THE VENOUS EFFECTS OF NITRIC OXIDE DONORS AND THE
PHOSPHODIESTERASE TYPE V INHIBITOR, ZAPRINAST

by

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Abstract

It is well-documented that agents which release nitric oxide (NO) or inhibit the degradation of cGMP promote vasodilatation by interacting with the endogenous L-arginine/NO pathway. There are few published *in vivo* studies examining the actions of these agents on the venous circulation. Although the venous system is not as extensively studied as the arterial system, it plays a significant role in the regulation of circulatory homeostasis. By altering the activity of venous smooth muscle, capacitance vessels maintain venous return and cardiac output. Similar to its arterial counterpart, venous smooth muscle activity can be altered passively according to inflow pressure or volume, or actively via reflex- or neurohumoral-mediated mechanisms. Drugs that influence venous smooth muscle tone or reflex control of the venous system have profound effects on venous return, cardiac output and blood pressure. Venodilatation unequivocally contributes to the therapeutic effectiveness of sodium nitroprusside and nitroglycerin in the management of hypertensive emergencies and chronic heart failure, respectively. It should be noted that not all vasodilator drugs dilate capacitance vessels. Hydralazine, for example, is an efficacious arterial dilator which lacks venodilator action. Therefore, better knowledge of the *in vivo* venous actions of vasodilators is essential to improving treatment strategies in cardiovascular pathology.

The current thesis investigated the effects of five nitrovasodilators, diethylamine/nitric oxide (DEA/NO) complex, S-nitroso-N-acetylpenicillamine (SNAP), nitroxy(η^5-cyclopentadienyl)-dinitrosylchromium (CpCr(NO)$_2$(ONO)), sodium nitroprusside (SNP) and nitroglycerin (NTG), as well as the phosphodiesterase type V inhibitor, zaprinast, on mean arterial pressure (MAP), arterial resistance (R_a), cardiac output (CO), heart rate (HR), mean circulatory filling pressure (MCFP) and venous resistance (R_v) in groups of thiobutabarbital-

anaesthetized rats under basal conditions, and in the presence of mecamylamine (3.7 μmol kg$^-$^{1}) and noradrenaline (7.3 nmol kg^{-1} min^{-1}). Mecamylamine and noradrenaline were used to suppress autonomic reflexes and elevate venomotor tone, respectively. This protocol enhances the assessment of the venodilator activity of drugs. Experimentally, MCFP is the mean vascular pressure that would exist following circulatory arrest and instantaneous redistribution of blood throughout the circulation. Thus, MCFP is conceptually the driving force of venous return at the level of the venules. Total body venous resistance (R_v) is best estimated by the ratio of (MCFP − right atrial pressure) to CO. In intact rats, zaprinast (ED$_{40}$ and ED$_{80}$ doses, 1.5, 3.0 mg kg^{-1} min^{-1}, respectively) and SNP (8.0, 64.0 μg kg^{-1} min^{-1}) dose dependently reduced MAP and R_a, but did not alter CO and R_v. Both increased HR, with the effect of zaprinast less than that of SNP. In ganglion-blocked rats with elevated venomotor tone, zaprinast and SNP elicited dose-dependent reductions in MAP, MCFP, R_a and R_v. Both increased CO, with the effect of zaprinast greater than that of SNP at the low dose. Zaprinast but not SNP reduced HR. It was concluded that zaprinast, similar to SNP, dilates both resistance and capacitance vessels in ganglion-blocked rats with restored vasomotor tone. Zaprinast but not SNP has a direct, negative chronotropic effect on the heart.

In the second series of experiments, all the thiobutabarbital-anaesthetized rats were pretreated with mecamylamine (3.7 μmol kg^{-1}, i.v. bolus) and continuously infused with noradrenaline (6.8 nmol kg^{-1} min^{-1}) for the reasons mentioned above. DEA/NO (ED$_{30}$, ED$_{80}$ and ED$_{100}$, 4, 32 and 256 μg kg^{-1} min^{-1}, respectively), SNAP (4, 32 and 256 μg kg^{-1} min^{-1}), CpCr(NO)$_2$(ONO) (4, 32 and 256 μg kg^{-1} min^{-1}), SNP (8, 32 and 128 μg kg^{-1} min^{-1}) and NTG (0.2, 0.8 and 6.4 μg kg^{-1} min^{-1}) caused similar dose-dependent increments in CO. HR was not altered by any of the five nitrovasodilators. All five drugs dose-dependently reduced both MAP and R_a with efficacy: DEA/NO $\approx$ SNAP $\approx$ CpCr(NO)$_2$(ONO) $\approx$ SNP > NTG. DEA/NO,

SNAP, $CpCr(NO)_2(ONO)$ and SNP but not NTG lowered MCFP with efficacy: DEA/NO > SNAP > $CpCr(NO)_2(ONO)$ $\approx$ SNP. All five drugs reduced R_v with efficacy: DEA/NO $\approx$ SNAP $\approx$ $CpCr(NO)_2(ONO)$ $\approx$ SNP > NTG. Therefore, the hypotensive, arterial and venous dilator actions of DEA/NO, SNAP and $CpCr(NO)_2(ONO)$ are comparable to those of SNP but greater than those of NTG. It would be of interest to evaluate the therapeutic potential of these new NO donors as alternatives for SNP and NTG in the management of cardiovascular dysfunction in future studies.

Key words: zaprinast; cGMP-selective phosphodiesterase; NO/nucleophile complexes; S-nitrosothiols; organotransition-metal dinitrosyl complexes; sodium nitroprusside; nitroglycerin; capacitance vessels; mean circulatory filling pressure; venous resistance

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1. Introduction

1.1 L-Arginine/Nitric Oxide Pathway

Furchgott & Zawadzki (1979) first discovered the release of a factor from the vascular endothelium which causes relaxation of the surrounding smooth muscle. The identity of this endothelium-derived relaxing factor (EDRF) remained unclear until two laboratories independently reported that EDRF was analogous to nitric oxide (Ignarro et al., 1987a; Palmer et al., 1987). Nitric oxide (NO) has since been the focus of intensive biomedical research. There is voluminous information in the literature suggesting that NO plays a role in many physiological and pathophysiological processes which include cardiovascular regulation, host defence and immunological reactions, neurotransmission, excitotoxicity, hypertension, myocardial infarction, atherosclerosis, impotence and many others (Moncada et al., 1991).

In the vasculature, it is well-documented that endogenous vasodilators such as acetylcholine and bradykinin (Furchgott, 1984; Cachofeiro et al., 1992), hypoxia (Pohl et al., 1988), pulsatile flow and shear stress (Pohl et al., 1986; Rubanyi et al., 1986) stimulate the constitutive form of nitric oxide synthase (eNOS) in endothelial cells, resulting in the synthesis of NO from the guanido nitrogen atoms of L-arginine. Calcium and a number of cofactors, namely, NADPH, flavin mononucleotide, flavin adenosine dinucleotide, tetrahydrobiopterin and calmodulin are required for and mediate the formation and release of NO (Singer & Peach, 1982; Moncada & Higgs, 1995). The presence of eNOS has been demonstrated in platelets, and the endothelium of conduit arteries, microvessels and veins from many species, including human (Moncada & Higgs, 1995). NO released from endothelial cells diffuses into nearby vascular smooth muscle cells and binds to the heme moiety of soluble guanylate cyclase (Craven & DeRubertis, 1978). The activation of guanylate cyclase results in the formation of

cyclic guanosine monophosphate (cGMP), which is associated with protein kinase G activation (Lincoln & Cornwell, 1993) and altered phosphorylation of various smooth muscle proteins (McDaniel et al., 1993). These biochemical events include decreased phosphorylation of myosin light chain, sequestration of intracellular Ca^{2+}, reduction of Ca^{2+} entry from the extracellular space and of Ca^{2+} release from intracellular stores as well as inhibition of inositol-1,4,5-triphosphate formation (Pfitzer et al., 1984; Collins et al., 1986; Twort & van Breemen, 1988; Lang & Lewis, 1989), any one of which will lead to vascular smooth muscle relaxation (Murad, 1994; Figure 1). The chemical instability (half-life ~ 3-50 s) of NO ensues that its biological effects remain localized. NO is spontaneously converted to nitrate and nitrite in the presence of oxygen, inactivated by oxyhaemoglobin, or destroyed by superoxide anion (Ignarro, 1989). The actions of cGMP are terminated by conversion to 5'GMP by phosphodiesterases (see section 1.3). Under basal conditions, endothelial cells continuously produce and release NO, which is involved in the maintenance of blood flow and pressure. Other physiological, cardiovascular functions of NO include the inhibition of platelet aggregation and adhesion, induction of platelet disaggregation, inhibition of mitogen release from platelets (Barrett et al., 1989) and of proliferation of vascular smooth muscle cells (Cornwell et al., 1994), modulation of myocardial contractility (Brady et al., 1993; Gross et al., 1996; Flesch et al., 1997) and inhibition of renin release (Vidal et al., 1988). In addition to its peripheral actions, NO has also been shown to decrease sympathetic nerve activity and blood pressure by acting on the cardiovascular regulatory centre in the brain (Carbreba & Bohr, 1995).

The differences in the vascular smooth muscle L-arginine/NO pathway of arteries and veins have been widely reported although the results are highly divergent and contradictory.
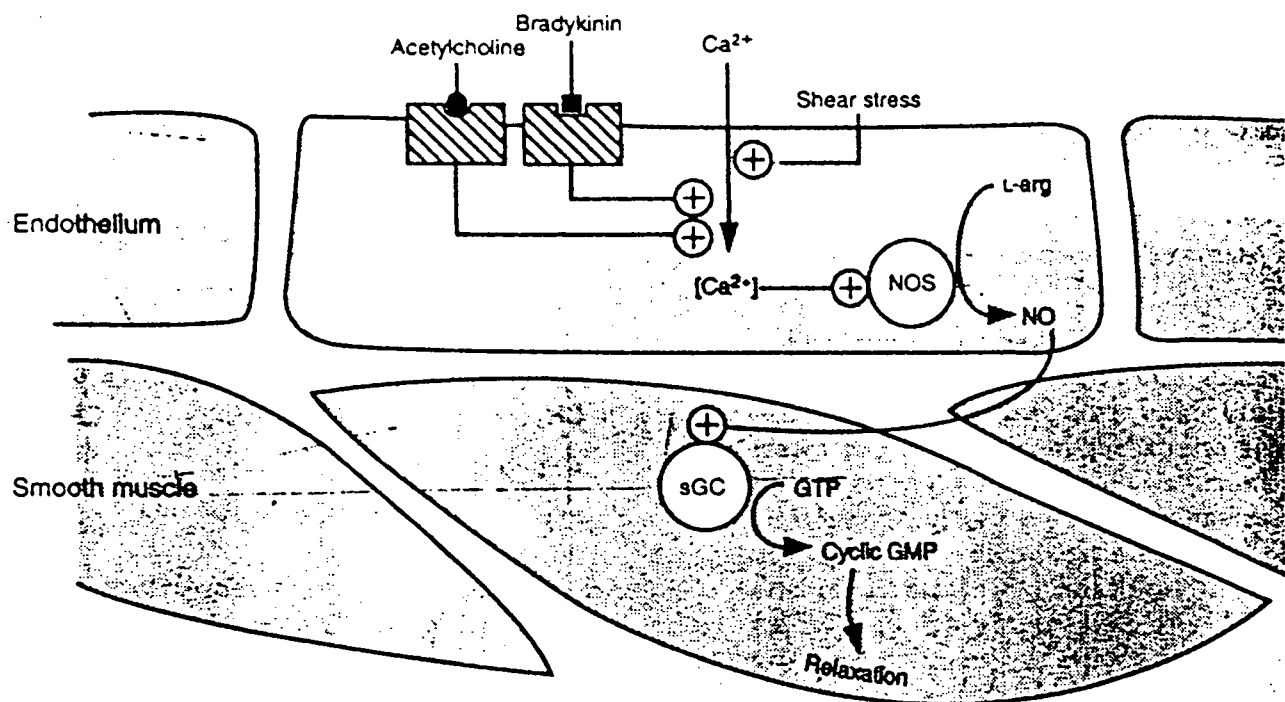
Figure 1   Shear stress or receptor activation of vascular endothelial cells by bradykinin or acetylcholine results in an influx of Ca^{2+}. The consequent increase in intracellular Ca^{2+} stimulates constitutive nitric oxide synthase (eNOS). The nitric oxide (NO) formed from L-arginine (L-arg) then diffuses to nearby smooth muscle cells, in which it stimulates soluble guanylate cyclase (sGC), resulting in enhanced synthesis of cyclic GMP from guanosine triphosphate (GTP). The increase in cyclic GMP in smooth muscle cells leads to vasorelaxation (adapted from Moncada & Higgs, 1993).

For instance, contracted canine saphenous veins dilate less in response to acetylcholine than femoral arteries (Seidel & LaRochelle, 1987). NO release is easily demonstrated from fresh arterial, but not venous tissues (Moncada et al., 1988). Venous tissues (Seidel & LaRochelle, 1987) and the venous circulation of humans *in vivo* (Vallance et al., 1989) were shown to have a lower basal release of NO relative to the arterial side. These observations may be attributed to the lesser ability of the venous endothelium to generate NO or the lesser sensitivity of the venous smooth muscle to NO. In contrast, McGrath et al. (1990) reported that the basal release of NO in veins is more pronounced than in arteries from the same vascular bed, possibly due to the low partial pressure of oxygen in the veins and venules. However, it should be noted that the aforementioned studies are not strictly comparable because different vascular preparations from different species were used. Nonetheless, the intrinsic differences of the arterial and venous L-arginine/NO pathway are likely to contribute in part to the differential responses of resistance and capacitance vessels to exogenous NO donors (section 1.2).

Given the active involvement of NO in circulatory homeostasis, it is apparent that a defect in the L-arginine/NO pathway and the consequent loss or change of NO-mediated dilator tone would lead to cardiovascular dysfunction. Indeed, in experimental models of hypertension, several groups have reported reduced endothelium-dependent relaxation (Winquist et al., 1984; De Mey & Gray, 1985; Luscher et al., 1987; Otsuka et al., 1988), and in some cases accompanied by reduced levels of cGMP (Otsuka et al., 1988). The impairment of endothelium-dependent relaxation has been correlated with the level and duration of hypertension in different strains of spontaneously hypertensive rats (Sunano et al., 1989). The vasodilator response to acetylcholine infusion into the brachial artery is reduced in patients with essential hypertension (Linder et al., 1990; Panza et al., 1990). A reduction in NO release

from the vascular endothelium or a decrease in endothelium-dependent relaxation has been reported in atherosclerotic vascular tissues obtained from rabbits (Coene et al., 1985; Guerra et al., 1989) and in human atherosclerotic coronary arteries (Forstermann et al, 1988). Clinical studies in humans suggested that hypertension is associated with impaired generation of NO (Moncada & Higgs, 1993). Node et al. (1997) reported that the basal concentration of NO in the plasma is reduced in individuals with essential hypertension. Hypertension can produce structural damage in endothelial cells. Decreased NO production might result from abnormal handling of intracellular Ca^{2+} and a consequent reduction in eNOS activity (Dominiczak & Bohr, 1995). Alternatively, increased production of superoxide anions, which rapidly inactivate NO, has been shown to be a characteristic feature of experimental hypertension (Grunfeld et al., 1995; Tschudi et al., 1996). Moreover, damage to the vascular endothelium during percutaneous transluminal coronary angioplasty or arterial and venous coronary bypass results in impaired vasodilation post-operatively. In patients with coronary artery disease, flow-dependent coronary vasodilation is markedly attenuated (Searle & Sahab, 1992). Impairment of NO formation not only predisposes blood vessels to constriction but also favours platelet adhesion, aggregation and the subsequent release of vasoconstrictors that exacerbate the tendency to vasospasm (Moncada et al., 1988). Consequently, the treatment of those diseases in which NO production or function is impaired should involve the direct or indirect delivery of NO from exogenons sources, the stimulation of receptors linked to the L-arginine/NO pathway or the potentiation of the actions of endogenous NO and/or cGMP.

1.2 Nitric Oxide Donors

Nitrovasodilators have been used for many decades in the management of cardiovascular disorders. They all act as prodrugs to release NO after administration (Figure

2). Although the scientific literature reported the existence of at least 30 classes of NO donors, only the organic nitrates (e.g., nitroglycerin, NTG) and nitrites (e.g., amyl nitrite), and sodium nitroprusside (SNP) are in common clinical use (Young, 1997). Nitrates are used to treat stable and unstable angina, coronary vasospasm, myocardial infarction, chronic heart failure, pulmonary hypertension, and in fibrinolytic therapy, percutaneous coronary angioplasty and for complications due to cardiac catheterization (Mondada et al., 1988). The beneficial effects of nitrates result from their ability to reduce preload via venodilatation, increase coronary flow to ischaemic area of the heart via vasodilatation of large epicardial arteries or collateral vessels, increase arterial and myocardial compliance, and inhibit vascular smooth muscle growth and myocyte hypertrophy (Pang, 1994; Moncada & Higgs, 1995). SNP is mainly used for lowering blood pressure in hypertensive emergencies because of its rapid action, potency and the ease of use in manipulating blood pressure via alteration of infusion rate (Young, 1997).

