REGULATION OF THE SPLANCHNIC VASCULAR RESISTANCE

BY

M. PAULA MACEDO

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS
FACULTY OF MEDICINE, UNIVERSITY OF MANITOBA

ISBN 0-612-13330-3

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REGULATION OF THE SPLANCHNIC VASCULAR RESISTANCE

BY

M. PAULA MACEDO

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

ACKNOWLEDGEMENTS

One that says that she is happy and is able to think can be called extremely strong. My time in the department has been a happy one and the training program has increased my capacity for scientific thought, both combining to make me a stronger person. Along with happiness, good fortune has to be present, and without any doubt the day I first went to the lab where I developed all the work for this thesis was a day of good fortune. I wish to express sincere thanks to Wayne who is the best supervisor that anyone can have and I could not have done it without him. His positivism and enthusiasm towards science and life was one of the best lessons I learned in all these years. His guidance in research and his insights into the philosophy of science are praiseworthy.

Although I greatly appreciate the generous availability of time that Clive Greenway provided for me, the quality of time was the greatest gift from a scientist of his calibre. Most of all I give immense thanks to Clive for his confidence in me.

I am grateful to Dallas Legare for his dedication and superb technical training. Karen Sanders was not only the person that prepared manuscripts, letters and application forms but someone that made me feel at home with her daily friendly smile (is it not time for a cigarette yet?). I thank Egon Brockhausen for his assistance over the years. I would like to thank the other PhD students in my lab, Hongsheng Xie, Hui Wang and Chao Han for their camaraderie. Sincere thanks to Miguel Escobar for his friendship and the never-ending discussions of the relevance of my *in vivo* versus his *in vitro* studies, knowing that I was always right. I would

like to thank all the members of my thesis committee, Drs Clive Greenway, Bob McNeill, Gerry Minuk, and Frank Burczynski for their support in this training program. A special thanks to Drs Elsa Gomes and Aranda da Silva for their initial support at the beginning of my scientific career. Financial support for this study was provided by the Heart and Stroke Foundation of Canada.

Friends are precious gifts and, even though I do not mention them all in this thesis I am grateful to all of them. I will be always in debt to Elsa and Roberto for their support and belief in me. A special thanks to Madalena and Pedro, the Leite, Teixeira and Xavier families.

My life was always profoundly affected in a positive way by the strength in character of the women in my family, my Mother, aunt Ilda and grandmother Rosa and for that I feel blessed. I am deeply indebted to my Mother.

Last, but not least, any words could not express my gratitude to Valeriano who has enriched my life with patience, encouragement, friendship and love. Thank you.

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ABSTRACT

The studies presented in this thesis addressed several aspects of the hepatic and mesenteric vascular bed. Briefly, for these *in vivo* studies, cats were anesthetized and the carotid artery and femoral vein were cannulated to measure systemic arterial and central venous pressures. After laparotomy, either the superior mesenteric artery (SMA) or hepatic artery (HA) were connected to the femoral arteries through an external circuit that allowed regulation of the blood flowing into the SMA or HA.

The hypothesis that vasoconstriction is modulated by nitric oxide (NO), only if shear stress is allowed to increase, was tested. Shear stress was increased by holding blood flow constant during vasoconstriction induced by nerve stimulation or norepinephrine infusion. In the presence of the nitric oxide synthase inhibitor, L-NAME, a potentiation of the vasoconstriction induced by nerve stimulation occurred and was reversed by L-arginine both in the intestine and liver. When shear stress was held constant, blockade of NO release did not affect response to either nerve stimulation or norepinephrine infusion. When norepinephrine was infused, but shear stress was allowed to rise, L-NAME potentiated the constriction for the portal vein and HA but not for the SMA. These results suggest a shear stress-dependent pre-junctional modulation of vasoconstriction by NO in the intestine but a post-junctional regulation in the liver.

The intrinsic ability of an organ to minimize blood flow changes over a wide range of perfusion pressure is known as autoregulation. It was hypothesized that

NO modulates autoregulation. In the intestine, NO antagonizes autoregulation over an autoregulatory range (70-140 mmHg) but has no effect at a non-autoregulatory range (40-70 mmHg). However, this mechanism is not present in the liver.

