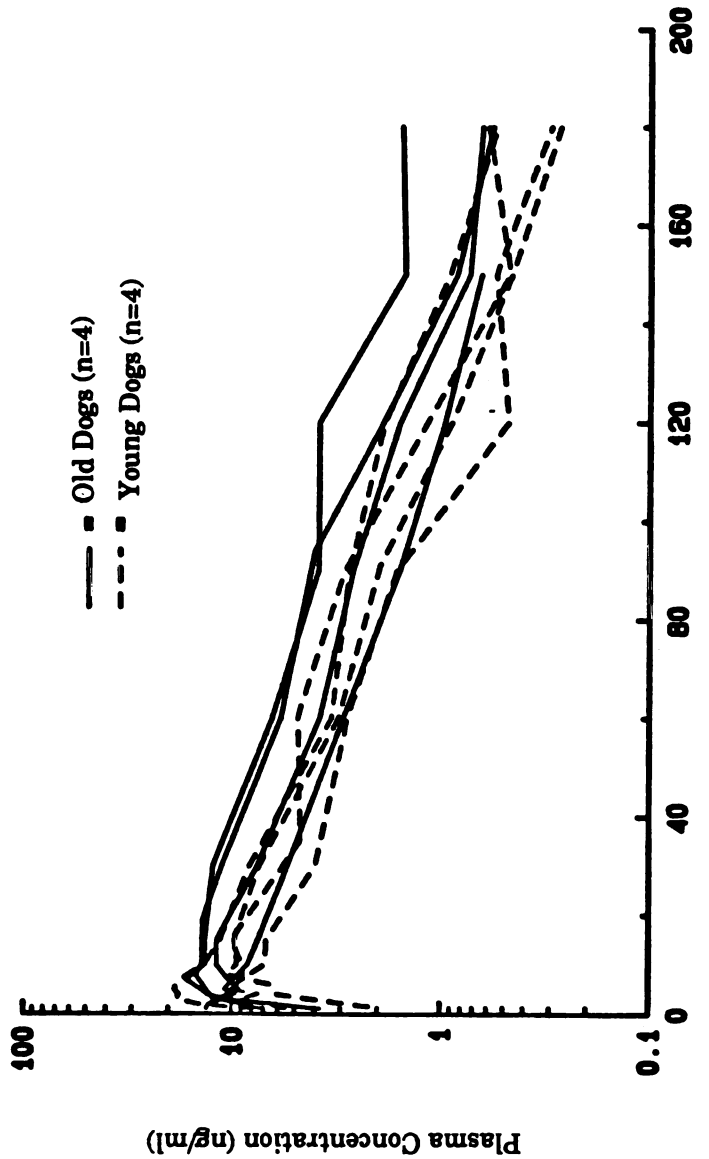

Figure VI-3: Plasma concentrations of 1,3-GDN in dogs following 0.1 mg/kg GTN iv bolus doses.

Table VI-1: GTN pharmacokinetic parameters in old and young dogs following 0.1 mg/kg iv bolus doses

	Old Dogs		Young Dogs	
	Mean	S.D.	Mean	S.D.
		AUC (ng*min/ml)		
GTN	160	37	123	74
1,2-GDN	3370	590	3090	75
1,3-GDN	836	276	592	142
		CLapp (L/min/kg)		
GTN	0.652	0.161	1.08	0.64
		Vapp,ss (L/kg)		
GTN	9.66	4.60	16.2	5.5

In old and young dogs, Clapp was 0.652 ± 0.161 L/min/kg and 1.08 ± 0.64 L/min/kg, respectively (Table VI-1). Apparent clearance in the old dogs seemed lower than that obtained in young dogs but the high variation observed in the young dog group precluded attainment of any statistical significance.

3. Apparent Volume of Distribution at Steady-State (Vapp,ss)

Vapp,ss was calculated using the noncompartmental moment method described by Benet and Galeazzi(1979):

$$Vapp,ss = Dose * (AUMC) / (AUC)^2$$

Vapp,ss was calculated as 9.66 ± 4.60 L/kg and 16.2 ± 5.5 L/kg for the old and young dogs, respectively (Table VI-1). As with value for clearance , the mean Vapp,ss in old dogs was less than that obtained in young dogs, but again the high standard deviation in both groups do not accord any significance to this difference.