The release of NO from SNP is believed to be spontaneous, whereas that from NTG and other organic nitrates require the presence of thiols (e.g., cysteine and glutathione) and/or enzymatic cleavage (Harrison & Bates, 1993). It is well-documented that SNP and NTG have differential effects on the arterial and venous vasculatures (see section 1.4). SNP efficaciously dilates both capacitance and resistance vessels. In humans, SNP reduces forearm vascular resistance and increases forearm venous compliance (Miller et al., 1976). NTG, on the other hand, preferentially dilates veins versus arterioles. The resultant venous pooling and reduced venous return, together with pulmonary vasodilatation (Cyong et al., 1976), would explain the decreased ventricular filling pressure, end-diastolic volume and heart size, and the resultant reduction in myocardial oxygen consumption, all of which are beneficial to patients with coronary heart diseases. In conscious intact rats pretreated with the ganglion blocker,
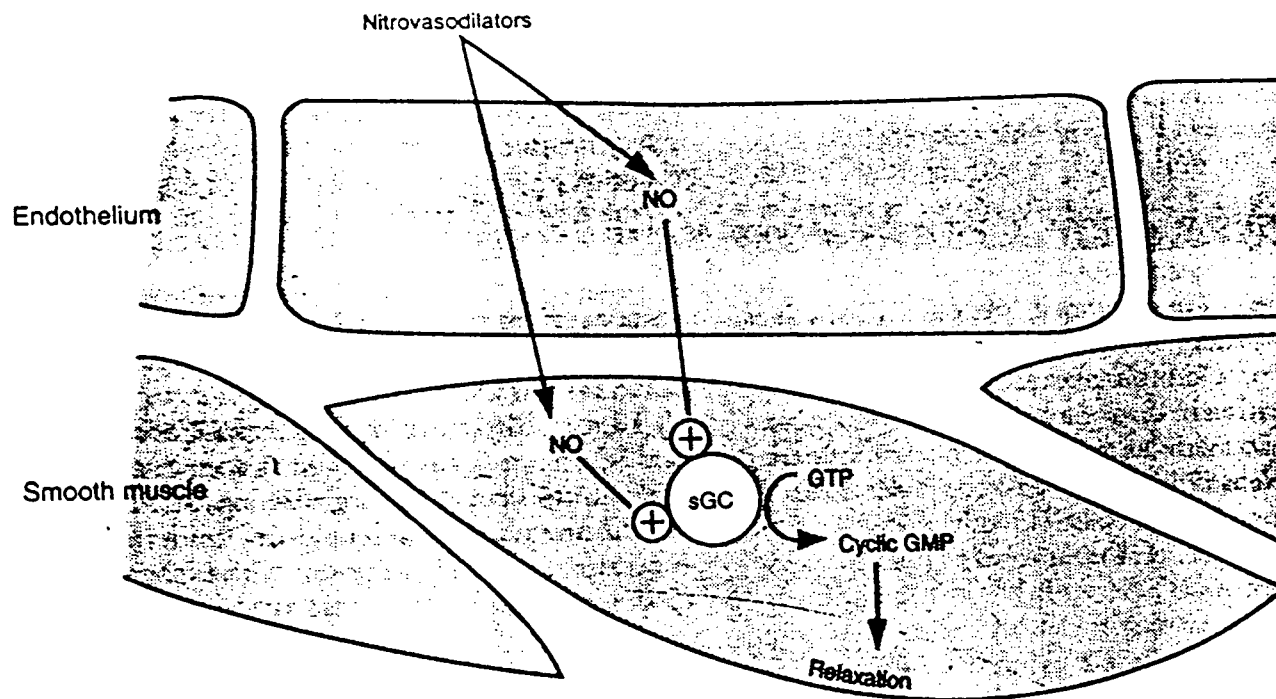
Figure 2 Nitrovasodilators such as sodium nitroprusside and nitroglycerin release nitric oxide (NO), spontaneously and/or through enzymatic reactions. The liberated NO stimulates soluble guanylate cyclase (sGC) in vascular smooth cells leading to relaxation (adapted from Moncada & Higgs, 1993).

hexamethonium, NTG slightly reduced mean arterial pressure but substantially decreased mean circulatory filling pressure (Pang, 1994). Maximal venodilatation was attained at a lower plasma concentration of NTG than arteriolar dilatation in healthy male volunteers (Imhof et al., 1980), suggesting that NTG is a more potent vasodilator of capacitance than resistance vessels. However, it should be noted that the relative effectiveness of NTG in dilating arteries and veins depends very much on the route of drug administration and the experimental conditions. In conscious supine human subjects (Imhof et al., 1980) and anaesthetized patients (Gerson et al., 1982), sublingual or intravenously infused NTG is more effective in dilating veins than arteries. On the contrary, intravenous (i.v.) bolus injections of NTG caused marked dilatation of arteries and arterioles but not capacitance vessels in conscious dogs (Nonaka et al., 1990). The effects of NTG on systemic arterial resistance and forearm venous tone were compared to those of SNP in patients undergoing cardiopulmonary bypass surgery (Gerson et al., 1982). I.V. infusion of NTG reduced forearm venous tone but only slightly decreased systemic vascular resistance; conversely, SNP reduced both parameters. Both drugs have drawbacks: cyanide toxicity and drug tolerance remain a concern with prolonged administrations of SNP and NTG, respectively. The undesirable pharmacological actions of the clinically available nitrovasodilators have fuelled the continuous development of new NO donors. The three compounds of interest, S-nitroso-N-acetylpenicillamine, diethylamine/nitric oxide complex and nitroxy(η^5-cyclopentadienyl)-dinitrosylchromium, in this thesis will be discussed in the following paragraphs. The chemical structures of the three agents and those of SNP and NTG are shown in Figure 3.

S-nitroso-N-acetylpenicillamine (SNAP) is a synthetic, chemically stable S-nitrosothiol. It was reported that S-nitrosothiols occur naturally in plasma and other body fluids as S-nitrosoglutathione (Gaston et al., 1993) and as the S-nitrosothiols of other sulfur-
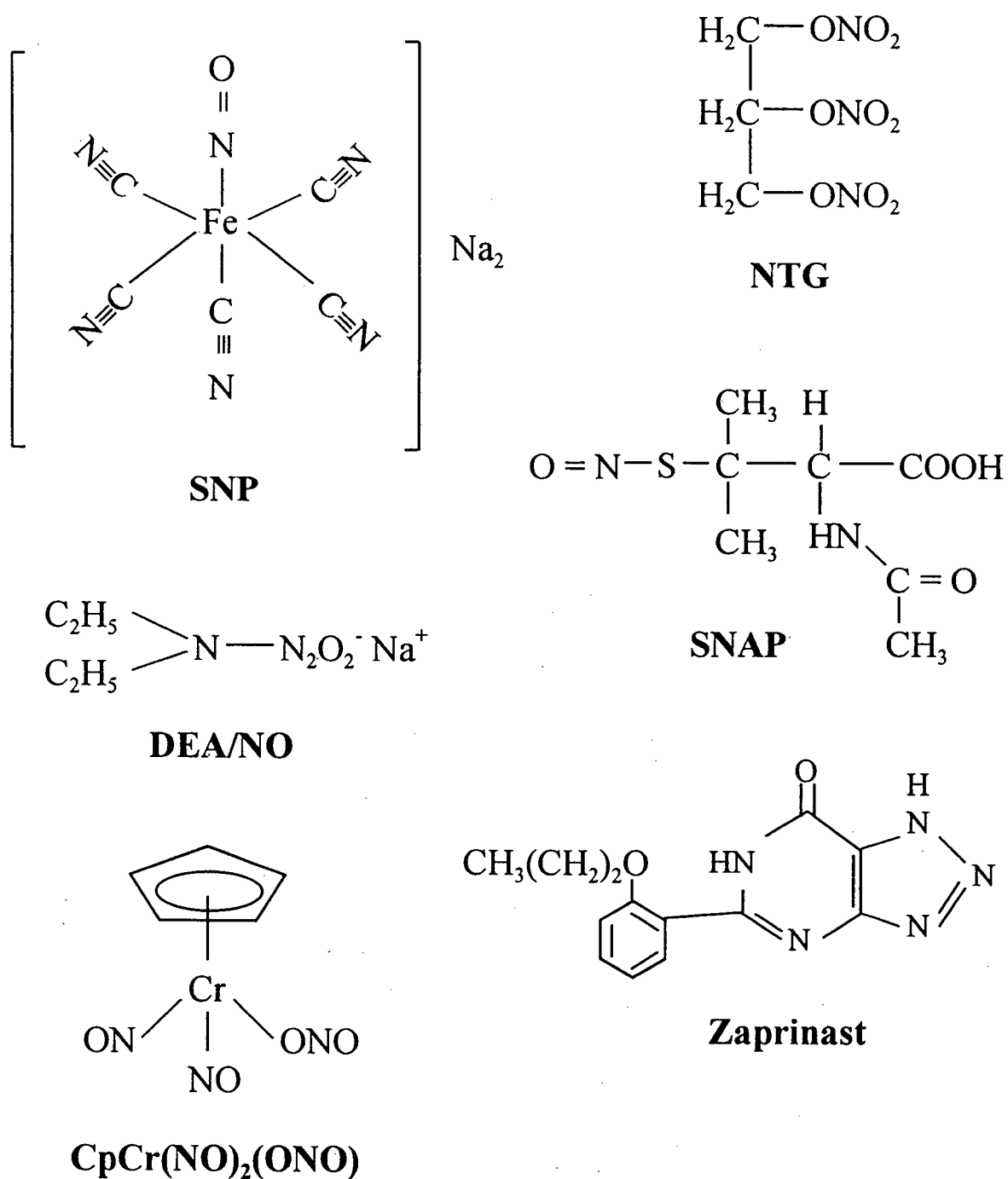
Figure 3 Chemical structures of sodium nitroprusside (SNP), nitroglycerin (NTG), diethylamine/nitric oxide (DEA/NO) complex, S-nitroso-N-acetylpenicillamine (SNAP), nitroxy(η^5-cyclopentadienyl)-dinitrosylchromium (CpCr(NO)$_2$(ONO)) and zaprinast.

containing proteins, the predominant species of which is S-nitrosoalbumin (Stamler et al., 1992). The concentration of NO in blood or plasma under normal circumstances is believed to be in the 1 nM range. Stamler et al. (1992) suggested that plasma S-nitrosothiols might serve as a reservoir for NO, effectively buffering its concentration. SNAP and other S-nitrosothiols have been shown to be potent vascular smooth muscle relaxants *in vitro* (Henry et al., 1989; Mathews & Kerr, 1993) and vasodilators *in vivo* (Ignarro et al., 1981). Needleman & Johnson (1973) first demonstrated that NTG-induced relaxation of preconstricted aortic strips was dependent upon the presence of tissue sulfhydryl groups. Later, it was shown that the vasodilator activity of organic nitrates and nitrites as well as SNP could be in part attributed to the formation within vascular smooth muscle cells of active, unstable, intermediate S-nitrosothiols which rapidly decompose to liberate NO (Ignarro et al., 1981; Ignarro & Kadowitz, 1985).

Venturini et al. (1993) demonstrated that vascular smooth muscle contains a depletable store of a vasodilator, most likely S-nitrosothiols, which is light-activated and restored by SNAP and NTG. Alternatively, S-nitrosothiols may interact with reducing entities such as thiols or heme-proteins on the cell surface (Stamler et al., 1992), undergo a denitrosation step and that the released NO then enters the cells (Travis et al., 1996). SNAP has been shown to cause similar decreases in systemic arterial pressure and vascular resistance relative to SNP and NTG following i.v. injections into anaesthetized cats (Ignarro et al., 1981). Furthermore, Bauer & Fung (1991) demonstrated that in rats with congestive heart failure, SNAP is a more potent *in vivo* vasodilator and has more arterial dilator action relative to NTG, and is less prone to produce left ventricular tolerance. In conscious rabbits, self-tolerance to SNAP did not develop when infused continuously for 24 hr (Shaffer et al., 1992).

Diethylamine/nitric oxide complex (DEA/NO), a NO donor which belongs to the novel

class of compounds known as the nucleophile/NO adducts or NONOates, was synthesized by Maragos et al (1991). This class of agents contains an anionic $N_2O_2^-$ group which is not found in other classes of nitrovasodilators. The spontaneous loss of the functional group produces up to two molecules of NO. They are generally stable as solids and highly soluble in aqueous media; the rate and extent of NO generation depends only on pH, temperature and the identity of the nucleophile residue to which the $N_2O_2^-$ group is attached. The NONOates were studied for their ability to spontaneously release NO in aqueous solution and for possible vasoactivity in the isolated rabbit aorta (Maragos et al., 1991; Morley et al., 1993). The investigators demonstrated that there is a strong correlation between the rate of NO release from these compounds upon introduction into the biological system and their vasorelaxant potency, both of which can be reliably adjusted over a wide range by the choice of the carrier nucleophile. Among these NONOates, DEA/NO exerts the most potent and fastest vasorelaxing effect, and has the highest molar generation of NO (Diodati et al., 1993a). In the rabbit aorta preconstricted with noradrenaline, the half-life, EC_{50} (effective concentration to produce half-maximal relaxation response) and E_{max} (maximal percent relaxation of noradrenaline-induced contraction) of DEA/NO are 2.1 min, 0.19μM and -85%, respectively; the response to DEA/NO rapidly peaked (maximum at 5 min) and receded during the 60-min observation period (Morley et al., 1993). Furthermore, DEA/NO was reported to cause comparable reductions in blood pressure and systemic arterial resistance as did SNP and NTG in anaesthetized rabbits (Diodati et al, 1993b) and conscious lambs (Vanderford et al, 1994).

Nitroxy(η^5-cyclopentadienyl)dinitrosylchromium, [CpCr(NO)$_2$(ONO)], is a prototype of the organotransition-metal nitrosyl complexes, which contain NO directly linked to metal centres via M-NO linkages (Richter-Addo & Legzdins, 1988, 1992). The electron density of the metal centres and the nature of the ancillary ligands determine the rate of NO release and

chemical stability of these agents. In preliminary studies conducted in Dr. Pang's laboratory (unpublished data), organotransition-metal nitrosyl complexes, including $CpCr(NO)_2(ONO)$, have been shown to dose-dependently relax phenylephrine-preconstricted rat aorta and lower arterial blood pressure in conscious rats.

1.3 Phosphodiesterase Type V and Zaprinast

The initial purification and characterization of phosphodiesterase activity was reported by Butcher & Sutherland (1962). At least thirty different phosphodiesterases have now been identified in mammalian tissues and cells. These different isoenzyme forms can be subdivided into seven distinct families based on their genetic and functional characteristics, as follows: Ca^{2+}/calmodulin-dependent phosphodiesterases (PDE I), cGMP-stimulated phosphodiesterases (PDE II), cGMP-inhibited phosphodiesterases (PDE III), cAMP-specific phosphodiesterases (PDE IV), cGMP-specific phosphodiesterases (PDE V), photoreceptor phosphodiesterases (PDE VI), and the high-affinity cAMP-specific phosphodiesterases in yeast, designated HCP1 (PDE VII) (Beavo et al., 1994). Many of the isozymes are differentially expressed and regulated in different cell types (Beavo & Reifsnyder, 1990). Studies using ion exchange column chromatography have identified the presence of phosphodiesterases I, II, III, IV and V in vascular smooth muscle (Polson & Strada, 1996). Phosphodiesterases represent a principle mechanism by which the actions of the intracellular messengers, cGMP and cAMP, are terminated. It is well-documented that both cyclic nucleotides play significant roles in the regulation of vascular smooth muscle tone. Elevations of cGMP and/or cAMP in vascular smooth muscle cells are associated with relaxation via lowering of intracellular Ca^{2+} concentrations. The inhibition of cGMP and/or cAMP hydrolysis is therefore expected to promote vasodilatation. Phosphodiesterase inhibitors have been examined for use as

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vasodilators, bronchodilators, cardiotonic agents and anticoagulants in the treatment of a wide range of clinical disorders such as hypertension, heart failure and asthma (Beavo & Reifsnyder, 1990). The current thesis will only discuss phosphodiesterase type V and its inhibitor, zaprinast (Figure 3).

Phosphodiesterases type V (PDE V) metabolize only cGMP, and are calmodulin-independent. They are localized in vascular smooth muscle cells of a variety of arteries and veins of numerous species, including human (see Polson & Strada, 1996 for review). The most widely used PDE V inhibitor is zaprinast. *In vitro* studies have demonstrated that zaprinast increased the concentrations of cGMP but not cAMP in isolated rat (Lugnier et al., 1986) and rabbit (Ahn et al., 1989; Weishaar et al., 1990) aortae, attenuated phenylephrine-induced contractions of bovine intrapulmonary artery and vein (Ignarro et al., 1987b), and caused relaxations of phenylephrine-precontracted rat and rabbit aortae (Martin et al., 1986) and dog saphenous vein (Villanueva et al., 1991), as well as prostaglandin $F_{2\alpha}$-precontracted porcine coronary artery (Merkel et al., 1992). Acute i.v. infusion of zaprinast into anaesthetized rats reduced blood pressure via reduction of total peripheral resistance (Trapani et al., 1991; Dundore et al., 1992). In addition, chronic administration of zaprinast reduced blood pressure in spontaneously hypertensive rats (McMahon et al., 1989). Zaprinast has also been reported to reverse haemodynamic tolerance to NTG *in vitro* (Pagani et al., 1993) and *in vivo* (De Garavilla et al., 1996) consequent to the restoration of changes in intracellular cGMP levels.

1.4 The Venous System

The peripheral circulation is composed of two major systems, the arterial and venous circuits. In contrast to the voluminous information on the arterial system in the scientific

literature, little is known about the venous system. As shown in Figure 4, approximately 65-75% of the blood resides in the venous side of the circulation (Shepherd & Vanhoutte, 1979). The systemic veins which contain smooth muscle in their walls permitting active modulation of venous capacity are termed capacitance vessels. As supposed to the postcapillary venous system, the arterial system contains only ~10% of the blood volume (Figure 4). The regulation of systemic vascular resistance resides in the precapillary arterioles, which are termed resistance vessels. The total cross-sectional area and volume of veins ($2.4x10^5$ μ^2 and 0.32 mm^3) as measured in bats are much greater than those of arteries ($1.8x10^4$ μ^2 and 0.059 mm^3, respectively) (Wiedeman, 1963). Since the majority of the blood volume resides in the venous circulation, even small alterations in its capacity can result in great changes in venous return and cardiac output. Therefore, it is evident that the venous system plays a significant role in the control of circulatory homeostasis. The circulation is a closed circuit thereby requiring that venous return and cardiac output be matched under steady-state conditions. Cardiac output or venous return is determined by both vascular and cardiac factors which include arterial and venous resistances, arterial and venous compliances, blood volume, myocardial contractility and heart rate (Greenway, 1982). The major function of capacitance vessels is to maintain venous return and therefore, cardiac output, by altering the activity of venous smooth muscle. Similar to its arterial counterpart, venous smooth muscle activity can be altered passively according to inflow pressure or volume, or actively via reflex- or neurohumoral-mediated mechanisms (Numao & Iriuchijima, 1977; Rothe & Gaddis, 1990). It should be noted that veins are less affected by metabolic factors but more affected by sympathetic nerve activity, relative to the arterial resistance vessels. Drugs that influence body venous tone or reflex control of the venous system have profound effects on venous return, cardiac output and blood pressure (Pang, 1994). Within the venous circulation, the bulk of the blood volume (~75%)

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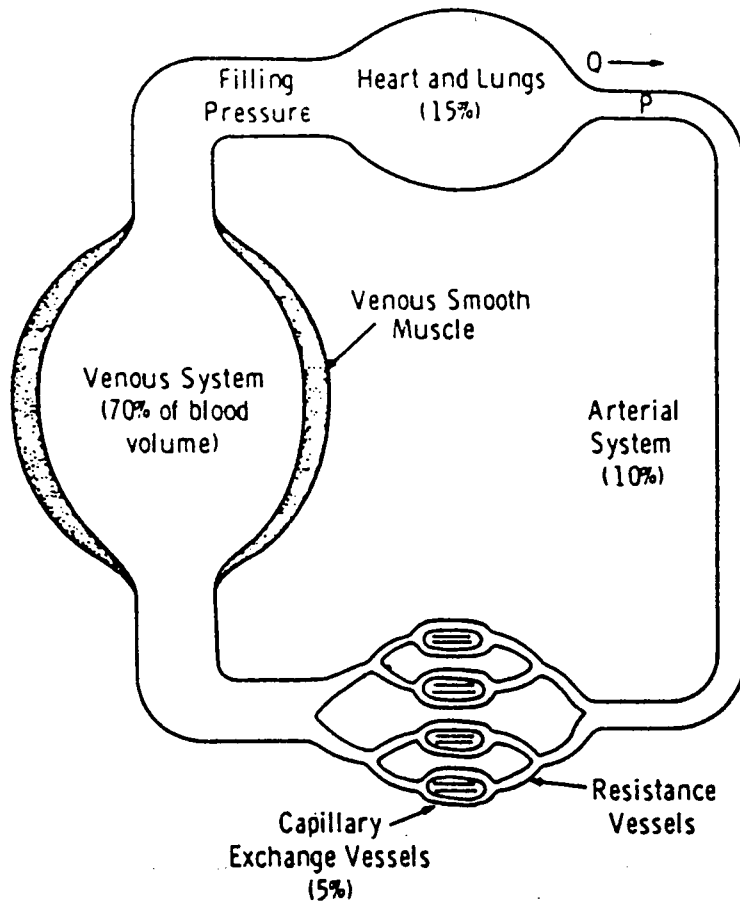
Figure 4   Distribution of blood volume in the central and systemic circulations. The capacity and pressure in the venous reservoir is regulated by contraction and relaxation of smooth muscle in the venous wall so that filling pressures may be regulated relatively independent of flow, by changes in venous resistance, according to $P = Q \times R$, where P = pressure, Q = flow, and R = resistance (adapted from Miller et al., 1982).

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resides in the small veins and venules, making these vessels the primary sites of control of venous (venomotor) tone. Total body venous tone is determined by venous capacitance, compliance and resistance. Briefly, vascular capacity is the total blood volume in the circulation at a specific pressure and is the sum of unstressed and stressed volumes. Unstressed volume represents ~60-70% of the total blood volume and is haemodynamically inactive (Rothe, 1983). Its function is to fill the circulation to maximum capacity without increasing transmural pressure. The capacity of blood vessels and the activity of vascular smooth muscles control the size of unstressed volume. Venous capacitance is defined as the relationship between blood volume and distending pressure at the level of the venules; whereas venous compliance represents a quantitative measure of the elasticity of the venous bed and is the ratio of the change in volume to the concomitant change in transmural distending pressure (see Pang, 1994 for details). Venous resistance, the focus of this thesis, will be discussed in greater detail herein.

Venous resistance controls the pressure gradient between upstream and downstream venous pressures and venous return. Venous pressure measured by catheters implanted into a large vein represents downstream venous pressure which does not accurately reflect upstream venous pressure. As small veins and venules are the predominant sites of venous resistance, compliance and capacitance, upstream venous pressure should be measured. To obtain venous resistance of any organ, one should measure blood flow as well as the driving upstream pressure at the level of the venules which is technically difficult to do, with the exception of the hepatic bed where portal venous pressure can be measured to give the driving pressure of the hepatic venous bed (Greenway & Lautt, 1989). In the hepatic bed, it has been shown that a pressure gradient of 4 to 15 mmHg exists between the portal vein and the inferior vena cava at the exit junction of the hepatic vein (Greenway & Lautt, 1989). In general, venous plateau

pressure during circulatory arrest (whereby upstream and downstream pressures equilibrate; see section 2.2) is used to estimate upstream venous pressure (Deschamps & Magder, 1992; Pang, 1994). Although venous resistance is lower than arterial resistance, it is still an important determinant of cardiac output. An increase in venous resistance reduces flow and increases upstream distending pressure depending on the upstream compliance, while simultaneously decreases downstream pressure. The pooling of blood in the venules reduces venous return and cardiac output. Conversely, a reduction in venous resistance increases flow and facilitates venous return which in turn increases cardiac output. It should be emphasized that the venous bed is sufficiently large that small changes in diameter result in large changes in capacitance affording accommodation of increased flow at lower pressures. Since flow is the ratio of pressure to resistance, it follows that flow will increase at reduced pressures if resistance falls proportionally more than pressure (Miller et al., 1982).

Total body venous resistance is best estimated by the ratio of (mean circulatory filling pressure – right atrial pressure) to cardiac output (Pang, 1994). Experimentally, mean circulatory filling pressure is the mean vascular pressure that would exist following circulatory arrest and instantaneous redistribution of blood throughout the circulation (Guyton et al., 1973). Thus, it is conceptually the driving force of venous return at the level of the venules. Changes in mean circulatory filling pressure, at a constant blood volume, reflect predominantly alterations in venous tone (Rothe, 1993; Pang, 1994). Despite the importance of the venous system in haemodynamic regulation, venous resistance and total body venous tone are little studied due to technical difficulties associated with the measurement of pressure and flow inside small veins and venules. It should be noted that *in vitro* experiments utilizing isolated venous tissues (usually large veins), which are devoid of neural and humoral influence, may not be representative of smaller veins and venules *in vivo*. Several techniques

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are available to assess total body venous tone in whole animals, including the mean circulatory filling pressure method for the determination of whole body venous tone, the constant cardiac output-reservoir technique for the measurement of vascular compliance or capacitance and the linear variable differential transformer technique for estimating the diameter of a human dorsal hand vein, among others. The mean circulatory filling pressure method, used in the experiments of this thesis, has been employed to evaluate the effects of drugs on total body venous tone in both anaesthetized and conscious animals (Pang. 1994), and will be described in detail in section 2.2.

Venomotor tone is altered in various cardiovascular disorders such as autonomic dysfunction, haemorrhage, hypertension and heart failure (Pang, 1994). There is evidence indicating that venous compliance is decreased and mean circulatory filling pressure increased in some forms of experimental hypertension (Samar & Coleman, 1979; Kooman et al., 1992). A reduction in venous compliance may be an adaptive mechanism to increase venous return in early stages of hypertension (Pang, 1994). In heart failure, sympathetic outflow as well as the levels of circulating vasoconstrictors (catecholamines, angiotensin II, arginine vasopressin, endothelin) are increased to compensate for the reduced cardiac output and blood pressure. Patients with coronary heart disease or dilated cardiomyopathy in the supine position were shown to have reduced forearm blood flow and venous compliance, and increased forearm vascular resistance (Todo et al., 1986). Gay et al. (1986) demonstrated that mean circulatory filling pressure was elevated in conscious rats with chronic heart failure induced by coronary ligation. In the normal and healthy heart, predominant venodilation often decreases cardiac output due to reduced preload. On the contrary, in the failing ventricle with markedly elevated filling pressures, despite a reduction in preload, venous return is maintained at adequate levels to increase cardiac output if there is a concomitant decrease in resistance. Therefore, agents

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with predominantly venodilator actions are maximally beneficial in the setting of markedly elevated cardiac filling pressures (Miller et al., 1982). If a venodilator drug also dilates arterioles, stroke volume and cardiac output would increase consequent to the reduction in the resistance to left ventricular ejection. It should be noted that not all vasodilator drugs dilate capacitance vessels. Hydralazine (D'Oyley, 1989) and the potassium channel blocker, pinacidil (Waite et al., 1995), for example, are efficacious arterial dilators which lack venodilator actions. Given the importance of the functional coupling between the arterial and venous systems in the control of cardiac output, it follows that drugs which dilate both resistance and capacitance vessels are likely to be more effective than those which dilate only one type of vessel in the management of hypertension and chronic heart failure.

1.5 Objectives

Despite the significance of the venous circulation in the control of blood pressure and cardiac output (section 1.4), there are relatively few reports on the effects of drugs on total body venous tone, mean circulatory filling pressure and venous resistance. This is in sharp contrast to the voluminous information on the actions of vasoactive agents on arterial pressure and resistance. Technical difficulties associated with venous studies likely preclude research in this area. Information on body venous tone in whole animals with intact cardiovascular reflexes cannot be obtained from *in vitro* studies using isolated venous preparations or perfused venous beds which lack neural and hormonal modulating mechanisms (Pang, 1994). Moreover, large veins are the principal venous vasculature used in *in vitro* bath studies and information obtained may not be representative of that in small veins and venules, which are primary sites of control of venous capacitance, compliance and resistance *in vivo* (section 1.4). Since venodilatation unequivocally contributes to the therapeutic effectiveness of sodium

nitroprusside (SNP) and nitroglycerin (NTG) in the management of hypertensive emergencies and chronic heart failure, respectively, better knowledge of the *in vivo* venous actions of nitrovasodilators is essential to improving treatment strategies in cardiovascular pathology.

The vascular effects of zaprinast and S-nitroso-N-acetylpenicillamine (SNAP) have been extensively investigated, however, little is known about their actions on the venous circulation *in vivo*. Diethylamine/nitric oxide (DEA/NO) complex and nitroxy(η^5-cyclopentadienyl)-dinitrosylchromium (CpCr(NO)$_2$(ONO)) are novel NO donors which have not been studied in detail. To my knowledge, there are no published *in vivo* reports examining the effects of these drugs on mean circulatory filling pressure and venous resistance. This thesis consists of two parts: a) the effects of zaprinast on mean circulatory filling pressure and venous resistance were studied and compared to those of SNP in anaesthetized rats under basal conditions, and in the presence of mecamylamine and noradrenaline, and b) the arterial and venous actions of DEA/NO, SNAP and CpCr(NO)$_2$(ONO) relative to those of SNP and NTG were concurrently investigated in anaesthetized rats in the presence of mecamylamine and noradrenaline. Mecamylamine and noradrenaline were used to suppress autonomic reflex and elevate venomotor tone, respectively, both of which facilitate the assessment of the venodilator activity of drugs.

My objectives were to study the haemodynamic profiles of zaprinast, DEA/NO, SNAP and CpCr(NO)$_2$(ONO), evaluate their arterial and venous selectivity and dilator efficacies, compare those to the clinically approved SNP and NTG, and discuss the therapeutic potential of these new agents as alternatives for SNP and NTG in the management of cardiovascular dysfunction.

2. Methods and Materials

2.1 Surgery and Instrumentation

Male Sprague-Dawley rats, weighing 400-500 g, were permitted access to food and water *ad libitum*. All experiments were conducted in compliance with the *Guidelines for Laboratory Animal Care* approved by the Animal Care Committee of the University of British Columbia.

The rats were anaesthetized with thiobutabarbital (100 mg kg^{-1} i.p.). Body temperature was maintained at 37±1 °C with a rectal probe and a heat lamp attached to a Thermistemp Temperature Controller (Model 71; Yellow Spring Instrument Co. Inc. OH, USA). A polyethylene (PE50) catheter was introduced into the left iliac artery to record mean arterial pressure via a pressure transducer (P23DB, Gould Statham, CA, USA). Heart rate was derived electronically from the upstroke of the arterial pulse pressure by a Grass 7P4G tachograph. Additional catheters were implanted into the left ventricle via the right carotid artery and the right iliac artery for the injection of radioactively-labelled microspheres and the withdrawal of a reference arterial blood sample (Wang et al., 1995; see section 2.4 for details), respectively. The vehicle or drugs were administered through cannulae inserted into the right iliac vein and the left external jugular vein. The inferior vena cava was also cannulated via the left iliac vein to measure central venous pressure by another pressure transducer (P23DB, Gould Statham). Although central venous pressure can be obtained from both intrathoracic and intra-abdominal sites, readings from the inferior vena cava are more accurate than those from the superior vena cava, as they are less influenced by respiratory movements (Pang, 1994). A saline-filled, balloon-tipped catheter was advanced into the right atrium through the right external jugular vein. The correct positioning of the balloon was tested by transiently inflating the balloon,

which when correctly placed, resulted in a simultaneous decrease in mean arterial pressure to 20-25 mmHg and an increase in central venous pressure within 5 s of circulatory arrest (section 2.2). Mean arterial pressure, heart rate and central venous pressure were continuously monitored and displayed on a Grass Polygraph (Model RPS 7C8). It should be noted that baseline central venous pressure (in the absence of circulatory arrest) is not an index of body venous tone since central venous pressure represents pressure within a large vein, the resultant of a pressure drop at both arterioles and venules (Pang, 1994). The rats were given 30 min to stabilize before baseline values of mean arterial pressure, heart rate, mean circulatory filling pressure (section 2.2) and cardiac output (section 2.3) were obtained.

2.2 The Mean Circulatory Filling Pressure (MCFP) Method

The MCFP technique, developed by Yamamoto et al. (1980), was used. As mentioned earlier, a balloon-tipped catheter was positioned at the right atrium. Circulation was stopped by injecting saline into the balloon, resulting in a simultaneous decrease in mean arterial pressure to 20-25 mmHg and an increase in central venous pressure to a plateau value within 4-5 s of circulatory arrest before hypotension-induced, reflex-mediated venoconstriction, which occurred at ~ 11 s after the cessation of flow, took place. Steady-state readings of mean arterial pressure and central venous pressure were noted at 4-5 s after inflation of the atrial balloon. The actual tracing from one of the experiments is shown in Figure 5. To avoid rapid equilibration of arterial and venous pressures during circulatory arrest, the arterial pressure contributed by the small amount of trapped arterial blood was corrected by the following equation: MCFP = VPP + 1/60 (FAP-VPP), where MCFP represents upstream venous pressure at the level of the venules, FAP and VPP denote the final arterial pressure and venous plateau pressure, respectively, and 1/60 represents the ratio of arterial to venous compliance.
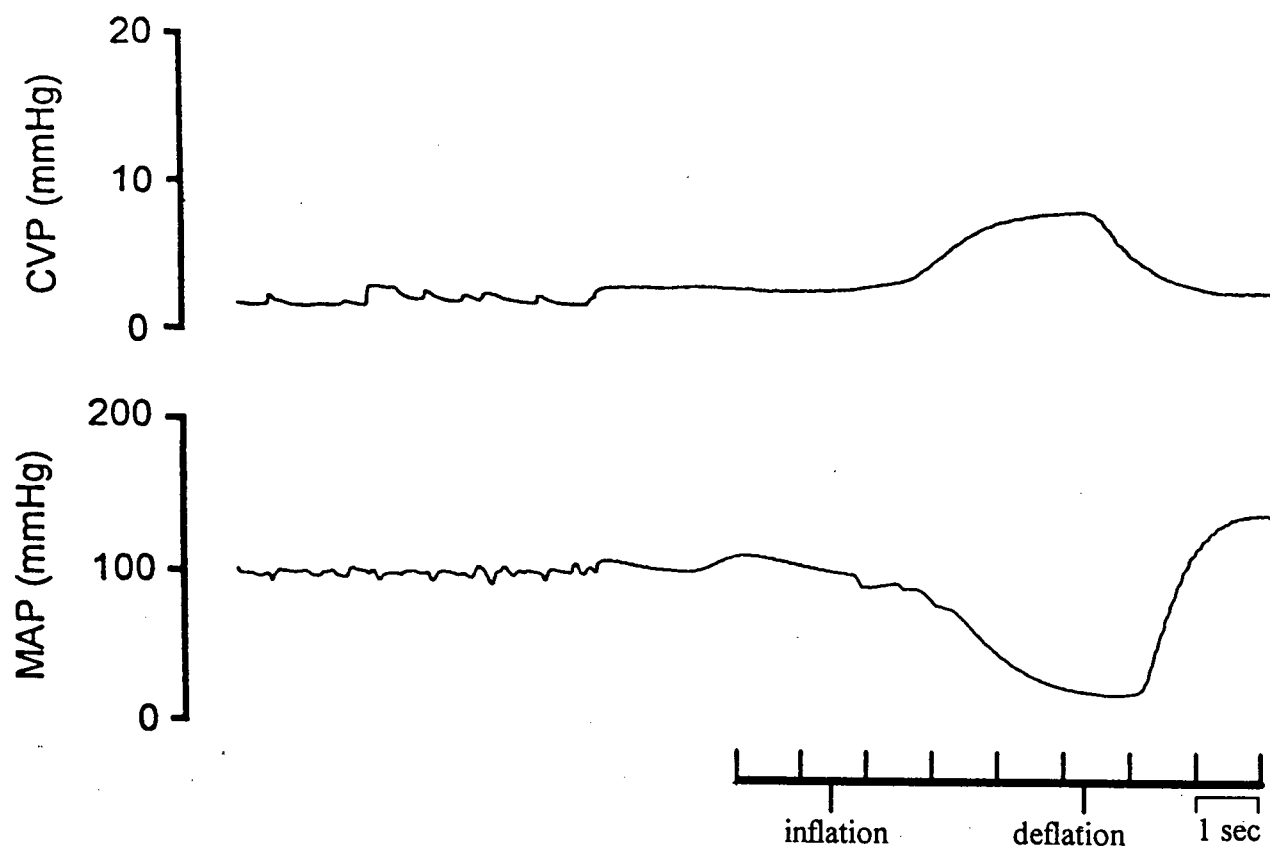
Figure 5 A Grass Polygraph tracing. Central venous pressure (CVP) and mean arterial pressure (MAP) are shown in the upper and lower panels, respectively. The readings were taken in the presence of mecamylamine (3.7 μmol kg^{-1}) and continuous noradrenaline infusion (6.8 nmol kg^{-1} min^{-1}). Upon inflation of the atrial balloon, MAP decreased to 25 mmHg, which is known as the final arterial pressure (FAP), and CVP rose to a plateau of ~8 mmHg, which is known as the venous plateau pressure (VPP). The balloon was then immediately deflated. Mean circulatory filling pressure can be calculated from FAP and VPP.

Although the MCFP method is presently the best available method for the estimation of body venous tone, it is not without limitations. By definition, all pressures in the circulation should be equal during circulatory arrest. In reality, it has been shown that there is a lack of equilibration between central venous pressure and portal venous pressure in various species under various experimental conditions. However, the error involved is so small that even a lack of complete equilibration does not invalidate the MCFP method (Tabrizchi & Pang, 1992; Pang, 1994).

2.3 Measurement of Cardiac Output

The microsphere technique for measuring cardiac output, known as the reference sample method, was first used by Hoffbrand & Forsyth (1969) and subsequently verified by Archie et al. (1973). In essence, the method involves the use of small, radioactive plastic spheres (microspheres) slightly to several times larger than erythrocytes. These microspheres are injected into the circulation, mixed with the blood and are carried to the periphery. Their size is so chosen that they cannot cross capillaries of vascular beds and are trapped in the tissues. If the total amount of radioactivity injected into the animal is known, then cardiac output equals reference flow multiplied by the ratio of total injected radioactivity to arterial radioactivity (Archie et al., 1973; see section 2.6) provided that the following requirements are met: namely, adequate mixing of the microspheres before the first branching of the arterial system, an adequate number of microspheres injected and collected in the reference sample for statistical accuracy, erythrocyte-like behaviour in the circulation, ~100% trapping in the peripheral tissues in one circulation and therefore minimal recirculation, and finally avoidance of significant disturbance of blood flow (Archie et al., 1973; Hof, 1982).

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In the following experiments, a well-stirred suspension (100 μl) containing 20,000-25,000 microspheres (15 μm diameter), labelled with Cobalt-57 (Du Pont Canada Inc., Ont., Canada), was injected and flushed over 10 s into the left ventricle at the end of the 30 min-equilibration period and 8 or 10 min (see section 2.4) after the i.v. infusion of a drug or vehicle. At 10 s before the injection of each set of microspheres, a blood sample was withdrawn (Harvard infusion/withdrawal pump) from the right iliac arterial cannula into a heparinized saline-filled syringe at 0.35 ml min^{-1} for 45 s. The blood removed was slowly injected back to the rats immediately after the counting of radioactivity at 80-160 keV using a 1185 Series Dual Channel Automatic Gamma Counter (Nuclear-Chicago, IL, USA) with a 3 inch NaI crystal.

2.4 Experimental Protocol

Two series of experiments were conducted. In the first series, rats were randomly divided into six groups (n=6 each). Immediately after baseline measurements of cardiovascular variables, three groups of rats were infused with the vehicle (0.05 N NaOH), zaprinast (ED$_{40}$ and ED$_{80}$ doses, 1.5 and 3.0 mg kg^{-1} min^{-1}, respectively) or sodium nitroprusside (SNP; ED$_{40}$ and ED$_{80}$ doses, 8.0 and 64.0 μg kg^{-1} min^{-1}, respectively) for 12 min each dose. ED$_{40}$ and ED$_{80}$ doses were chosen with reference to preliminary results in intact, thiobutabarbital-anaesthetized rats under similar experimental conditions (data not shown), and represented the doses of zaprinast or SNP which caused 40% and 80% of decrease in mean arterial pressure from their respective maximums, respectively. Cardiac output followed by mean circulatory filling pressure measurements were taken 10 min after the infusion of a drug or vehicle, at the plateau response to each drug. A recovery period of 12 min, during which infusion was stopped, was allowed between doses. The effects of the vehicle, zaprinast and

SNP were also studied in another three groups of rats given i.v bolus injection of mecamylamine (3.7 μmol kg^{-1}) followed by continuous infusion of noradrenaline (7.3 nmol kg^{-1} min^{-1}) to elevate vasomotor tone. The dose of mecamylamine used was previously found to block ganglionic transmission effectively for more than 2 hr (Wang & Pang, 1991).