B. Hemodynamic Responses

1. Systolic Blood Pressure Decrease (SPD)

Mean values of SPD versus time plots for the young and old dog groups are illustrated in Figure VI-4. In the old dogs the mean (± standard deviation) peak decrease (Dmax), time to the peak (Tmax) and area under the pharmacodynamic response curve (AUPRC) were 60 ± 24 mm Hg, 30 ± 18 sec and 1050 ± 660 mm Hg * min, respectively, whereas in the young dog group the mean values of Dmax, Tmax and AUPRC were 56 ± 24 mm Hg, 43 ± 44 sec and 798 ± 290 mm Hg * min, respectively (Table VI-2). Comparison of Dmax, Tmax and AUPRC

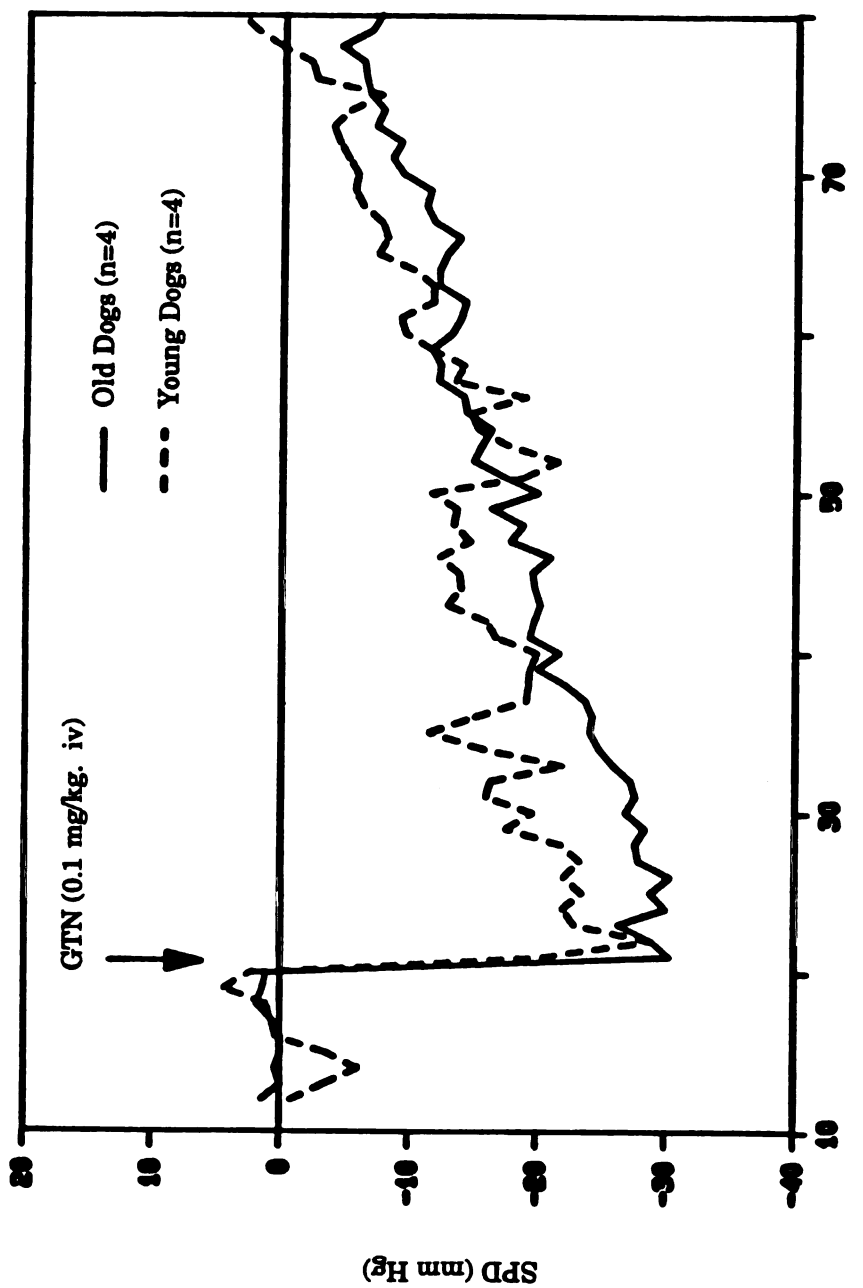

Figure VI-4: Systolic blood pressure decreases following GTN iv dosing (0.1 mg/kg) in conscious dogs.