In the second series of experiments, rats were randomly assigned to six groups (n=6 each). Immediately after baseline measurement of haemodynamic parameters, all groups of rats were given i.v. bolus injections of mecamylamine (3.7 μmol kg^{-1}) followed by i.v. infusion of noradrenaline (6.8 nmol kg^{-1} min^{-1}) at 10 min later. After another 10 min, each group of rats were infused with either the vehicle (0.9% NaCl), diethylamine/nitric oxide complex (DEA/NO; 4, 32 and 256 μg kg^{-1} min^{-1}), S-nitroso-N-acetylpenicillamine (SNAP; 4, 32 and 256 μg kg^{-1} min^{-1}), nitroxy(η^5-cyclopentadienyl)dinitrosylchromium (CpCr(NO)$_2$(ONO), 4, 32 and 256 μg kg^{-1} min^{-1}), SNP (8, 32 and 128 μg kg^{-1} min^{-1}) or nitroglycerin (NTG; 0.2, 0.8 and 6.4 μg kg^{-1} min^{-1}) for 10 min each dose. In our preliminary studies, dose-response curves were constructed for the five nitrovasodilators under similar experimental conditions (data not shown). The doses of each drug which reduced mean arterial pressure by 30% (ED$_{30}$) and 80% (ED$_{80}$) from their respective maximums and the lowest dose that maximally reduced mean arterial pressure (ED$_{100}$) were selected for the present study. Cardiac output followed by mean circulatory filling pressure measurements were taken 8 min after the infusion of a drug or vehicle, at the plateau phase of response to each drug. A recovery period of 5 min, during which infusion was terminated, was allowed between doses.

2.5 Drugs

Zaprinast was a gift from Sanofi Recherche (France). Diethylamine/nitric oxide complex sodium, S-nitroso-N-acetylpenicillamine and Inactin were obtained from Research

Biochemicals International (MA, USA). Mecamylamine hydrochloride and noradrenaline hydrochloride were purchased from Sigma Chemical Co. (MO, USA). Sodium nitroprusside was obtained from Fisher Scientific Co. (NJ, USA). Zaprinast was dissolved in 0.05 N NaOH, all other drugs were dissolved in normal saline (0.9% NaCl) and prepared fresh daily. Nitroglycerin Injection USP was purchased from David Bull Laboratories Pty. Ltd. (Victoria, Australia) and diluted with 0.9% NaCl before use.

2.6 Calculations and Data Analysis

Cardiac output (CO, ml min^{-1}), arterial resistance (R$_a$, mmHg min ml^{-1}) and venous resistance (R$_v$, mmHg min ml^{-1}) were calculated according to the following equations:

$$CO = \frac{\text{rate of withdrawal of blood} \times \text{total injected c.p.m.}}{\text{c.p.m. in withdrawn blood}}$$

$$R_a = \frac{MAP}{CO}$$

$$R_v = \frac{MCFP - CVP}{CO}$$

Due to the technical difficulty of monitoring right atrial pressure in small animals, central venous pressure (CVP) rather than right atrial pressure was used to estimate pressure gradient to venous return (mean circulatory filling pressure − right atrial pressure). This is legitimate as mean CVP is nearly identical to mean right atrial pressure (Rothe, 1993).

All results are presented as mean ± s.e.mean. Comparisons were made with one way analysis of variance/covariance (ANOVA/ANCOVA) followed by Duncan's multiple range test, with $P < 0.05$ as the criterion for statistical significance. Profile/trend analysis (curve

analysis) was used to compare dose-dependency of responses with the statistical package,

SYSTAT v. 5.03 (SYSTAT Inc., IL, USA) (see Wang et al., 1995).

3. Results

3.1 Haemodynamic Effects of Zaprinast

a) Intact Rats

Baseline values of mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), mean circulatory filling pressure (MCFP), arterial resistance (R_a) and venous resistance (R_v) among the six groups of rats were not significantly different from each other (Table 1). The vehicle (i.e., time-control) did not cause significant changes in any haemodynamic parameters (Figures 6-11). The ED_{40} and ED_{80} doses of zaprinast and SNP caused similar dose-dependent reductions of MAP and R_a (Figure 6). Both drugs increased HR (Figure 7), with the tachycardiac effects of zaprinast less than those of SNP, but did not significantly alter CO (Figure 7) and R_v (Figure 8) relative to the corresponding readings in the time-control group. MCFP was increased by both doses of zaprinast but only the high dose of SNP (Figure 8).

b) Ganglion-Blocked Rats

Injections of mecamylamine into three other groups of rats caused similar reductions of MAP, HR, CO and MCFP but insignificant changes in R_a and R_v (Table 1). The subsequent infusion of noradrenaline caused similar increases in MAP, HR, MCFP, R_a and R_v but did not alter CO in all groups (Table 1). Relative to the respective baselines, the combination of ganglionic blockade and noradrenaline increased MAP, HR, MCFP, R_a and R_v but reduced CO (Table 1).

In ganglion-blocked rats, the vehicle did not significantly alter any haemodynamic readings but tended to increase HR, R_a and R_v, and decrease CO with the passage of time (Figures 9-11). Both doses of zaprinast and SNP caused greater ($P<0.05$) dose-dependent reductions in MAP relative to the changes in intact rats, with the depressor responses to

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Table 1 Pooled values (mean±s.e.m.) of baseline haemodynamic parameters in intact rats (n=18) and rats treated with i.v. bolus injections of mecamylamine (mec) followed by i.v. infusion of noradrenaline (NA) (n=18).

	MAP (mmHg)	HR (beats min^{-1})	CO (ml min^{-1})	MCFP (mmHg)	Ra (mmHg min ml^{-1})	Rv (mmHg min ml^{-1})
Intact rats						
baseline	94±2	355±10	96±6	4.3±0.1	1.00±0.05	0.035±0.002
Ganglion-blocked rats						
baseline	95±2	358±9	102±5	4.2±0.1	0.95±0.05	0.031±0.001
mec	74±3 [a]	328±8 [a]	80±8 [a]	3.5±0.1 [a]	0.97±0.09	0.033±0.003
NA	122±4 [a,b]	419±9 [a,b]	82±4 [a]	5.6±0.2 [a,b]	1.50±0.10 [a,b]	0.057±0.004 [a,b]

[a] Significantly different (P<0.05) from the corresponding baseline readings prior to any drug treatment.

[b] Significantly different (P<0.05) from the corresponding readings after mec injections.

Figure 6   Effects (mean ± s.e.m.) of i.v. infusion of zaprinast (1.5 and 3.0 mg kg^{-1} min^{-1} shown as ZAP-L and ZAP-H, respectively), sodium nitroprusside (8.0 and 64.0 μg kg^{-1} min^{-1}) as SNP-L and SNP-H, respectively) or equivalent volumes of vehicle (0.05 N NaOH) on mean arterial pressure (MAP) and arterial resistance (R_a) in three groups of intact rats (n = 6 each). All measurements were obtained 10 min after the beginning of infusion of a drug or vehicle. [a] Significantly (P<0.05) different from the corresponding values in the vehicle group.

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Figure 7   Effects (mean ± s.e.m.) of i.v. infusion of zaprinast (1.5 and 3.0 mg kg^{-1} min^{-1} shown as ZAP-L and ZAP-H, respectively), sodium nitroprusside (8.0 and 64.0 μg kg^{-1} min^{-1}) as SNP-L and SNP-H, respectively) or equivalent volumes of vehicle (0.05 N NaOH) on heart rate (HR) and cardiac output (CO) in three groups of intact rats (n = 6 each).   All measurements were obtained 10 min after the beginning of infusion of a drug or vehicle. [a] Significantly (P<0.05) different from the corresponding values in the vehicle group. [b] Significantly different (P<0.05) from the corresponding values in the SNP group.

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Figure 8   Effects (mean ± s.e.m.) of i.v. infusion of zaprinast (1.5 and 3.0 mg kg^{-1} min^{-1} shown as ZAP-L and ZAP-H, respectively), sodium nitroprusside (8.0 and 64.0 μg kg^{-1} min^{-1}) as SNP-L and SNP-H, respectively) or equivalent volumes of vehicle (0.05 N NaOH) on mean circulatory filling pressure (MCFP) and venous resistance (R$_v$) in three groups of intact rats (n = 6 each). All measurements were obtained 10 min after the beginning of infusion of a drug or vehicle. [a] Significantly (P<0.05) different from the corresponding values in the vehicle group. [b] Significantly different (P<0.05) from the corresponding values in the SNP group.
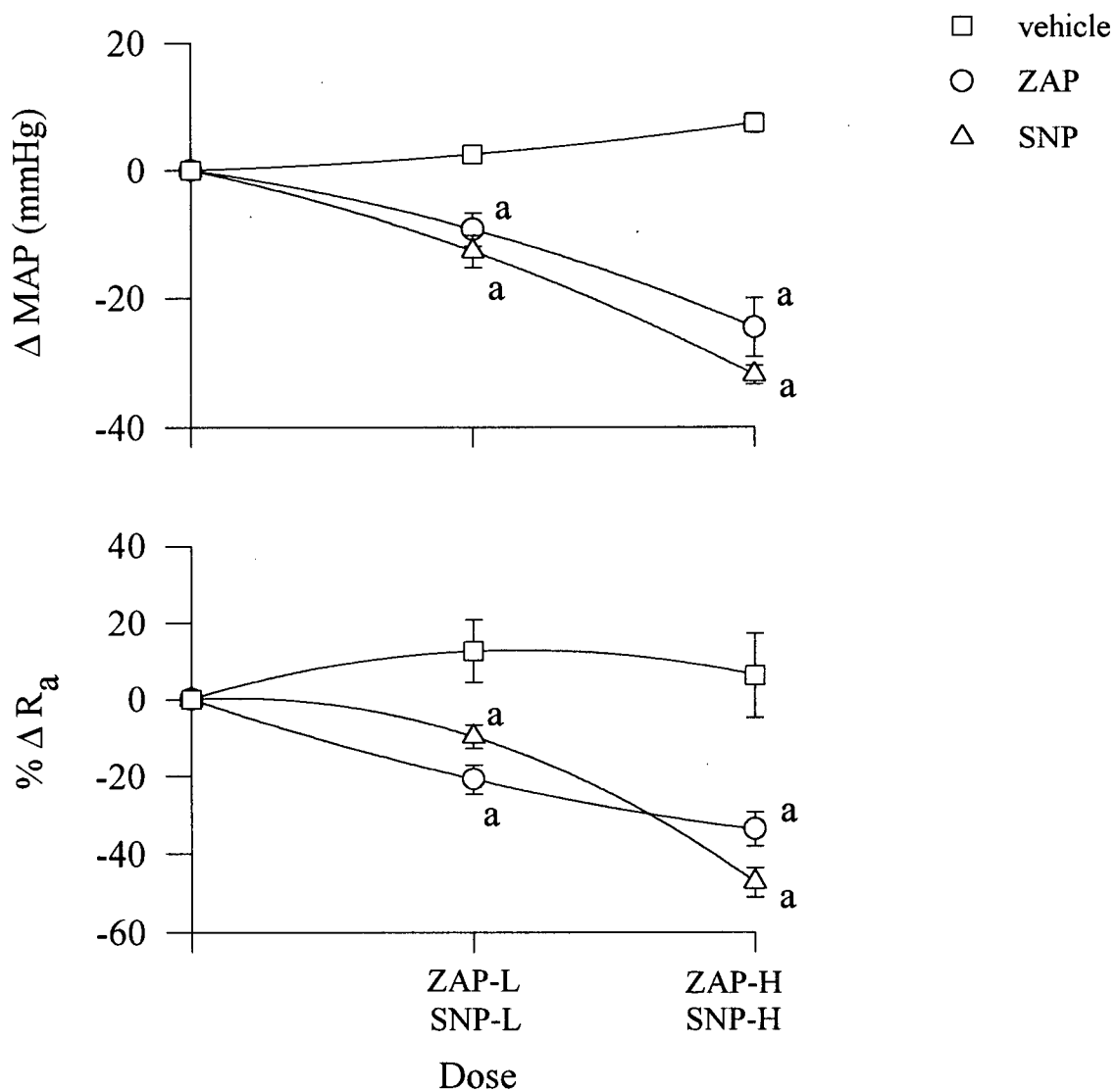
Figure 9   Effects (mean ± s.e.m.) of i.v. infusion of zaprinast (1.5 and 3.0 mg kg^{-1} min^{-1} shown as ZAP-L and ZAP-H, respectively), sodium nitroprusside (8.0 and 64.0 µg kg^{-1} min^{-1}) as SNP-L and SNP-H, respectively) or equivalent volumes of vehicle (0.05 N NaOH) on mean arterial pressure (MAP) and arterial resistance (R$_a$) in three groups of rats (n = 6 each) pretreated with mecamylamine (3.7 µmol kg^{-1}) and continuously infused with noradrenaline (7.3 nmol kg^{-1} min^{-1}). All measurements were obtained 10 min after the beginning of infusion of a drug or vehicle. [a] Significantly (P<0.05) d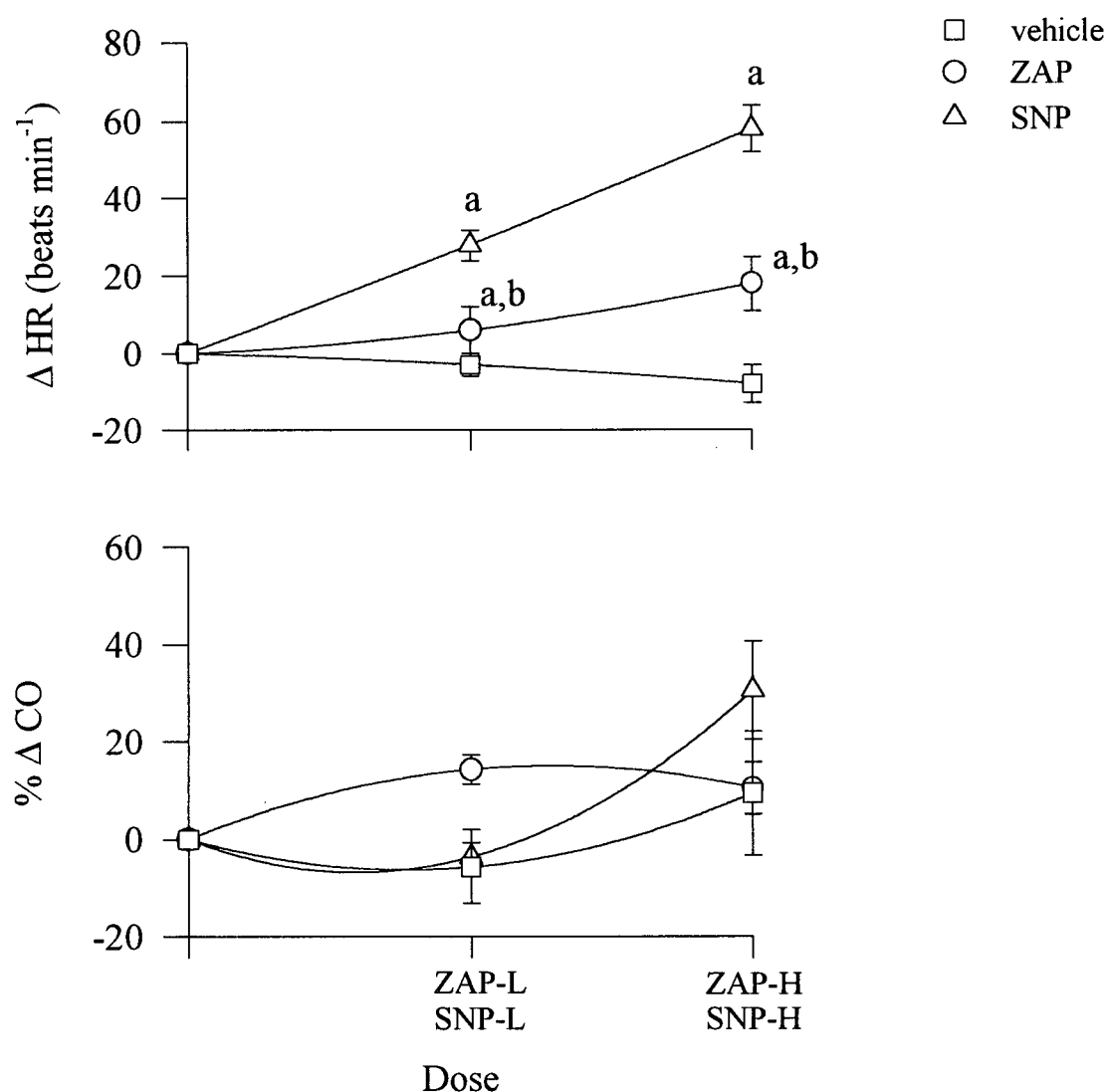ifferent from the corresponding values in the vehicle group. [b] Significantly different (P<0.05) from the corresponding values in the SNP group.

Figure 10 Effects (mean ± s.e.m.) of i.v. infusion of zaprinast (1.5 and 3.0 mg kg^{-1} min^{-1} shown as ZAP-L and ZAP-H, respectively), sodium nitroprusside (8.0 and 64.0 µg kg^{-1} min^{-1}) as SNP-L and SNP-H, respectively) or equivalent volumes of vehicle (0.05 N NaOH) on heart rate (HR) and cardiac output (CO) in three groups of rats (n = 6 each) pretreated with mecamylamine (3.7 µmol kg^{-1}) and continuously infused with noradrenaline (7.3 nmol kg^{-1} min^{-1}). All measurements were obtained 10 min after the beginning of infusion of a drug or vehicle. [a] Significantly (P<0.05) different from the corresponding values in the vehicle group. [b] Significantly different (P<0.05) from the corresponding values in the SNP group.
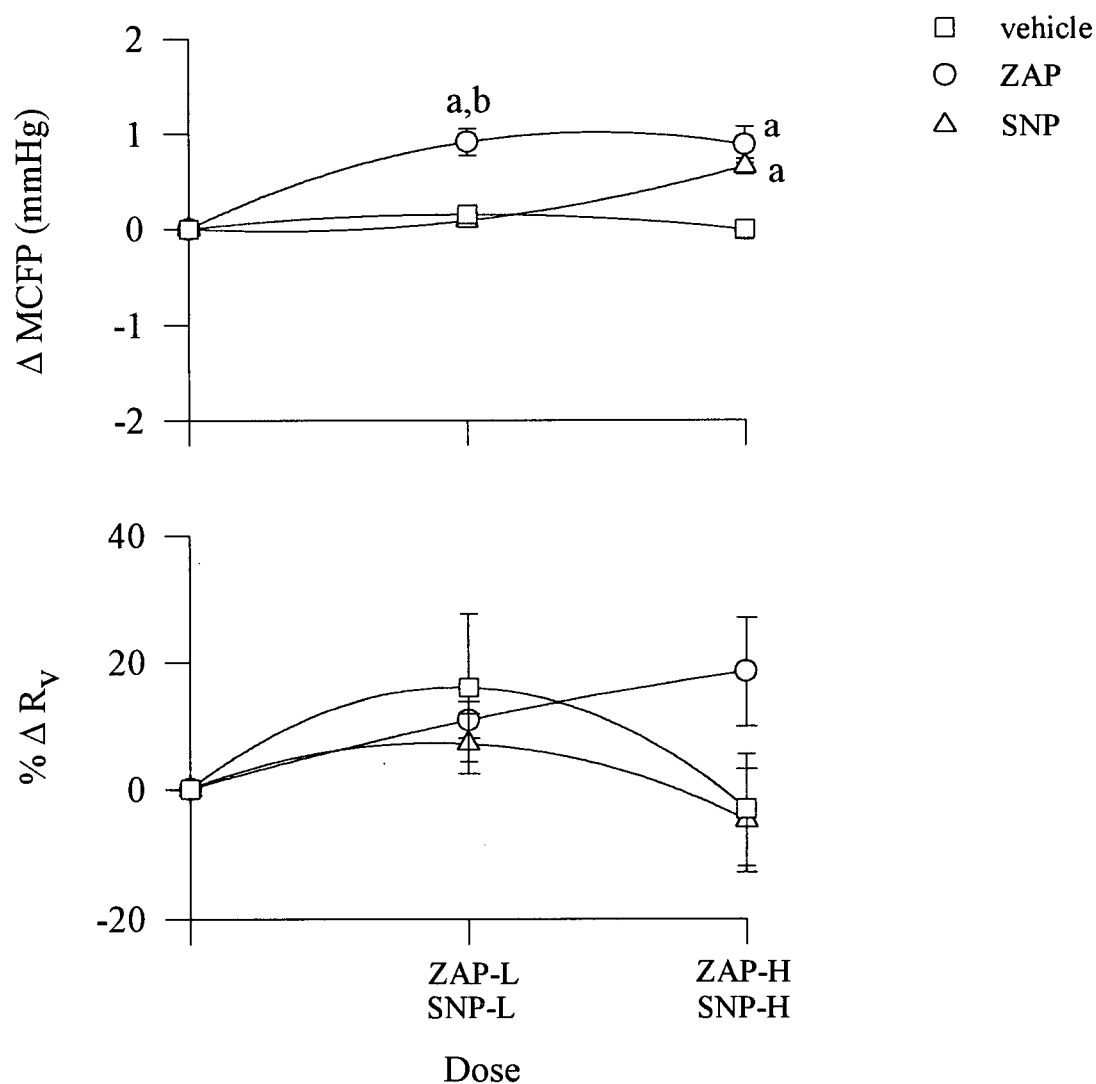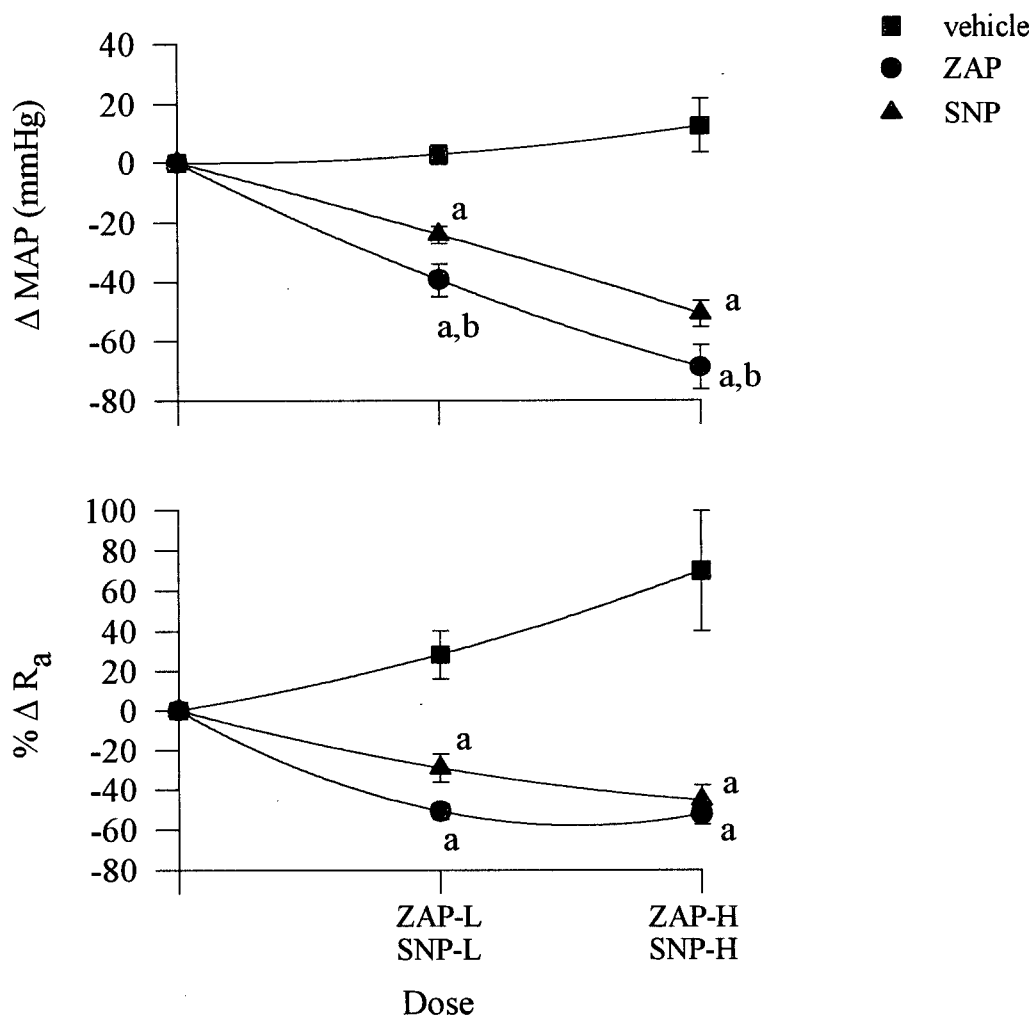
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Figure 11 Effects (mean ± s.e.m.) of i.v. infusion of zaprinast (1.5 and 3.0 mg kg^{-1} min^{-1} shown as ZAP-L and ZAP-H, respectively), sodium nitroprusside (8.0 and 64.0 µg kg^{-1} min^{-1}) as SNP-L and SNP-H, respectively) or equivalent volumes of vehicle (0.05 N NaOH) on mean circulatory filling pressure (MCFP) and venous resistance (R$_v$) in three groups of rats (n = 6 each) pretreated with mecamylamine (3.7 µmol kg^{-1}) and continuously infused with noradrenaline (7.3 nmol kg^{-1} min^{-1}). All measurements were obtained 10 min after the beginning of infusion of a drug or vehicle. [a] Significantly (P<0.05) different from the corresponding values in the vehicle group.

zaprinast slightly greater than those to SNP (Figure 9). HR was dose-dependently decreased by zaprinast but unaltered by SNP (Figure 10). Both drugs caused dose-dependent reductions in R_a (Figure 9) and R_v (Figure 11). The low doses, but not the high doses, of zaprinast and SNP caused similar reductions in MCFP (Figure 11). Zaprinast and SNP also increased CO; CO response to the low dose of zaprinast ($46\pm8\%$) was greater than that to SNP ($18\pm9\%$) (Figure 10).

3.2 *Cardiovascular Actions of DEA/NO, SNAP and CpCr(NO)₂(ONO)*

Table 2 shows the values (mean±s.e.m.) of haemodynamic parameters for the six groups of rats at baseline, at steady-state response to i.v. bolus injections of mecamylamine ($3.7~\mu$mol kg^{-1}) and to i.v. infusion (6.8 nmol kg^{-1} min^{-1}) of noradrenaline. Baseline values of MAP, CO, HR, MCFP, R_a and R_v were not significantly different among the six groups. I.V. bolus injections of mecamylamine reduced MAP, HR, CO and MCFP, but did not significantly alter R_a and R_v. The subsequent infusion of noradrenaline significantly increased MAP, HR, MCFP, R_a and R_v, but did not change CO. Therefore, the combination of ganglionic blockade and noradrenaline, relative to the corresponding pre-treatment baselines, increased MAP, HR, MCFP, R_a and R_v but decreased CO.

The vehicle (time-control) did not significantly alter any of the haemodynamic parameters (Figures 12-14). DEA/NO, SNAP, CpCr(NO)₂(ONO), SNP and NTG caused similar dose-dependent increments in CO, but had no significant effects on HR at all three doses (Figure 13). MAP was dose-dependently and similarly reduced by DEA/NO, SNAP and SNP (Figure 12). CpCr(NO)₂(ONO) caused the least reductions in MAP at the ED₃₀ and ED₈₀ doses relative to DEA/NO, SNAP and SNP. At the ED₁₀₀ dose, CpCr(NO)₂(ONO) produced similar decrement in MAP to SNAP and SNP, but lesser decrement than did DEA/NO. NTG

Table 2 Values (mean±s.e.m.) of mean arterial pressure (MAP), cardiac output (CO), heart rate (HR), mean circulatory filling pressure (MCFP), arterial resistance (R_a) and venous resistance (R_v) for the six groups (n=6 each) of rats at baseline, at steady-state response to i.v. bolus injections of mecamylamine (3.7 μmol kg⁻¹) and i.v. noradrenaline infusion (6.8 nmol kg⁻¹ min⁻¹) are presented in the first, second and third row, respectively.

	Vehicle	DEA/NO	SNAP	CpCr(NO)₂(ONO)	SNP	NTG
MAP (mmHg)	92±2	91±2	91±3	90±1	95±2	93±3
	66±4 [a]	63±3 [a]	68±3 [a]	71±2 [a]	69±2 [a]	66±4 [a]
	110±3 [a,b]	109±3 [a,b]	119±4 [a,b]	118±1 [a,b]	117±5 [a,b]	112±3 [a,b]
CO (ml min⁻¹)	95±6	97±6	90±3	92±4	93±4	101±7
	75±5 [a]	77±8 [a]	69±7 [a]	65±4 [a]	77±3 [a]	76±4 [a]
	70±4 [a]	58±6 [a]	57±5 [a]	55±4 [a]	67±4 [a]	68±5 [a]
HR (beats min⁻¹)	362±10	359±11	348±9	362±9	363±12	375±14
	329±7 [a]	325±12 [a]	320±6 [a]	344±9 [a]	328±8 [a]	345±12 [a]
	403±9 [a,b]	387±15 [a,b]	402±6 [a,b]	403±5 [a,b]	415±10 [a,b]	393±15 [a,b]
MCFP (mmHg)	3.9±0.3	4.1±0.2	3.7±0.2	3.6±0.1	3.8±0.2	4.0±0.3
	3.1±0.2 [a]	3.3±0.1 [a]	2.9±0.2 [a]	2.9±0.1 [a]	3.2±0.3 [a]	3.0±0.2 [a]
	5.3±0.4 [a,b]	5.9±0.3 [a,b]	5.3±0.2 [a,b]	5.1±0.2 [a,b]	5.4±0.5 [a,b]	4.9±0.4 [a,b]
R_a (mmHg min ml⁻¹)	0.98±0.04	0.95±0.05	1.02±0.04	0.99±0.04	1.02±0.03	0.94±0.06
	0.88±0.04	0.86±0.06	1.02±0.09	1.11±0.06	0.90±0.04	0.82±0.07
	1.60±0.09 [a,b]	2.02±0.30 [a,b]	2.21±0.26 [a,b]	2.23±0.17 [a,b]	1.78±0.13 [a,b]	1.65±0.12 [a,b]
R_v (mmHg min ml⁻¹)	0.030±0.004	0.032±0.002	0.030±0.003	0.029±0.002	0.030±0.002	0.031±0.004
	0.028±0.002	0.032±0.004	0.030±0.005	0.030±0.002	0.028±0.003	0.026±0.002
	0.061±0.006 [a,b]	0.090±0.011 [a,b]	0.080±0.008 [a,b]	0.076±0.006 [a,b]	0.066±0.007 [a,b]	0.057±0.004 [a,b]

[a] Significantly (P<0.05) different from the corresponding baseline readings prior to any drug treatment. [b] Significantly (P<0.05) different from the corresponding readings after mecamylamine injection.

Figure 12 Effects (mean ± s.e.mean) of i.v. infusions of DEA/NO (4, 32 and 256 μg kg^{-1} min^{-1} shown as effective depressor doses, ED$_{30}$, ED$_{80}$ and ED$_{100}$, respectively), SNAP (4, 32 and 256 μg kg^{-1} min^{-1}), CpCr(NO)$_2$(ONO) (4, 32 and 256 μg kg^{-1} min^{-1}), SNP (8, 32 and 128 μg kg^{-1} min^{-1}), NTG (0.2, 0.8 and 6.4 μg kg^{-1} min^{-1}) or equivalent volumes of vehicle (0.9% NaCl) on mean arterial pressure (MAP) and arterial resistance (R$_a$) in six groups of rats (n = 6 each) pretreated i.v. with mecamylamine (3.7 μmol kg^{-1}) and noradrenaline (6.8 nmol kg^{-1} min^{-1}). All measurements were obtained 8 min after the infusion of a drug or vehicle. [a] Significantly different (P<0.05) from the corresponding values in the vehicle group. [b] Significantly different from the corresponding values in the NTG group. [e] Significantly different from the corresponding values in the CpCr(NO)$_2$(ONO) group.

Figure 13 Effects (mean ± s.e.mean) of i.v. infusions of DEA/NO (4, 32 and 256 μg kg^{-1} min^{-1} shown as effective depressor doses, ED$_{30}$, ED$_{80}$ and ED$_{100}$, respectively), SNAP (4, 32 and 256 μg kg^{-1} min^{-1}), CpCr(NO)$_2$(ONO) (4, 32 and 256 μg kg^{-1} min^{-1}), SNP (8, 32 and 128 μg kg^{-1} min^{-1}), NTG (0.2, 0.8 and 6.4 μg kg^{-1} min^{-1}) or equivalent volumes of vehicle (0.9% NaCl) on heart rate (HR) and cardiac output (CO) in six groups of rats (n = 6 each) pretreated i.v. with mecamylamine (3.7 μmol kg^{-1}) and noradrenaline (6.8 nmol kg^{-1} min^{-1}). All measurements were obtained 8 min after the infusion of a drug or vehicle. [a] Significantly different (P<0.05) from the corresponding values in the vehicle group.
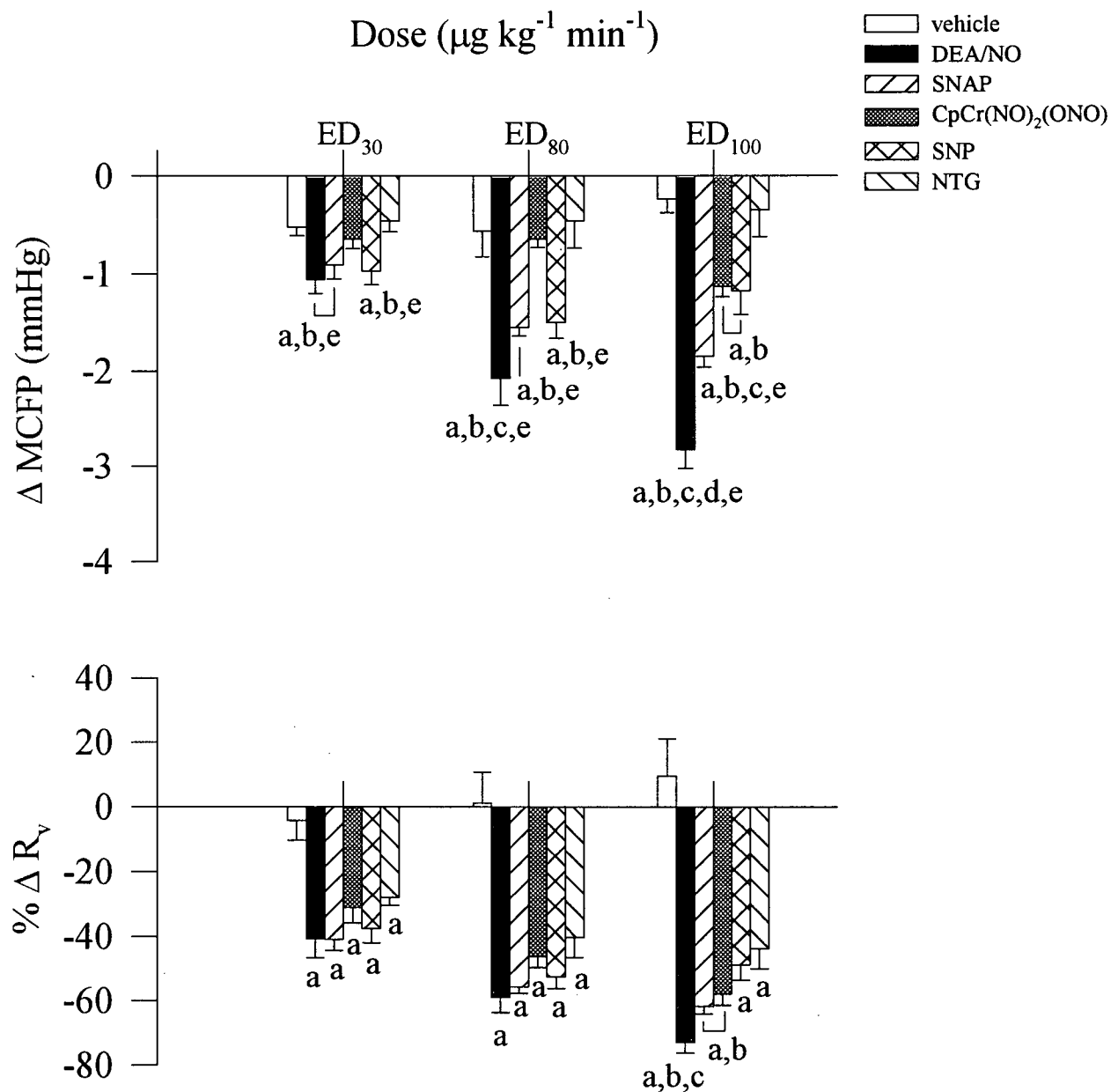
Figure 14 Effects (mean ± s.e.mean) of i.v. infusions of DEA/NO (4, 32 and 256 μg kg⁻¹ min⁻¹ shown as effective depressor doses, ED_{30}, ED_{80} and ED_{100}, respectively), SNAP (4, 32 and 256 μg kg⁻¹ min⁻¹), CpCr(NO)₂(ONO) (4, 32 and 256 μg kg⁻¹ min⁻¹), SNP (8, 32 and 128 μg kg⁻¹ min⁻¹), NTG (0.2, 0.8 and 6.4 μg kg⁻¹ min⁻¹) or equivalent volumes of vehicle (0.9% NaCl) on mean circulatory filling pressure (MCFP) and venous resistance (R_v) in six groups of rats (n = 6 each) pretreated i.v. with mecamylamine (3.7 μmol kg⁻¹) and noradrenaline (6.8 nmol kg⁻¹ min⁻¹). All measurements were obtained 8 min after the infusion of a drug or vehicle. [a] Significantly different (P<0.05) from the corresponding values in the vehicle group. [b] Significantly different from the corresponding values in the NTG group. [c] Significantly different from the corresponding values in the SNP group. [d] Significantly different from the corresponding values in the SNAP group. [e] Significantly different from the corresponding values in the CpCr(NO)₂(ONO) group.
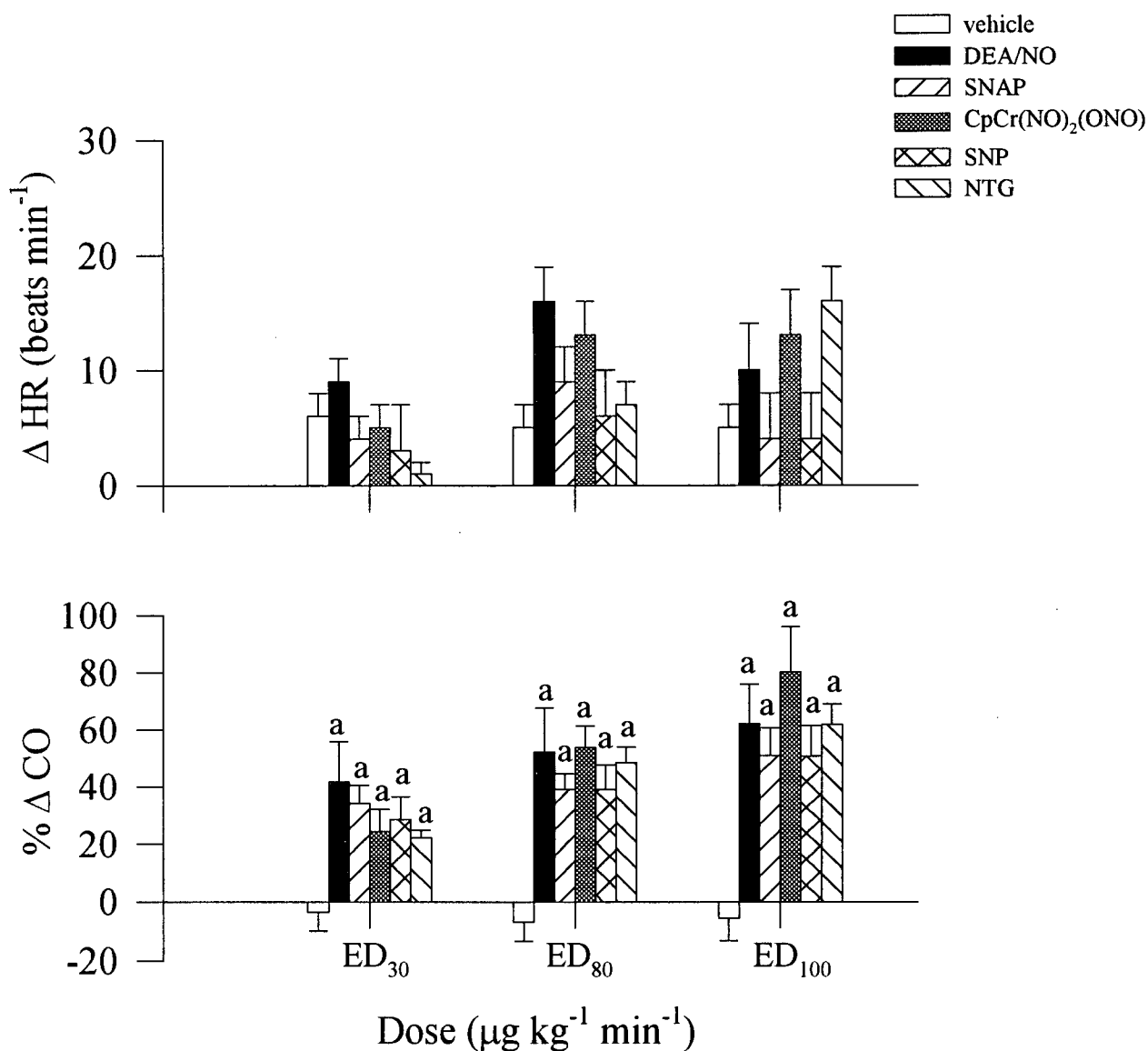
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did not significantly lower MAP except at the highest dose (Figure 12). While DEA/NO, SNAP, $CpCr(NO)_2(ONO)$, SNP and NTG elicited comparable reductions in R_a at the ED_{30} doses, DEA/NO, SNAP, $CpCr(NO)_2(ONO)$ and SNP caused greater reductions in R_a than did NTG at higher doses (Figure 12). Curve analysis revealed that DEA/NO, SNAP, $CpCr(NO)_2(ONO)$ and SNP caused significantly greater reductions of R_a than did NTG. The onset and duration of action of the five nitrovasodilators were rapid (within s) and short, respectively, as MAP returned to pre-drug level soon after the infusion was terminated (data not shown).