Table VI-2: Hemodynamic responses to iv GTN (0.1 mg/kg) in conscious dogs

		Old Dogs			Young Dogs		
		Dmax (mm Hg) or (bpm)	Tmax (sec)	AUPRC (mm Hg*min) or (bpm*min)	Dmax (mm Hg) or (bpm)	Tmax (sec)	AUPRC (mm Hg*min) or (bpm*min)
SBP	Mean	61	30	1050	56	43	798
	S.D.	24	18	660	24	44	290
MBP	Mean	37	31	230	38	21	192
	S.D.	6	9	98	4	4	60
HRC	Mean	146	30	823	134	43	273
	S.D.	27	1	454	9	13	187

indicated that GTN did not depress systolic blood pressure differently between these two groups. Although AUPRC in the old dogs may be higher than young dogs, the large standard deviations in these two groups makes any conclusion impossible.

2. Mean Blood Pressure Decrease (MPD)

The old dogs exhibited an average peak decrease of 37 ± 12 mm Hg, while the young dogs yielded a mean value of 38 ± 8 mm Hg. Times to the peak decrease were 31 ± 18 sec and 21 ± 8 sec in old and young dogs, respectively. AUPRCs for MPD were 230 ± 196 mm Hg * min and 192±12 mm Hg * min in the old and young dogs, respectively. Figure VI-5 presents the mean blood pressure decrease versus time plots. There were no differences between old and young dogs, in terms of any of these parameters.

3. Heart Rate Change (HRC)

Both old and young dogs exhibited a substantial heart rate increase after nitroglycerin dose administration. In the old dog group the peak increase (mean ± SD = 146 ± 54 bpm) was observed at 30 ± 2 sec after GTN administration, yielding an AUPRC of 823 ± 908 bpm * min. In the young dog group the peak increase (134 ± 18 bpm) was observed at 43 ± 26 sec after GTN administration giving an AUPRC of 273 ± 374 bpm * min. Heart rate change as a function of time is illustrated in Figure VI-6. The responses in peak increase and Tmax were very similar between old and young dogs. The mean AUPRC in the old dog group was about 3 times greater than the mean obtained in the young dog group since the duration of the heart rate increase in the old dogs was longer than that in young dogs. However, the high standard deviations observed in each group make this conclusion uncertain.