DEA/NO, SNAP and SNP dose-dependently lowered MCFP, $CpCr(NO)_2(ONO)$ reduced MCFP only at the highest dose; whereas NTG did not alter MCFP at any dose (Figure 14). Curve analysis demonstrated that DEA/NO caused greater reductions of MCFP than did SNAP, $CpCr(NO)_2(ONO)$ and SNP. DEA/NO, SNAP, $CpCr(NO)_2(ONO)$, SNP and NTG caused dose-dependent reductions in R_v (Figure 14). Curve analysis showed that the venodilator effects of DEA/NO, SNAP, $CpCr(NO)_2(ONO)$ were similar to those of SNP but greater than those of NTG.

4. Discussion

4.1 Zaprinast Has Arterial and Venous Dilator, and Negative Chronotropic Actions

The results of this thesis show that the ED_{40} and ED_{80} doses of zaprinast and SNP caused similar dose-dependent reductions in mean arterial pressure and arterial resistance in intact rats, i.e., both drugs reduced mean arterial pressure by decreasing arterial resistance, since cardiac outputs were unchanged. As expected with vasodilator drugs (Pang, 1994), both caused greater depressor responses following the impairment of autonomic reflexes with mecamylamine and the elevation of vasomotor tone with i.v. infusion of noradrenaline. The hypotensive responses to zaprinast were slightly greater than those to SNP. Hypotensive responses to both in the intact and areflex animals were also due to reduced arteriolar resistance, since cardiac outputs were not reduced. The vasorelaxant and depressor actions of zaprinast *in vitro* and *in vivo*, respectively, are well-documented and have been attributed to the rises of cGMP concentrations in vascular smooth muscle cells. Zaprinast dose-dependently increased cGMP levels in isolated human, bovine and rat aortae at a concentration (1 μM) near its IC_{50} for inhibition of phosphodiesterase type V; while cAMP levels were unaltered with concentrations of zaprinast as high as 1 mM (Lugnier et al., 1986). Similarly, Souness et al. (1989) and Ahn et al. (1989) also demonstrated that zaprinast (1-100 μM) caused endothelium-dependent relaxations and induced dose-dependent increases in cGMP but not cAMP in rat and rabbit aortae. Furthermore, zaprinast (10-30 μM) potentiated the relaxant activities of agents whose actions are mediated by cGMP such as NTG (Martin et al., 1986; Ishibashi et al., 1995; Satake et al., 1996) and SNP (Harris et al., 1989), but not those working via cAMP such as isoproterenol (Martin et al., 1986) *in vitro*. Phenylephrine-induced contractions of bovine intrapulmonary artery and vein (Ignarro et al., 1987b) and dog

saphenous vein (Villanueva et al., 1991) as well as prostaglandin $F_{2\alpha}$-induced contractions of porcine coronary artery (Merkel et al., 1992) were attenuated by zaprinast (1 nM-100 μM). Intravenous bolus injections of zaprinast (3-30 mg kg^{-1}) dose-dependently increased plasma, aortic and urine cGMP levels and decreased mean arterial pressure in anaesthetized and conscious, spontaneously hypertensive rats (Merkel et al., 1992; Dundore et al., 1991, 1993), and rats with renal artery ligation (Anderson & Drew, 1997). In anaesthetized dogs (Weishaar et al., 1990), normotensive (Trapani et al., 1991) and spontaneously hypertensive (McMahon et al., 1992) rats, i.v. bolus injections (0.03-3 mg kg^{-1}) or infusion (0.03-3 mg kg^{-1} min^{-1}) of zaprinast dose-dependently reduced blood pressure and total peripheral resistance. Plasma levels of cGMP in anaesthetized spontaneously hypertensive rats infused with zaprinast were shown to increase in parallel with the depressor action of the drug (McMahon et al., 1992). Dundore et al. (1992) reported that in conscious rats, hindquarter and mesenteric resistance were significantly decreased by zaprinast at lower doses (10 and 18 mg kg^{-1}), renal resistance was reduced only at the highest dose (30 mg kg^{-1}). Taken together, these findings support the notion that zaprinast exerts its vasorelaxant effects *in vitro* and hypotensive effects *in vivo* by inhibiting phosphodiesterase type V and promoting cGMP-dependent vasodilatation.

In this study, zaprinast elicited less tachycardia than SNP in intact rats. The tachycardiac responses to SNP were eliminated by ganglionic blockade indicating that the heart rate responses were due to hypotension-induced reflex activation of the sympathetic nervous system. Contrary to SNP, zaprinast (1.5 and 3 mg kg^{-1} min^{-1}) reduced heart rate following ganglionic blockade suggesting that the drug was negatively chronotropic. Direct negative chronotropism may explain why equihypotensive doses of zaprinast caused less tachycardia than did SNP in intact rats. The negative chronotropic action of zaprinast may be beneficial in the management of myocardial ischaemia. Heart rate is a major determinant of

myocardial tolerance to ischaemia. Tachycardia causes imbalance between oxygen supply and demand, thus bradycardic drugs may possess anti-ischaemic effect. Indeed, zaprinast (10 mg kg^{-1}) has been shown to reduce heart rate during myocardial ischaemia induced by overdrive pacing in conscious rabbits (Szilvassy et al., 1993). However, Dundore et al. (1992) and Anderson & Drew (1997) reported that heart rate was unaltered by zaprinast (3-30 mg kg^{-1} or 3 mg kg^{-1} bolus followed by 3 mg kg^{-1} min^{-1} infusion) in conscious rats with and without renal artery ligation. In the isolated rat heart (Pabla et al., 1995a) and guinea pig right atria (Noguchi et al., 1998), 0.1 mM and 0.01 mM of zaprinast had no significant effect on heart rate. Inconsistencies in heart rate responses to zaprinast could be explained by the different preparations, species or doses of zaprinast used. More importanly, the effects of cGMP, the accumulation of which is enhanced by zaprinast, on heart rate has yet to be clarified. The membrane-permeable analogues of cGMP, dibutyryl-cGMP (42 mM) and 8 bromo-cGMP (1 mM) have been demonstrated to slow beating in cultured rat cardiac cells (Krause et al., 1972; Balligand et al., 1993). The release of cGMP induced by atrial natriuretic peptide exerted a negative chronotropic effects in the isolated rat atria (Favaretto et al., 1997). Conversely, 8-bromo-cGMP (0.5-2 mM) increased heart rate in the guinea pig sinoatrial node/atrial preparation (Musialek et al., 1997). Patch-clamping experiments using cardiac pacemaker cells should provide information on the types of ion channels involved in the cGMP-mediated regulation of heart rate and resolve the present controversy.

Neither zaprinast nor SNP reduced mean circulatory filling pressure or venous resistance in intact rats. Mean circulatory filling pressure was significantly elevated by both doses of zaprinast and the high dose of SNP. Following ganglionic blockade, zaprinast as well as SNP similarly reduced mean circulatory filling pressure and venous resistance suggesting dilatation of capacitance vessels. The high dose of either zaprinast or SNP did not lower mean

circulatory filling pressure and venous resistance further suggesting that maximum reductions of the two parameters had been achieved at the low dose. These observations indicate that the zaprinast- and SNP-induced increases in mean circulatory filling pressure in intact rats were due to hypotension-induced reflex venoconstriction. Dr. Pang's laboratory has previously shown that endogenous sympathetic tone must be abolished in order to reveal the venodilator activity of hypotensive drugs such as verapamil (Waite et al., 1988), NTG (D'Oyley et al., 1989), isoprenaline (Abdelrahman & Pang, 1990) and calcitonin gene-related peptide (Abdelrahman & Pang, 1992) in conscious and anaesthetized rats. The suppression of sympathetic nerve activity was also indicated in the study by Hirakawa et al. (1992), who demonstrated that the phosphodiesterase type III inhibitor, milrinone, reduced mean circulatory filling pressure and venous resistance in spinal-anaesthetized dogs continuously infused with adrenaline. It should be noted that not all vasodilator agents reduce mean circulatory filling pressure. Hydralazine caused a steep rise in mean circulatory filling pressure in intact rats and no change in mean circulatory filling pressure in ganglion-blocked rats (D'Oyley et al., 1989) suggesting a lack of venodilator action. Pinacidil, a potassium channel opener, did not reduce mean circulatory filling pressure or venous resistance in intact or ganglion-blocked rats (Waite et al., 1995). Atrial natriuretic peptide has also been shown to cause minimal venodilatation as indicated by its lack of effect on mean circulatory filling pressure in spinal cord-transected rats given noradrernaline to maintain vascular tone (Trippodo et al., 1986). The results of this study show that in ganglion-blocked rats with elevated vasomotor tone, zaprinast has similar dilator activity as SNP in resistance and capacitance vessels.

Cardiac output was not altered by either zaprinast or SNP in intact rats. In areflexic rats, both doses of zaprinast and SNP increased cardiac output; zaprinast at the low dose

caused markedly greater increment of cardiac output than did SNP. The increase in cardiac output elicited by both drugs were likely due to reductions in flow resistance, arterial and venous resistances, thereby facilitating venous return. The greater increase in cardiac output (despite bradycardia which should reduce cardiac output) caused by the low dose of zaprinast relative to SNP was likely secondary to the greater, though statistically insignificant, reductions in arterial and venous resistances elicited by zaprinast. The lesser increase in cardiac output at the high dose of zaprinast was probably due to the lesser decrement in venous resistance.

Trapani et al. (1991) reported that i.v. infusion of zaprinast (1 and 2 mg kg^{-1} min^{-1}) reduced mean arterial pressure and total peripheral resistance in anaesthetized intact rats as well as rats with autonomic blockade due to atropine and propranolol. However, in contrast to my results on cardiac output and heart rate, zaprinast (1 and 2 mg kg^{-1} min^{-1}) increased cardiac output but did not alter heart rate in intact rats, and increased cardiac output as well as heart rate in areflex rats. These discrepancies could be attributed to the differential experimental conditions in the two studies. The acute placement of an electromagnetic flow probe around the ascending aorta for the measurement of cardiac output in the Trapani study required more invasive surgery than the relatively non-invasive surgical procedure needed for the injection of microspheres in my study. It is well documented that open-chest surgery causes the release of large quantities of vasopressor agents such as catecholamines, angiotensin II and vasopressin (McKenzie et al., 1967; McNeil & Pang, 1982; Pang, 1983; Anand, 1986; Udelsman & Holbrook, 1994), as indicated by the higher baseline blood pressure and total peripheral resistance, 112 mmHg and 0.593 mmHg ml^{-1} min kg, respectively, in the Trapani study versus 94 mmHg and 0.450 mmHg min^{-1} min kg in my study. With elevated vasomotor tone, zaprinast caused greater reductions in mean arterial pressure and total peripheral resistance,

which in turn, led to increases in cardiac output in intact rats. In this sense, the haemodynamic status of intact rats in Trapani's study resembled those of the areflex rats continuously infused with noradrenaline in this study. Furthermore, the methods of autonomic blockade were different between the two studies. The observed increase in heart rate in areflex rats of the Trapani study was likely due to incomplete blockade of the autonomic nervous system by the selected doses of atropine and propranolol. The reflex tachycardia might have masked the direct negative chronotropic effect of zaprinast. My experiments used the ganglion blocker, mecamylamine, which has been shown to block ganglionic transmission effectively for more than 2 h (Wang & Pang, 1991).

The present findings indicate that zaprinast equally dilates both resistance and capacitance vessels *in vivo*. The arterial and venous dilator efficacies of zaprinast are comparable to those of SNP which is one of the most efficacious vasodilator agents known. It is of interest that zaprinast was reported to reverse nitroglycerin-induced tolerance (Pagani et al., 1993; De Garavilla et al., 1996) and protect the heart against overdrive pacing-induced myocardial ischaemia (Szilvassy et al., 1995). The vascular profile of zaprinast and other members of phosphodiesterase type V inhibitors should be investigated in cardiovascular diseases.

To summarize, zaprinast dose-dependently reduced mean arterial pressure and arterial resistance in both anaesthetized intact and areflex rats. Zaprinast did not alter venous resistance and cardiac output, and slightly increased heart rate and mean circulatory filling pressure in intact rats. In areflex rats, zaprinast significantly decreased mean circulatory filling pressure, venous resistance as well as heart rate. The vasodilator activity of zaprinast is similar to that of SNP.

4.2 Arterial Versus Venous Actions of DEA/NO, SNAP and CpCr(NO)₂(ONO)

The results of this thesis show that i.v. infusion of DEA/NO, SNAP and CpCr(NO)$_2$(ONO), similar to SNP, caused dose-dependent reductions in mean arterial pressure and arterial resistance, and increases in cardiac output in areflex rats with elevated vasomotor tone. The hypotensive effects of CpCr(NO)$_2$(ONO) were significantly less than those of DEA/NO, SNAP and SNP at the ED$_{30}$ and ED$_{80}$ doses, but similar to those of DEA/NO, SNAP and SNP at the highest dose. All four drugs lowered blood pressure by decreasing arteriolar resistance, since cardiac output readings were not reduced. Intravenous infusion of NTG dose-dependently decreased arterial resistance but did not significantly reduce mean arterial pressure except at the highest dose. The arteriolar dilator effects of NTG were significantly less than those of DEA/NO, SNAP, CpCr(NO)$_2$(ONO) and SNP. The reduced ability of i.v. infused NTG to lower mean arterial pressure is therefore a consequence of its lesser arteriolar dilator action. Heart rate was not affected by any of the five drugs. Since the rats were ganglion-blocked with mecamylamine, the lack of effect on heart rate indicates that these drugs do not have any direct chronotropic action. Cardiac output was similarly increased by all five compounds. The increases in cardiac output were the result of reduced arterial and venous resistances. However, the possibility that the tested nitrovasodilators also exerts positive inotropic action on the heart cannot be excluded. In fact, the effects of NO on myocardial contractility remain controversial (see later).

In accordance with the findings of this thesis, Diodati et al (1993a) demonstrated that DEA/NO (0.2-8 µg kg^{-1}) was equipotent to SNP in lowering mean arterial pressure and systemic vascular resistance in anaesthetized rabbits, although neither DEA/NO nor SNP affected cardiac output. Heart rate was unaltered by both drugs. Again, the reduction in blood pressure was not associated with a fall in cardiac output, indicating that the hypotensive effects

of both agents were not due to negative inotropy but arteriolar dilatation. The effect of DEA/NO was short-lived, with peak reductions in blood pressure and systemic vascular resistance at 1 min and no significant effects 5 min after i.v. injection. In anaesthetized rats, DEA/NO (16-155 μg kg^{-1}) decreased mean arterial pressure and heart rate, and increased arteriolar diameter (Shan et al., 1997). DEA/NO (1-2 μg kg^{-1} min^{-1}) has also been reported to dose-dependently lower mean arterial pressure and systemic vascular resistance without altering heart rate or cardiac output in intact newborn lambs (Vanderford et al., 1994). *In vitro* studies show that neither the nitric oxide synthase inhibitor, L-NMMA, nor the absence of intact endothelium, had any significant effect on the vasorelaxant potency of DEA/NO in rabbit thoracic aorta, suggesting that the drug does not mediate vasorelaxation by stimulation of eNOS. Moreover, DEA/NO induced substantial rises in cGMP, the effect of which was attenuated by methylene blue (Morley et al., 1993). Therefore, it is apparent that the mechanism of action of DEA/NO involves spontaneous release of NO, which in turn, stimulates guanylate cyclase in vascular smooth muscle cells resulting in cGMP accumulation and vasorelaxation.