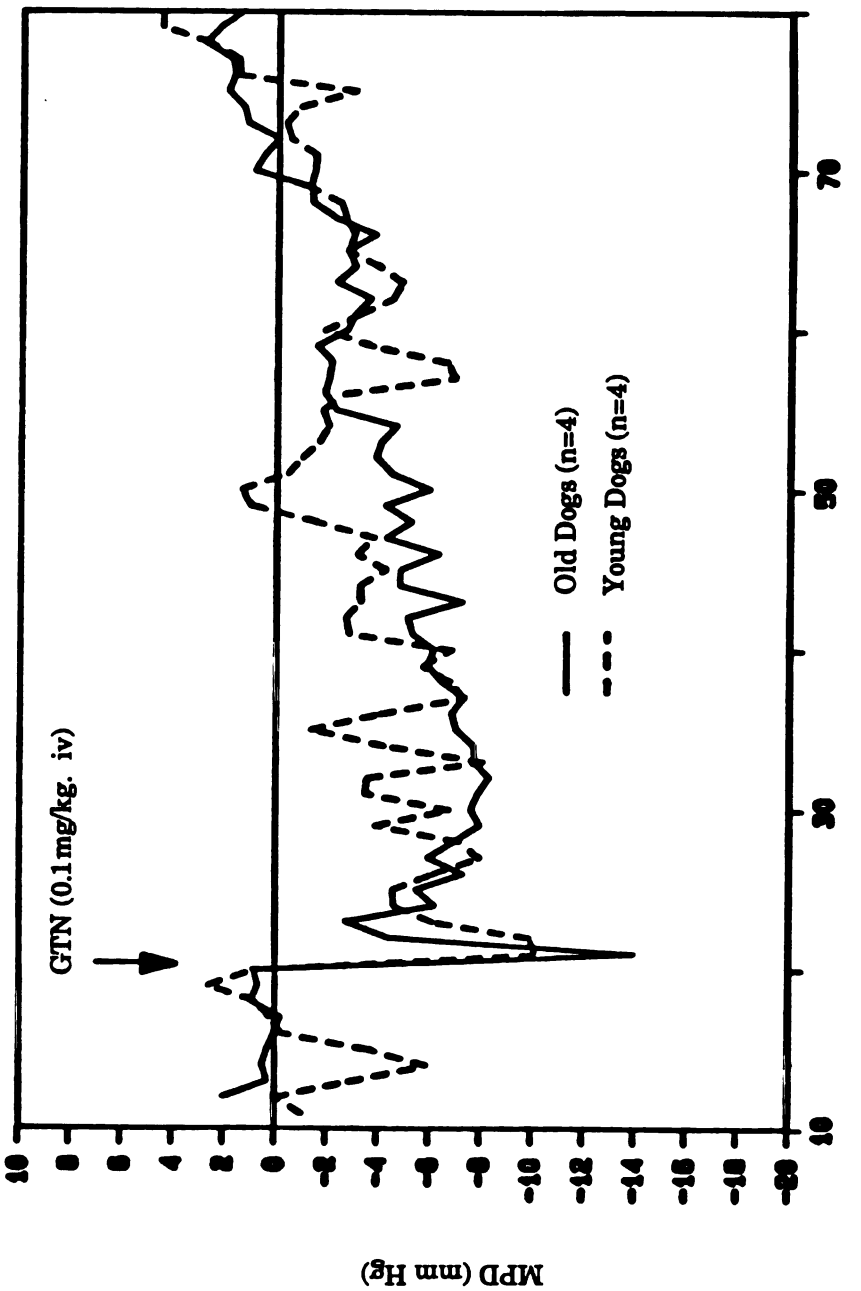

Figure VI-5: Mean blood pressure decreases following GTN iv dosing (0.1 mg/kg) in conscious dogs.