SNAP is also a known NO releaser (Feelisch & Noack, 1987; Travis et al., 1996) and a well-documented vasodilator *in vitro* and *in vivo*. In bovine coronary artery (Ignarro et al., 1981), intrapulmonary artery and vein (Edwards et al., 1983) as well as rabbit aorta and mesenteric artery (Mathews & Kerr, 1993), SNAP (0.1-10 μM) increased cGMP levels which preceded the onset of vasorelaxation. Intravenous bolus injections of SNAP (3-1000 μg kg^{-1}) in anaesthetized cats dose-dependently and similarly reduced systemic arterial pressure and vascular resistance as did SNP and NTG, without altering cardiac output (Ignarro et al., 1981). In conscious rats, SNAP (5 μg kg^{-1} min^{-1}) caused hypotension, tachycardia and reduction of stroke index (Gardiner et al., 1993). SNAP and NTG were shown to be equipotent

vasodilators in dogs (Lee et al., 1990) and rabbits (Shaffer et al., 1992). In accord with my findings, Zanzinger et al. (1996) demonstrated that during noradrenaline infusion (0.001 μg kg^{-1} min^{-1}), SNAP (25 μg kg^{-1}) decreased total peripheral resistance in anaesthetized pigs following vagotomy and ganglionic blockade. In conscious rats with congestive heart failure, SNAP (5-10 μg min^{-1}) significantly reduced both left ventricular end-diastolic and peak systolic pressures, suggesting that SNAP decreases both preload and afterload (Bauer & Fung, 1991). NTG, on the other hand, is virtually devoid of effects on left ventricular peak systolic pressure under similar experimental conditions, consistent with the predominant venodilator action of the drug. In contrast to my results, Vanderford et al. (1994) showed that SNP reduced mean arterial pressure more than DEA/NO, SNAP and NTG during U46619-induced pulmonary hypertension. Differences among the various studies could be due to variations in species, doses, modes of drug administration and experimental conditions. SNAP resembled SNP and NTG in both the onset (immediate) and duration (1-3 min) of the hypotensive response (Ignarro et al. 1981), consistent with the observations of my experiments (data not shown).

Contrary to DEA/NO, there is evidence indicating that the vasorelaxant activities of SNAP and other S-nitrosothiols are not entirely dependent on the spontaneous decomposition of these compounds to NO. Also, it appears that entry of these compounds into vascular smooth muscle cells is not mandatory for the manifestation of their effects (Kowaluk & Fung, 1990; Mathews & Kerr, 1993). Travis et al. (1996) recently reported that both the L- and D-isomers of SNAP decompose equally to NO, but the former is a more potent generator of cGMP than the latter in cultured porcine aortic smooth muscle cells. Furthermore, lower concentration (0.1 μM) of L-SNAP significantly elevates cGMP levels without decomposing to NO suggesting that the extracellular or intracellular release of NO is not the only

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mechanism by which SNAP generates cGMP. The steroisomeric configuration appears to be a crucial determinant of the ability of SNAP to generate cGMP. The authors therefore proposed the possibility of a membrane bound S-nitrosothiol recognition site or receptor which is linked to particulate guanylate cyclase. Alternatively, L- and D-SNAP may differentially interact with atrial natriuretic peptide receptors which are particulate guanylate cyclases (Travis et al., 1996).

The vasorelaxant activities of nitrovasodilators seem to involve not only cGMP-dependent but also cGMP-independent pathways. It has been reported that NO and cGMP stimulate Ca^{2+}-ATPase activity (Cornwell et al., 1991) and decrease intracellular Ca^{2+} concentrations by inhibiting Ca^{2+} entry through voltage-gated Ca^{2+} channels in vascular muscle cells (Blatter & Wier, 1994). The NO-mediated elevation of cGMP levels may stimulate Na^+/K^+-ATPase promoting vasorelaxation (Rapoport, 1986). Conversely, Gupta et al. (1994) showed that NO stimulated this enzyme directly without increasing cGMP. Another mechanism by which nitrovasodilators cause relaxation is the activation of the Ca^{2+}-dependent K^+ (BK_{Ca}) channel. Although multiple classes of K^+ channels are expressed at varying densities in vascular smooth muscle, BK_{Ca} channels are the predominant subtype (Bolton & Beech, 1992). Carrier et al. (1997) illustrated that relaxation of rat mesenteric microvessels induced by SNAP and SNP was impaired by the blockade of the BK_{Ca} channel, or inhibition of protein kinase G (PKG), suggesting that nitrovasodilator-mediated relaxant responses involve at least in part cGMP-dependent phosphorylation events and subsequent opening of BK_{Ca} channels and membrane hyperpolarization. Indeed, NTG was shown to cause relaxation in rabbit aorta via cGMP-mediated activation of BK_{Ca} channels (Ishibashi et al., 1995). The vasorelaxant effects, but not the increase in cGMP, produced by NTG were sensitive to inhibition by the BK_{Ca} channel blockers, charybdotoxin and iberiotoxin. Zanzinger et al.

(1996) demonstrated that charybdotoxin or iberiotoxin increased total peripheral resistance, reduced venous and pulmonary compliance, and diminished SNAP-induced vasodilatation in the absence or presence of continuous noradrenaline infusion in anaesthetized areflex pigs, indicating that activation of BK_{Ca} channels is an important mechanism by which NO attenuates the constrictor tone of resistance and capacitance vessels *in vivo*. On the other hand, there is evidence showing that NO directly stimulates BK_{Ca} channels and produces relaxations in rabbit carotid arteries (Najibi et al., 1994) and thoracic aorta (Bolotina et al., 1994) without increasing cytosolic cGMP levels. Interestingly, Plane et al. (1998) reported that NO-evoked relaxation of the rabbit carotid artery can be mediated by three distinct mechanisms: a) a cGMP-dependent, voltage-independent pathway, b) cGMP-mediated smooth muscle repolarizaion and c) cGMP-independent, charybdotoxin-sensitive smooth muscle repolarization. The relative contribution of these pathways to changes in vascular tone depends on the source of NO. Relaxation and repolarization to both authentic NO and endothelium-derived NO in isolated rabbit carotid artery appear to be mediated by parallel cGMP-dependent and -independent pathways. Conversely, relaxation to NO donors SIN-1 and SNAP appear to be mediated entirely via cGMP-dependent pathway. The NO-induced, cGMP-independent membrane repolarization most likely involves the activation of BK_{Ca} channels (Bolotina et al., 1994; Plane et al., 1998). It is noteworthy that in hypercholesterolemic rabbits, cGMP-mediated relaxation is impaired while normal smooth muscle relaxation to both endothelium-derived and authentic NO is maintained via stimulation of charybdotoxin-sensitive K^+ channels (Najibi et al., 1994; Najibi & Cohen, 1995). The authors therefore suggested that under physiological conditions ~70% of the ability of NO to induce vasorelaxation persists in the absence of a change in smooth muscle membrane potential; but under pathological conditions, cGMP-independent repolarization may provide

the dominant route for arterial dilatation. It can be concluded that SNAP and possibly other S-nitrosothiols cause vasodilatation via multiple and more complex mechanisms relative to DEA/NO.

The chrontropic and inotropic actions of nitrovasodilators and nitric oxide have yet to be elucidated. Reports in the literature are highly divergent and contradictory. Besides, different cardiac preparations, species, NO donors and experimental conditions were employed in different studies making direct comparison difficult. The effects of NO on heart rate have not been extensively investigated relative to those on myocardial contractility. The findings of this thesis indicate that none of DEA/NO, SNAP, SNP, NTG or $CpCr(NO)_2(ONO)$ exhibited any direct chronotropic activity. Myocardial contractile function was not measured in the experiments. In agreement with my results, heart rate was unaltered by SNP in isolated rat ventricular myocytes (Balligand et al., 1993). Somatic delivery of the human eNOS gene into spontaneouly hypertensive rats via i.v. injection led to a sustained lowering of blood pressure without any significant changes in heart rate (Lin et al., 1997). In isolated atria of the rat, low concentrations of SIN-1 did not affect heart rate whereas high concentrations reduced heart rate (Kennedy et al., 1994). Pabla & Curtis (1995b) noted significant bradycardia in response to SNP (10 μM) in isolated rat hearts both before the onset of ischaemia and throughout the ischaemic period (60 min); interestingly, bradycardia was also evident after NO synthase inhibition by L-NAME.

In patients investigated for chest pain, heart rate was decreased during global intracoronary infusion of SNP at a dose ($\leq$ 4 μg min^{-1}) that was previously shown to be devoid of systemic effects (Paulus et al., 1994). Conversely, De Marco et al. (1995) demonstrated that SNP (147 μg min^{-1}) increased heart rate in heart transplant recipients (Levine et al., 1986) prior to sympathetic reinnervation suggesting that NO donors might stimulate pacemaker

activity independent of arterial baroreflex.

SNAP and SNP caused significant dose-dependent increases in heart rate at low concentrations (nanomolar to micromolar); but unlike SNP, SNAP did not produce a persistent negative chronotropic effect at higher concentrations (millimolar) in guinea pig sinoatrial node/atrial preparation (Musialek et al., 1997). The same authors also demonstrated that the positive chronotropy was due to stimulation of hyperpolarization-activated inward current (I_f) via NO/cGMP pathway in cardiac pacemaker cells. Taken together, it is likely that NO itself and nitrovasodilators affect cardiac pacemaker activity in a dose-dependent biphasic fashion.

Heart rate usually increases concomitantly with the hypotensive response to systemically administered NO donors. The tachycardia is most likely due to reflex activation of the sympathetic nervous system. However, there is evidence indicating that some NO donors may exert some of their cardiovascular effects via centrally-mediated mechanisms. For instance, Nurminen & Vapaatalo (1996) reported that intracerebroventricular (i.c.v.) administration of SNP (7-56 μg kg^{-1}) increased heart rate substantially and had no significant effect on blood pressure. The same doses were hypotensive when given intravenously in anaesthetized rats. In contrast, Ma & Long (1992) demonstrated that i.c.v. SNP (3-10 μg kg^{-1}) altered neither blood pressure nor heart rate, indicating that SNP does not exert its cardiovascular actions by central mechanisms. Interestingly, i.c.v. NTG decreased mean arterial pressure and increased heart rate, but intracisternal injection of the drug caused hypotension and bradycardia; the reflex tachycardia to i.v. NTG was less than that to i.v. SNP. The investigators therefore suggested that the tachycardic and bradycardic effects mediated by the forebrain and medulla, respectively, may modify the reflex chronotropic response to NTG. The central mechanism of action of NTG appears to involve the increase in the synthesis and release of noradrenaline, which then activates central α_2-receptors resulting in hypotension

(Ma & Long, 1991a, 1991b). Inhibition of endogenous NO formation in various brain areas has been shown to increase blood pressure and sympathetic nerve activity in cats, rabbits and rats (Shapoval et al., 1991; Togashi et al., 1992; Cabrera & Bohr, 1995). While central adminstration of DEA/NO, SNP and NTG into the lateral cerebral ventricle decreased blood pressure in anaesthetized cats and rats (Ma & Long, 1992; Hedge et al., 1994; Carbrera & Bohr, 1995), SNAP slightly increased blood pressure in conscious rats (Ota et al., 1993). Variations in the heart rate responses to different NO donors may be due to their differential mechanisms of NO release (see later) and/or abilities to cross the blood brain barrier thereby modifying cardiovascular responses via centrally-mediated pathways.

With respect to myocardial contractility, some investigators observed positive inotropic responses induced by SNP (Diamond et al., 1977; Sys et al., 1993) while others observed the opposite caused by SNP and NO itself (Brady et al., 1993; Flesch et al., 1997). Having stated a dose-dependent biphasic chronotropic response to NO, it is possible that the inotropic response to NO may also exhibit the same characteristic. Indeed, Kodja et al. (1995, 1996) demonstrated that low concentrations of DEA/NO, SNAP and NTG (10 µM) produced small increases in cGMP and contractility while high concentrations (100 µM) generated marked increases in cGMP but reduced contractility in rat ventricular myocytes and isolated hearts. Similarly, Mohan et al. (1996) reported a concentration-dependent biphasic contractile response to SNAP, SNP as well as to 8-bromo-cGMP and zaprinast in cat papillary muscle. A low-dose intracoronary or i.v. infusion of DEA/NO, SNAP, NTG or cGMP analogues induced a significant positive inotropic effect in the absence of changes in loading conditions in anaesthetized dogs (Raff et al., 1970; Preckel et al., 1997) and cats (Leite-Moreira et al., 1994). So far, the precise mechanisms whereby NO and/or cGMP affect cardiac contractility are not completely understood although numerous hypotheses have been postulated. Sherman et al.

(1997) suggested that inhibition of the cGMP-stimulated cAMP phosphodiesterase (phosphodiesterase type II) at low NO/cGMP concentrations and stimulation of PKG at high NO/cGMP levels might explain at least in part the positive and negative inotropy, respectively. There is evidence indicating that PKG plays a role in the modulation of L-type Ca^{2+} channel activity (Thakkar et al., 1988; Mery et al., 1991). Kirstein et al. (1995) demonstrated that in human atrial myocytes SIN-1 (1 pM-10 nM) had a stimulatory effect on Ca^{2+} current (I_{Ca}) consequent to cGMP-induced inhibition of cGMP-inhibited phosphodiesterase (phosphodiesterase type III), however, I_{Ca} was suppressed at higher concentrations. The direction of myocardial contractile response to NO and cGMP is determined by multiple factors such as the concentration of NO donors used, the integrity of the endothelium, and the presence of cholinergic or adrenergic stimulation (Mohan et al., 1996).

As mentioned in section 4.1, the venodilator activity of a drug is best revealed in animals with inactivation of the sympathetic nervous system and/or elevation of venomotor tone (Tabrizchi & Pang, 1992; Pang, 1994), rats in the second series of experiments were all given mecamylamine to suppress autonomic reflex and infused with noradrenaline to elevate venomotor tone. Under these conditions, DEA/NO, SNAP as well as SNP dose-dependently reduced MCFP. CpCr(NO)$_2$(ONO) lowered MCFP only at the highest dose. NTG, however, did not alter MCFP at any dose. The reductions of MCFP by DEA/NO were significantly greater than those produced by SNAP, CpCr(NO)$_2$(ONO) and SNP. All five drugs lowered R_v relative to the vehicle, with no significant differences among them at the ED$_{30}$ and ED$_{80}$ doses. Curve analysis showed that DEA/NO, SNAP, CpCr(NO)$_2$(ONO) and SNP caused greater reductions of R_v than did NTG at the highest dose. My results indicate that DEA/NO is the most efficacious in lowering MCFP among the five tested compounds. The venodilator efficacies of DEA/NO, SNAP and CpCr(NO)$_2$(ONO) are comparable to those of SNP but

greater than those of NTG. The venodilator actions of DEA/NO and CpCr(NO)$_2$(ONO) are first reported in the current thesis and those of SNAP is occasionally reported in the literature. SNAP was more potent than NTG in causing relaxation in PGF$_{2\alpha}$-precontracted porcine vena cordis magna (Kojda et al., 1994). Zanzinger et al. (1996) reported that SNAP (25 µg kg^{-1}) reversed the decrease in venous compliance during noradrenaline infusion in anaesthetized pigs. SNAP (5 µg kg^{-1} min^{-1}) was also shown to lower central venous pressure in conscious rats (Gardiner et al., 1993). The venous versus arterial actions of NTG are greatly dependent on the route of administration as briefly mentioned in the Introduction. Depressor effects are generally more pronounced with i.v. bolus injections than with i.v. infusions. For example, i.v. bolus injections of NTG (25, 50 µg kg^{-1}) were more effective in lowering total peripheral resistance than mean circulatory filling pressure in anaesthetized dogs (Ito & Hirakawa, 1984); however, at higher doses (50-200 µg kg^{-1}) NTG also decreased mean circulatory pressure and venous resistance. D'Oyley et al. (1989) reported that NTG (0.4-13 µg kg^{-1} min^{-1}) lowered mean circulatory filling pressure in anaesthetized, hexamethonium-treated rats. It remains unclear why NTG-induced decreases in mean circulatory filling pressure were not observed in my experiments.

The differential abilities of the five NO donors to dilate veins may be attributed to the differences in the amount and/or rate of NO released from these agents. Using a chemiluminescence technique to monitor NO evolution, Morley et al. (1993) demonstrated that both DEA/NO and SNAP spontaneously released NO, whereas SNP and NTG generated negligible NO under similar conditions. The release of NO from DEA/NO was shown to be controlled and predictable following first order kinetics, in sharp contrast to the erratic release from SNAP. In fact, it has been shown that the NO-generating activity of SNAP might also involve a metabolic activation step similar to that of NTG, in addition to spontaneous release

(Kowaluk & Fung, 1990) and other mechanisms as mentioned above. The differential mechanisms of NO release, which in turn, determine the amount and rate of NO released from these compounds, may in part account for their differential venous effects. It remains unclear why these differences were not manifested in the arterial vasculature. One possibility is that capacitance vessels may be more sensitive than resistance vessels to small variations of NO concentration in situ.

The intrinsic differences in the L-arginine/NO pathway between arteries and veins have been held accountable for the differential vasodilator responses to NO donors. It is well-known that blood vessels of different anatomical origin, and even pre- and post-capillary vessels within the same vascular bed respond differently to the same pharmacological and physiological stimulus (Shepherd & Vanhoutte, 1975; Vanhoutte, 1978) suggesting the possibility of significant differences in the role of endothelium in blood vessels from different sites within the same species (Thom et al., 1987). In addition, the differential behaviour of arteries and veins are also dependent on the species from which the blood vessels are obtained, the concentration of the vasoactive substance used and the integrity of the endothelium. For instance, in pulmonary vasculature, endogenous NO-mediated relaxation plays a larger role in veins than in arteries in lambs and pigs (Bansal et al., 1993; Feletou et al., 1995), similarly modulates arterial and venous tone in cattles (Ignarro et al., 1988), and acts predominantly in arteries in ferrets and guinea pigs (Gao et al., 1995; Shi et al, 1997). Acetylcholine (ACh; 0.1-3 μM) produced endothelium-dependent relaxations in isolated human renal, colic, pulmonary, uterine, transverse cervical, brachial and coeliac branch ateries as well as saphenous veins but contractions in coronary arteries (Thom et al., 1987). De Mey & Vanhoutte (1982) reported the heterogeneous responses of several canine arteries and veins (pulmonary, splenic, femoral and saphenous) to different vasoactive substances. After preconstiction with noradrenaline

and in the presence of endothelium, isoprenaline (0.01-1 μM) relaxed all the veins but not the arteries, whereas ACh (0.03-1 μM) similarly and dose-dependently relaxed all the arteries, transiently and moderately relaxed femoral, saphenous and splenic veins but not pulmonary veins. Higher concentrations (1-100 μM) of ACh, however, caused contractions in all the veins but not the arteries. Therefore, the three canine systemic and pulmonary arteries appear to exhibit fairly homogenous endothelium-dependent relaxations in contrast to their venous counterparts during exposure to noradrenaline. All the veins but not the arteries contracted to ACh under basal conditions. Other qualitative differences in responsiveness to various stimuli between arteries and veins obtained from the same species include the presence of a relaxant effect of arachidonic acid in certain de-endothelialized arteries, the absence of contraction caused by anoxia in certain de-endothelialized veins, and the greater response of veins to the endothelium-independent relaxant effect of adenosine. The heterogeneity of endothelium-dependent responses to some of these stimuli may be due to the difference in the ability/quantity of endogenous NO production between arteries and veins, and/or in the sensitivity of arterial and venous smooth muscle to the released NO. Seidel & LaRochelle (1987) provided evidence that the latter explanation is unlikely using dog femoral artery and vein in a "sandwich" preparation. The de-endothelialized saphenous vein, when "sandwiched" with an intact femoral artery, relaxed at low ACh concentrations (0.001-0.1 μM); at high concentrations (0.1-1 μM), the contractile response to noradrenaline was obliterated. On the other hand, de-endothelialized femoral artery "sandwiched" with an intact saphenous vein did not relax in response to ACh. Furthermore, SNP equally relaxed the saphenous vein and femoral artery. Stimulated endogenous NO production measured as endothelium-dependent relaxation was substantially greater in arteries than in veins (De Mey & Vanhoutte, 1982; Luscher et al., 1988; Kojda et al., 1994). These findings suggest that the smaller relaxant

response observed in the saphenous vein is due to the inability of the venous endothelium to release adequate NO to produce a comparable magnitude of relaxation rather than that the venous smooth muscle cells are insensitive to NO. The differential responses to endothelium-dependent vasodilators between arteries and veins are not limited to dogs. Internal mammary arteries and veins, and saphenous veins obtained from patients undergoing coronary bypass surgery were studied (Luscher et al., 1988). Not surprisingly, endothelium-dependent relaxation in response to ACh (1 nM-100 μM) was weak in both veins with a maximal value of ~20% as opposed to ~85% in the artery, indicating heterogeneous endothelial reactivity in human arteries and veins. Again, SNP (1 nM-10 μM) induced similar magnitude of relaxation in all vessels. However, D'Orleans-Juste et al. (1992) noted a comparable release of NO from endothelial cells cultured from both bovine aorta and vena cava, arguing that the smaller endothelium-dependent relaxation in veins does not result from the reduced ability of venous endothelial cells to produce NO. The venous endothelium is fully functional with respect to NO release as demonstrated in the venous vasculature of the rat mesenteric bed (Warner, 1990) and in bovine intrapulmonary veins (Ignarro et al., 1987). Vallance et al. (1989) also described L-arginine-dependent NO synthesis in human veins *in vivo*. Taken together, the findings in these studies contradict those of the above and indicate that the reduced venous endothelium-dependent relaxant response may result from a more rapid degradation of endogenous NO or decreased response of the underlying smooth muscle cells to NO in the venous side of the circulation.