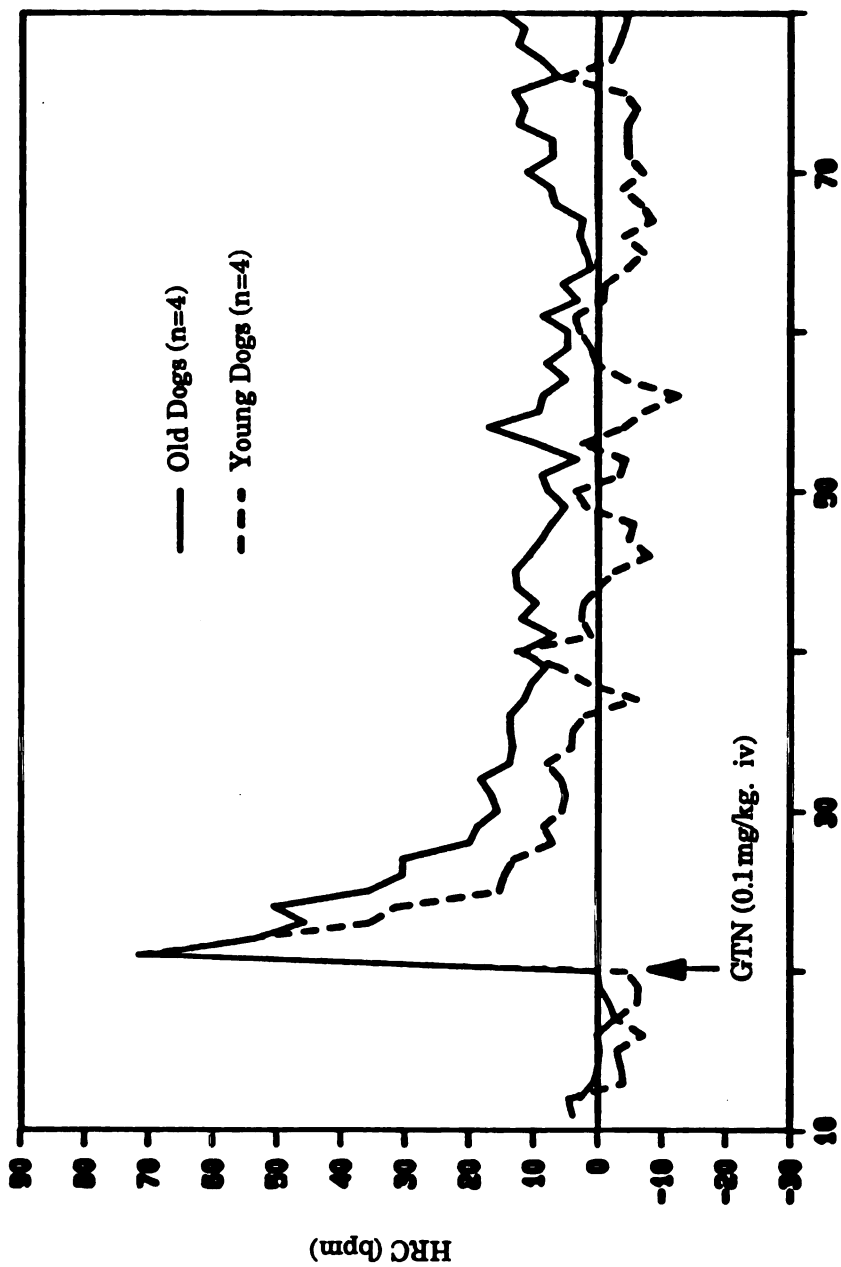

Figure VI-6: Heart rate changes following GTN iv dosing (0.1 mg/kg) in conscious dogs.

It is possible that the age span of the dogs may not have been large enough to observe any changes in pharmacokinetics and hemodynamics between the old and young dog groups. Since mongrel dogs, rather than pure bred dogs were studied, genetic factors might also contribute to the large variabilities observed. Therefore, a pure bred geriatric dog group (> 15 years) may be a more suitable model to consider.

CONCLUSIONS

In these studies no marked differences in either GTN pharmacokinetics or hemodynamic responses were observed when old and young dogs were compared at a GTN dose of 0.1 mg/kg. Since the inter- and intra-dog variabilities are very large, any subtle changes in pharmacokinetics or hemodynamics due to age would be very difficult to be detected.

CHAPTER VII

SUMMARY

Assay Method

A simple, sensitive gas chromatography-electron capture detection (GC-ECD) method which is capable of simultaneously determining the antianginal drug nitroglycerin and its dinitrate metabolites was developed. This method has many advantages compared to other reported methods. Its detection limits for GTN, 1,2-GDN and 1,3-GDN in plasma are 0.025, 0.1 and 0.1 ng/ml, respectively, which allows quantitation of the low levels of nitroglycerin and its dinitrate metabolites observed following therapeutic doses of nitroglycerin, as well as GDN levels after GDN doses to dogs. This method does not require two separate assays to measure GTN and GDN plasma levels as reported by Noonan et al. (1985) and Sioufi et al. (1987). The methylene chloride/pentane (3/7) solvent system employed in this method extracts less contaminants from plasma than other solvent systems, resulting in cleaner chromatograms and prolonged column life. This method was used to measure GTN, 1,2-GDN and 1,3-GDN plasma levels in all of the dog studies described here.

Pharmacokinetics

Remarkable variability in nitroglycerin pharmacokinetics and pharmacodynamics was observed in all of the dog studies. Biphasic decline were observed for nitroglycerin plasma levels following the iv bolus doses and after the cessation of infusion doses of GTN. The rapid decline phase exhibited a short half-life of about 4 min and represents the majority of the total AUC. The slow decline phase exhibited a longer half-life (>18 min) and only represents a small portion of the total AUC (<20%) after the low 0.025 mg/kg iv GTN dose.

Therefore, the short half-life is more significant and represents the great majority of the GTN clearance. However, for the high 0.25 mg/kg iv dose, the AUC corresponding to the longer half-life approached 40% of total AUC, a value which may not be insignificant in terms of either pharmacokinetics or pharmacodynamics. The importance of this longer half-life became obvious in attempting to reach steady-state conditions during infusion dosing. Here GTN steady-state plasma levels were not reached until about 60 min following intravenous infusions of GTN instead of 12-16 min (3-4 half-lives) as might be expected if the short half-life predominates. The apparent clearance of nitroglycerin in dogs is greater than the cardiac output and is much greater than the liver blood flow. Such high clearance can be attributed to extensive GTN tissue (i.e., liver, lung, muscle, blood vessels and blood, etc.) metabolism and uptake. The formation of 1,2-GDN and 1,3-GDN was rapid and extensive and the metabolites reached levels much higher than GTN after iv bolus, infusion and oral doses of GTN. Especially for oral GTN doses, the drug was extensively degraded to GDNs as a result of gastrointestinal and hepatic first-pass metabolism and only 1% of the drug was bioavailable. The GTN plasma levels declined rapidly and were not detectable after 15 min post-dose. Therefore, the measurement of GDNs is important in studying the pharmacokinetics and pharmacodynamics of GTN.

Apparent dose dependent pharmacokinetics of GTN were observed with CLapp decreasing following the 0.25 mg/kg iv bolus dose as compared to the low 0.025 mg/kg iv bolus dose and to the graded infusion doses (10, 30, 50, and 70 μg/min) in dogs #4, #5, and #8. These three dogs were used in all 6 studies. There was no significant difference between apparent clearances following low iv bolus doses and graded GTN infusions. The apparent clearance change seems not to be due to metabolite inhibition as Cossum et al. (1986) suggested, since in our studies no significant arterial and venous GTN clearance changes were observed after co-administration of GTN and GDN to dogs. The GDN plasma levels maintained in these interaction studies were more than two fold higher than the GDN Cmax obtained after the

high bolus GTN dose (0.25 mg/kg). Thus, the changes in apparent GTN clearance are most likely due to concentration dependent tissue uptake, saturable blood metabolism and/or a blood flow decrease. The results of our dog studies are contrary to those reported by Sutton and Fung (1984) and Morrison and Fung (1984) where isosorbide mononitrate inhibited the metabolism of isosorbide dinitrate in rats and the report of Cossum et al. (1986) where the glyceryl dinitrates significantly impaired GTN metabolism across the hind leg in anesthetized sheep. The metabolite inhibitory effects they observed are most likely due to perturbation of GTN or ISDN tissue uptake and replacement of the parent drug by GDNs or ISMNs.

The pattern of GTN metabolism changes as a function of the GTN dose as reflected in the ratio of 1,2-GDN to 1,3-GDN metabolites formed (i.e., 8.44 ± 1.94 for low iv bolus GTN doses and 3.41 ± 0.90 for high iv bolus GTN doses). This is reflected in the changes of apparent fractional clearances of GTN to 1,2-GDN and 1,3-GDN. The calculated clearances of GTN to form 1,2-GDN (CLm1) were similar for the four different GTN infusion rate and the low 0.025 mg/kg intravenous bolus GTN administration; however, CLm1 was significantly lower following the high 0.25 mg/kg intravenous bolus GTN dose ($p<0.02$, one-way ANOVA). There was no significant difference between the calculated clearances of GTN to form 1,3-GDN (CLm2) following low and high iv GTN bolus doses and the four different infusion rates. Changes in the 1,2-GDN/1,3-GDN ratio were also observed when the drug was given via different routes of administration (i.e., 1.61 ± 0.19 for oral doses and 5.78 ± 1.23 for infusion doses). It appears that the major portion of the nonlinear change in GTN clearance may result from the nonlinear change in the clearance of GTN to 1,2-GDN. The marked change in the 1,2-GDN/1,3-GDN ratio when the GTN dose was given orally suggests the possibility of different enzyme specificity for GTN metabolism to GDNs in different tissues.