If one or more of the above hypotheses indeed contribute to the differential responses of arteries and veins to endothelium-dependent vasodilators, then why do the responses of the two types of vessels to endothelium-independent vasodilators differ as observed in my experiments ? Kojda et al. (1994) demonstrated that relaxations of porcine vena cordis magna

to SNAP and NTG were significantly enhanced by endothelium denudation or pretreatment with L-NAME, revealing the substantial influence of the endothelium and endogenous NO on the vasodilator potency of nitrovasodilators. The same phenomenon was also observed in rat aorta (Shirasaki & Su, 1985) and mesenteric resistance arteries (Tesfamariam & Halpern, 1988), rabbit aorta and femoral artery (Pohl & Basse, 1987), porcine coronay arteries as well as human internal mammary arteries and saphenous veins (Luscher et al., 1989). This is further supported by Moncada et al. (1991), who demonstrated that systemic application of L-NAME substantially improved the response to NTG in anaesthetized rats. It seems logical to speculate that continuous basal NO production may desensitize soluble guanylate cyclase (Axelsson & Andersson, 1983; Schroder et al., 1988) and/or reduce the efficacy of cGMP (Kojda et al., 1994), thereby contributing to attenuation of SNAP- or NTG-induced vasorelaxant response in intact veins. Furthermore, prior exposure to high concentrations of exogenous NO derived from SNAP led to a 10-fold reduction in potency of NTG but only a 3-fold decrease in potency of SNAP, indicating the greater inhibitory effect of NO on the vascular bioactivation of NTG. Kojda et al. (1994) proposed that prolonged exposure to NO, whether originated from endogenous or exogenous sources, may inhibit its own action in vascular smooth muscle of the porcine vena cordis magna. Interestingly, a larger relaxation to ACh and exogenous NO was observed in pulmonary veins than arteries of lambs (Gao et al., 1995); on the other hand, 8-bromo-cGMP elicited similar degree of relaxation in arteries and veins suggesting that the sensitivity of both types of vessels to cGMP is comparable. The same study also demonstrated that NO induced a smaller increase in cGMP level in pulmonary arteries than in veins. A lower production of cGMP in pulmonary arteries compared to veins was evidently the result of the lower activity of soluble guanylate cyclase which might explain the smaller relaxant response in the arteries.

Pohl et al. (1986) showed that shear stress, which is much less in veins than in arteries, mainly determines endothelial NO production. Indeed, a lower basal release of NO and increased sensitivity to nitrovasodilators has been reported in venous tissue (Seidel & La Rochelle, 1987) and the venous circulation of humans *in vivo* (Vallance et al., 1989). The fact that nitrovasodilators are more potent in vessels with lower or absent basal synthesis of endogenous NO (Luscher et al., 1989; Yang et al., 1991) might be attributed to increased sensitivity of guanylate cyclase after loss of endogenous NO synthesis (Edwards et al., 1984; Moncada et al., 1991). NTG was shown to be more active on the saphenous vein than on the femoral artery of the dog (MacKenzie & Parratt, 1977). For instance, 1 μM NTG inhibited >50% but only <10% of noradrenaline-induced tone in the saphenous vein and the femoral artery, respectively. Even in concentrations in excess of 100 μM, NTG could only inhibit tone in arterial preparations by 50%; in contrast, noradrenaline-induced tone in venous smooth muscle was completely inhibited by a concentration of only 10 μM. The differential intensity of endothelial NO production in arteries and veins might influence the bioactivation of NTG leading to preferential venodilatation.

Recently, Kojda et al. (1998) conducted an elegant series of experiments to study the kinetics of NO release from DEA/NO, SNAP and NTG in association with the activation of soluble guanylate cyclase and vasorelaxation in an attempt to explain the preferential venodilator activity of NTG. Under physiological conditions (pH 7.4, 37°C, presence of O_2), DEA/NO (10 μM) rapidly decomposed and liberated the highest concentration of NO in 6-7 min, whereas SNAP (10 μM) showed a similar time course of NO release but liberated 4-5 times less NO relative to DEA/NO. Keefer et al. (1996) also provided evidence that DEA/NO releases 2 moles NO/mole of compound, whereas the mechanism of NO release from SNAP remains unclear. There was no detectable NO release from similar concentration of NTG

(Kojda et al., 1998). DEA/NO and SNAP dose-dependently activated soluble guanylate cyclase, with the activity of the latter about 10-fold lower than the former. Maximal stimulation of the enzyme by NTG was very low and occured at much higher concentration (> 100 μM). Preincubation of isolated porcine coronary arteries with DEA/NO, SNAP or NTG reduced vascular cGMP formation by and vasorelaxant responses to NTG. The duration of NO exposure appeared to be more important than the concentration of NO. In accordance, it has been shown that endogenous NO production by the vascular endothelium suppresses the vasodilator activity of NTG and other organic nitrates and that desensitization of guanylate cyclase is involved (Moncada et al., 1991; Kojda et al., 1994). Kojda et al. (1994) previously demonstrated that inhibition of vascular bioactivation of NTG by endogenous NO predominantly occurs in arteries, therefore, it follows that the preferential reduction of preload may be a consequence of a less pronounced inhibition of the bioactivation process in veins. This is consistent with a recent report which noted a higher production of NO from NTG in veins than in arteries in vivo (Mulsch et al., 1995). On the contrary, MacAllister et al. (1995) reported that SNP, NTG and SIN-1, three drugs with different mechanisms of biotransformation to NO, have similar arteriovenous profiles in human vessels in vitro and in vivo suggesting that preferential metabolism of NTG by venous tissue could not justify its venoselectivity and that NO itself might selectively dilate veins. Indeed, NO solutions was shown to have similar arteriovenous profiles to nitrovasodilators (Miller & Vanhoutte, 1989). The differences in the venous versus arterial actions of systemic administration of SNP, NTG and SIN-1 reported in clinical settings are not likely due to differential sensitivities of arteries and veins because all three agents caused significant venodilatation (Armstrong et al., 1975; Majid et al., 1980; Gerson et al., 1982). In these studies, large doses of SNP were given to supine patients in order to produce effects in resistance vessels, metabolism of SNP during its

passage through the arterial vascular bed reduced the concentration of the drug reaching the veins and consequently masking its venodilator activity. The *in vivo* haemodynamic differences between SNP and NTG have been well-documented. Armstrong et al. (1975) reported the balanced arterial and venous effects of SNP versus the preferential dilatation of veins by NTG in patients with acute myocardial infarction. In clinical congestive heart failure, sublingual NTG produced a greater decline in end-diastolic pressure than i.v. SNP, whereas SNP but not NTG reduced total peripheral resistance (Miller et al., 1976). Canine saphenous veins were more sensitive to NTG than the femoral and dorsal pedic arteries (MacKenzie & Parratt, 1977; Armstrong et al., 1980). Stiefel & Kreye (1984) compared the vascular profiles of SNP and NTG in isolated rabbit renal arteries and veins as well as in anaesthetized rats, and reported several interesting findings. First, SNP (0.01 nM-10 μM) and NTG (0.01 nM-1 μM) relaxed rabbit renal arteries and veins in a dose-dependent manner; the threshold concentration (EC_{10}) of both drugs was lower in veins than in arteries, whereas their EC_{50} values did not differ in both types of vessels. Second, on prolonged exposure (30 min), the relaxant response to both SNP and NTG faded partially. The degree of this fade was larger in arteries than in veins with NTG, but more evenly distributed in both arteries and veins with SNP. Finally, in anaesthetized rats, NTG (1-10 μg min^{-1}) infused into the femoral artery or intravenously elicited similar depressor responses indicating that no inactivation of NTG occurred during its passage through the capillary bed of the hindleg. In contrast, the hypotensive action of SNP (0.3-3 μg min^{-1}) was considerably weaker during intra-arterial than intravenous infusion. It was determined that only two-thirds of the infused dose of SNP reached the venous side of the circulation (Kreye & Reske, 1982). Therefore, *in vivo* venodilatation caused by SNP would be less pronounced than expected from its relaxant activity in isolated veins, suggesting that the somewhat greater *in vitro* sensitivity of venous preparations is outweighed by the lowered

concentration of SNP in venous blood. This hypothesis may account for the balanced effect of SNP on resistance and capacitance vessels *in vivo*.

Basal release of NO appears to vary considerably among blood vessel types and diameters. For example, greater basal release of NO was observed in bovine intrapulmonary artery and vein of smaller rather than larger diameters (Ignarro et al., 1987). The investigators noted the higher resting levels of cGMP and the difficulty to induce and maintain induced tone in endothelium-intact rings with smaller diameter. SNP (1 nM-10 μM) and NTG (1 nM-10 μM) relaxed dog coronary arteries to a greater extent than mesenteric arteries; in coronary arteries of different sizes, the relaxations caused by NTG was in the order of large > medium > small-size whereas those caused by SNP were not significantly different (Miwa & Toda, 1985). Studies by Sellke et al (1990) and Kurz et al. (1991) using a video-imaging technique in isolated microvessels and vessels on the epicardial surface in acute intact animal preparation showed that NTG caused dilatation of coronary vessels of 100-300 μm but little or no dilatation in those of 29-100 μm. In addition, Zhang et al. (1993) reported that coronary arterial conductance vessels were markedly more sensitive to the effects of NTG than resistance vessels, in support of the above findings. The vasodilatation induced by NTG was prolonged in the conductance vessels lasting from 100-320 s but brief (<20 s) in resistance vessels. The differential sensitivity of microvessels to NTG could be explained by a relatively lack of bioactivation of NTG consequent to a lack of enzymes necessary to convert NTG to a nitrosothiol compound or a relative lack of available sulfhydryl groups within the vascular smooth muscle of the smallest coronary microvessels. To further complicate matters, Ekelund (1994) reported that large arterial resistance vessels (>25 μm), arterioles (<25 μm) and veins obtained from the cat skeletal muscle vascular bed respond differently to endogenous and exogenous NO. SNP (0.5-32 μg kg tissue^{-1} min^{-1}), NTG (1-4096 μg kg tissue^{-1} min^{-1}) and NO

dissolved in saline (0.14-0.82 mg kg tissue^{-1} min^{-1}) administered close-arterially elicited a dose-dependent generalized dilatation in all three vascular sections (Ekelund, 1994), but endogenous NO had an almost selective dilator action on large arterial resistance vessels, little or no effect on small arterioles and veins (Ekelund & Mellander, 1990). To summerize, several factors may contribute to the regional differences in the vascular response to nitrovasodilators observed *in vivo*: a) differences in hypotension-induced reflex vasocontriction among different vascular beds, b) variations in regional sensitivities to these drugs consequent to differences in regional, basal release of NO and/or activity of guanylate cyclase, and c) differences in the concentration of the drug delivered to various tissues resulting from differences in regional blood flow.

Using an NO-trapping technique and cryogenic electron spin resonance spectroscopy, Mulsch et al. (1995) provided direct evidence of a close relationship between the formation of NO from NTG in vascular tissues and organs of anaesthetized rabbits and the concomitant vasodilatation. NTG was observed to produce NO at a significantly higher rate in the mesenteric bed and the vena cava than in the aorta and femoral artery, consistent with the preferential venodilator activity and efficient preload reduction of the drug (Ferrer et al., 1966; MacKenzie & Parratt, 1977; Loos et al., 1983). Also, more NO was formed in organs (especially liver, lung and kidney) than in blood vessels. There is evidence supporting the notion that NO release from NTG in organ tissues occur in nonvascular cells (Shroder, 1992). For instance, hepatocytes express high cytochrome P450 activity which has been shown to catalyze NO formation from NTG (McDonald & Bennett, 1993). Whether NTG-derived NO generated in nonvascular cells has any pharmacological consequences remains to be elucidated.

There are as yet no reports in the literature examining the cardiovascular actions of

CpCr(NO)$_2$(ONO) or other organotransition-metal nitrosyl complexes. In preliminary studies conducted in Dr. Pang's laboratory, the EC$_{50}$ (effective concentration to induce half-maximal relaxation response) and E$_{max}$ (maximal percent relaxation of phenylephrine-induced contraction) of CpCr(NO)$_2$(ONO) are 0.1 μM and -98%, respectively. I.V. bolus injections of CpCr(NO)$_2$(ONO) into conscious rats dose-dependently lower arterial blood pressure. The EC$_{50}$ (effective dose to produce half-maximal pressure reduction) and E$_{max}$ (maximal decrease in blood pressure) are 1.5 μg/kg and -45 mmHg, respectively. My results demonstrated that i.v. infusion of CpCr(NO)$_2$(ONO) equally dilates both resistance and capacitance vessels in anaesthetized, ganglion-blocked rats with elevated venomotor tone.

It is unclear why NTG did not show preferential venodilator action in my experiments. Instead, DEA/NO, SNAP, CpCr(NO)$_2$(ONO), SNP as well as NTG equally dilate both resistance and capacitance vessels. Reports in the literature provide ample evidence that different classes of nitrovasodilators possess variable pharmacokinetics and pharmacodynamics, both of which alone or in combination can have profound influence on the resultant cardiovascular responses observed *in vivo*. In summary, DEA/NO, SNAP, CpCr(NO)$_2$(ONO), SNP and NTG dose-dependently and similarly increased CO but did not alter HR at any dose. All five drugs dose-dependently reduced both MAP and R$_a$ with efficacy: DEA/NO $\approx$ SNAP $\approx$ CpCr(NO)$_2$(ONO) $\approx$ SNP > NTG. DEA/NO, SNAP, CpCr(NO)$_2$(ONO) and SNP but not NTG lowered MCFP with efficacy: DEA/NO > SNAP > CpCr(NO)$_2$(ONO) $\approx$ SNP. All five drugs reduced R$_v$ with efficacy: DEA/NO $\approx$ SNAP $\approx$ CpCr(NO)$_2$(ONO) $\approx$ SNP > NTG. Therefore, the hypotensive, arterial and venous dilator actions of DEA/NO, SNAP and CpCr(NO)$_2$(ONO) are comparable to those of SNP but greater than those of NTG.

5. Conclusion and Future Direction

All of the nitrovasodilators (DEA/NO, SNAP, CpCr(NO)$_2$(ONO), SNP and NTG) of interest as well as the phosphodiesterase type V inhibitor, zaprinast, have both arterial and venous dilator actions. Although the NO donors and zaprinast interfere with the endogenous L-arginine/NO pathway at different points, they converge at the cGMP level leading to vascular smooth muscle relaxation. However, NO liberated from nitrovasodilators, regardless of the mechanism of release, may also exert its action via cGMP-independent pathways.

In addition to vasodilatation, the newer NO donors, DEA/NO and SNAP, have been shown to exert other haemodynamic actions which are beneficial under certain pathophysiological conditions. For instance, i.v. administration of DEA/NO caused a significant platelet inhibitory effect which was comparable in potency with SNP, and more potent than aspirin in anaesthetized rabbits (Diodati et al., 1993b); the antiplatelet activity of DEA/NO was shown to be more effective than SNP in human blood *in vitro* in the same report. DEA/NO, by releasing NO, was demonstrated to obliterate proliferation and migration of injured vascular smooth muscle cells in culture (Sarkar et al., 1996). Interestingly, Baskin et al. (1996) showed that DEA/NO reduces the toxicity of cyanide in mice. It seems logical to speculate that the use of DEA/NO in combination with SNP may alleviate the problem of cyanide toxicity associated with prolonged administration of the latter.

SNAP, similar to DEA/NO, has also been shown to inhibit platelet aggregation, induce platelet disaggregation, inhibit fibrinogen binding to platelets via NO/cGMP-mediated mechanisms (Salas et al., 1994). The anti-aggregating and disaggregating potencies of SNAP are comparable to those of NO, but SNAP has a longer duration of action (at least 60 min). SNAP, unlike NTG, does not cause haemodynamic tolerance after continuous infusion in

anaesthetized rabbits (Shaffer et al., 1992), conscious intact (Booth et al., 1996) and chronic heart failure (Bauer & Fung, 1991) rats. Furthermore, NTG-tolerant animals are not tolerant to SNAP (Bauer & Fung, 1991; Shaffer et al., 1992; Serone et al., 1996). Sellke et al. (1990) showed that NTG dilates only large coronary arteries, whereas the S-nitrosothiol, S-nitrosocysteine produces vasodilatation in coronary vessels of all sizes. SNAP appears to provide a more balanced haemodynamic profile relative to NTG by decreasing both preload and afterload (Bauer & Fung, 1991). It has been suggested that balanced arteriovenous dilatation may contribute to long-term vasodilator efficacy and improvement in survival in congestive heart failure (Cohn et al., 1986). These findings indicate that the therapeutic potential of SNAP and other nitrosothiols as alternatives to NTG should be further examined. Also, specific delivery of NO to certain vessel types or tissues should be advantageous in cardiovascular disorders.

The heterogeneous behaviour of arteries and veins to physiological and pharmacological stimuli has important clinical implications. First, the patency rate of coronary bypass grafts obtained from the internal mammary artery is known to be greater than those from the saphenous vein (Grondin et al., 1984; Spencer, 1986). This may be attributed to the more effective release of endothelium-derived NO in mammary artery grafts in response to increase in blood flow, and to stimulation by ACh, platelet-derived products and thrombin. The released NO also inhibits platelet adhesion and aggregation, and consequently limits thrombus formation. Second, the differential venous and arterial responses to nitrovasodilators *in vivo* would dissimilarly affect cardiac preload and afterload as well as capillary pressure and fluid exchange (Ekelund, 1994). A greater release of endothelium-derived NO in arterial grafts would not only protect against vasospasm but also against the development of atherosclerotic changes that commonly develop in venous grafts (Grondin et al., 1984).

Future investigations should focus on the rate/extent and precise mechanisms of NO release from different classes of nitrovasodilators, and determine how these factors correlate with their arterial and venous dilator potencies/efficacies as well as other *in vivo* haemodynamic actions. It would be of interest to examine the cardiovascular profiles of the newer NO donors, DEA/NO, SNAP and CpCr(NO)$_2$(ONO), in experimental models of hypertension and congestive heart failure.

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