Following low and high intravenous bolus GTN doses, 76±19% and 86±21% of the dose may be accounted for, respectively, in terms of measurable dinitrate metabolites in the systemic circulation. The unexplained residual fractions of the GTN dose are most likely the result of sequential metabolism whereby the GDNs are further metabolized to glucuronides, GMNs and glycerol in the various eliminating organs before reentering the systemic circulation. Following oral GTN doses, 70±29% of the GTN dose may be accounted for in terms of measurable dinitrate metabolites in the systemic circulation. However, considering that the bioavailability of orally dosed GDNs is 0.626±0.220 for 1,2-GDN and 0.680±0.115 for 1,3-GDN and realizing that less than 1% of an oral GTN dose is available, one may consider that the GDNs formed via oral GTN dosing would not be completely available. Under these conditions 106 ± 18% of the GTN dose can be accounted for in terms of dinitrate metabolites. It appears that the residual 30% of the oral GTN dose was consecutively metabolized in the GI tract and liver. All of the GTN dose at steady-state during the GTN infusions can be accounted for by measured GDNs.

There was no apparent dose dependency of GTN within the infusion range studied (10-70 μg/min). Although only in one of four dogs did GTN Css increase proportionately with infusion rate, a linear relationship between Css of GTN and infusion rate was observed over all four dogs.

The GDNs exhibited much lower apparent clearances and volumes of distribution, greater bioavailability and considerably longer half-lives than that observed for nitroglycerin. The pharmacokinetic parameters obtained for 1,2-GDN and 1,3-GDN following iv bolus doses (0.25 mg/kg) of 1,2-GDN and 1,3-GDN were similar for both compounds. Following iv infusions (20 and 100 μg/min), the pharmacokinetics of 1,2-GDN and 1,3-GDN were consistent and linear over the dose range, however, significantly higher clearances were

observed as compared with the apparent clearances determined after the 0.25mg/kg iv bolus GDN dose indicating possible dose dependent pharmacokinetics for the GDNs.

There were no marked pharmacokinetic differences between young and old dogs in terms of GTN AUC, clearance, volume of distribution and GDNs AUCs after a 0.1 mg/kg GTN iv bolus dose was given to these two groups of dogs.

Pharmacodynamics

The change in systolic blood pressure was the most marked hemodynamic response observed when GTN and GDNs were administered to dogs. Nitroglycerin exhibited a more rapid onset (Tmax), higher response (Dmax) and shorter duration than those for GDNs in terms of the systolic blood pressure decrease (SPD). The pharmacokinetic/pharmacodynamic equilibration time for GTN in the dogs was extremely short (< 1 min), whereas the equilibration time for the GDNs was about 6min. Comparing maximum net systolic blood pressure decreases (Dmax) after intravenous doses, nitroglycerin is about 10 and 12 times more potent than 1,2-GDN and 1,3-GDN, respectively. However, the durations of the systolic blood pressure decrease caused by 1,2-GDN and 1,3-GDN were more prolonged than that for GTN and yield a larger AUCspd. This prolonged duration correlated well with the larger GDN plasma AUC. When the ratios of AUCspd/Dose are compared, 1,2-GDN was about twice as potent as GTN while 1,3-GDN was about equipotent with GTN. Therefore, comparison of pharmacodynamic potency based on single point efficacy measurements (such as Dmax) can be misleading and it is often better to base the potency comparison on integrated measures of effect.

Oral doses of GTN are pharmacologically active. Following oral GTN dosing, GTN plasma levels fall below the detectable limit 15 min post-dose, however, the SPD did not return to

baseline until 90 min or longer which correlates well with the time course of 1,2-GDN and 1,3-GDN plasma levels. This finding suggests that high oral doses of GTN did not saturate the GI tract and liver first-pass metabolism, allowing intact GTN to enter the systemic circulation and cause the systolic blood pressure decrease. Rather, the high levels of the GDNs play a major role in the systolic blood pressure decrease. Because the formation of 1,2-GDN and 1,3-GDN is rapid and extensive following GTN doses, the contribution of GDNs to GTN pharmacodynamics can not be ignored as we have demonstrated in Chapter IV. Following GTN infusions, the concentrations on the ascending limb of the SPDss vs plasma level plot are lower than those of the descending limb, i.e., reverse hysteresis (Figure IV-10). This phenomenon could be attributed to the formation of the active metabolites 1,2-GDN and 1,3-GDN. Indeed, this reverse hysteresis disappeared after the pharmacodynamic contributions due to 1,2-GDN and 1,3-GDN were subtracted from the total SPDss (Figure IV-17).

The systolic blood pressure decrease at steady-state following 20 μg/min infusions of 1,2-GDN and 1,3-GDN (yielding average Css measurements of 33.2 and 42.7 ng/ml, respectively), were very small. A significant systolic blood pressure decrease was observed after the GDN infusion rate was increased to 100 μg/min yielding average Css of 160 and 204 ng/ml for 1,2-GDN and 1,3-GDN, respectively. Comparing Css values between GTN and GDNs based on comparable SPDss, GTN was about 100 times more potent than 1,2-GDN and 1,3-GDN. However, the dinitrate metabolites formed after the GTN infusion may have partially contributed to the nitroglycerin potency.

In our 1,2-GDN and 1,3-GDN studies, it is interesting to note that based on the ratio AUCspd/AUCpl (Table III-17) the oral 1,2-GDN dose is about 1.5 times more potent than the iv 1,2-GDN dose and the oral 1,3-GDN dose is about 2.7 times more potent than the iv 1,3-GDN dose. These results support the findings reported by Chen et al. (1979;1981) in the

anesthetized cat that the major site of nitroglycerin-induced venous pooling is in the splanchnic circulation. Since orally dosed organic nitrates can reach the action site prior to passing through the liver, even though the organic nitrate levels in the systemic circulation are low due to GI tract and liver first-pass metabolism, the hemodynamic response can be similar to iv doses of organic nitrates.

There were no inhibition of nitroglycerin hemodynamics in dogs by the glyceryl dinitrates at the dose levels studied here. Comparing net systolic blood pressure decrease, net mean blood pressure decrease and net heart rate change, there were no marked differences between young and old dogs following 0.1 mg/kg iv bolus doses of GTN. Since the interdog and intradog variability is very large, any subtle changes in pharmacokinetics or hemodynamics due to age would be very difficult to detect. In addition, the age span may not have been large enough to observe any changes in pharmacokinetics and hemodynamics between the old and young dog groups.

Conclusions

GTN exhibited a shorter half-life, higher apparent clearance and volume of distribution and lower bioavailability than 1,2-GDN and 1,3-GDN. The pharmacokinetic parameters for 1,2-GDN and 1,3-GDN are similar to each other. The formation of GDNs after GTN doses is rapid and extensive.

Apparent dose dependent pharmacokinetics were observed with CLapp decreasing for GTN, 1,2-GDN and 1,3-GDN during the highest dose. The major portion of the nonlinear change in GTN clearance may result from the nonlinear change in the clearance of GTN to 1,2-GDN. The marked change in the 1,2-GDN/1,3-GDN ratio after oral GTN dosing suggests the possibility of different enzyme specificity for GTN metabolism to GDNs in different tissues.

An average 75% of the GTN dose can be accounted for by the measurable dinitrate metabolites in the systemic circulation with the residual fraction probably lost due to sequential metabolism in various eliminating organs.

Comparing Dmax, GTN is about 10-12 times more potent than GDN; however, when AUCspd/Dose is compared, 1,2-GDN is about twice as potent as GTN while 1,3-GDN is about equipotent with GTN. Orally dosed GTN is pharmacologically active, which probably results from the formation of the active dinitrate metabolites.

There is no inhibitory effect of the GDNs on either the pharmacokinetics or pharmacodynamics of GTN. There were no marked differences in pharmacokinetics and pharmacodynamics between young and old dogs.

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