NO has a variety of interactions with vasoactive agents and it was unclear if the vasodilatory effect of adenosine and isoproterenol on the HA and SMA would be modulated by the endothelial-dependent dilator. Maximal responses to adenosine and isoproterenol were determined using the decrease in perfusion pressure at constant flow as the index of vasodilation. In these studies, L-NAME potentiated the dilation induced by adenosine and isoproterenol in the SMA but had no effect on the HA. It is suggested that there is a highly organ-specific compensatory mechanism in which the absence of NO promotes potentiation of other vasodilators.

In the SMA, NO affected basal tone, caused pre-junctional inhibition of sympathetic nerves, antagonized other dilators and inhibited autoregulation. In the HA, NO did not alter basal tone or autoregulation, nor did it alter other vasodilator responses. It did cause shear stress-dependent post-junctional inhibition of vasoconstriction in both the HA and portal vein. Organ selectivity for vascular roles of NO was dramatic in these studies.

1. INTRODUCTION

1.1 NITRIC OXIDE EFFECT ON RESISTANCE VESSELS

1.1.1. Introduction

The last two decades have been preponderant times in understanding some of the concepts that illuminate the significance of the vascular endothelium. Sir John Vane was the promoter of this cascade of events with the first demonstration that vascular endothelium synthesizes and releases prostacyclin, a metabolite of arachidonic acid with importance as a thrombo-resistant agent and an endothelium-dependent vasodilator (Moncada et al., 1976). A pioneer study was that of Furchgott et al. (1980) who discovered that relaxation of isolated preparations of arteries by acetylcholine was dependent on the presence of an intact endothelial cell layer. The mechanism by which acetylcholine promotes the vasorelaxation remained unknown and the substance that mediated the vascular relaxation was termed endothelium-derived relaxing factor (EDRF). In the following years, studies showed that EDRF is similar to nitrovasodilators. EDRF increases cyclic GMP by activating guanylate cyclase (Rapoport & Murad, 1983). It is diffusible with a very short biological half-life (Griffith et al., 1984). The next milestone in this field was the discovery suggesting that nitric oxide (NO) is the endothelium-derived relaxing factor (Palmer et al., 1987). The importance of EDRF (nitric oxide) is well recognized, however the mechanisms by which it modulates vascular resistance and its physiological and pathological significance are still undergoing research.

1.1.2. Synthesis

The endothelial enzyme, nitric oxide synthase (NOS), responsible for the production of NO is activated by the calcium-calmodulin association leading to the production of NO and citrulline from L-arginine, its natural substrate. The NO formed stimulates the soluble guanylate cyclase (GC) to increase the cyclic GMP concentration in the generator and target cells (Moncada et al., 1991). So far three prototypical isoforms of NOS have been identified: neuronal, cytokine inducible and endothelial NOS (Moncada et al., 1991). Amongst the NOS family the endothelial NOS displays, as a unique feature, its N-terminal myristoylation. This rare attribute allows enzymes to be oriented near their endogenous substrates and to initiate or respond to compartmentalized cellular signals (Gordon et al., 1991). Suggestion was made that the N-terminal myristoylation allows the protein to be membrane bound, however a soluble endothelial NOS is also found in these cells (Hecker et al., 1994). Thus, either there are two different endothelial NOS isoenzymes or a single isoenzyme that is soluble or membrane bound depending on its conformation (Hecker et al., 1994). The understanding of the physiological implications of the different endothelial NOS is now relevant in conjunction with the knowledge that different stimuli (shear stress versus hormonal) activate the release of NOS.

The substrate for the NOS is the L- but not the D-arginine (Moncada et al., 1991). The formation of NO is dependent on nicotinamide adenine dinucliotide phosphate (NADPH) that acts as a NO donor. Oxygen is derived from molecular

oxygen and N^Ghydroxy-L-arginine is formed as an intermediate and citrulline as a coproduct (Moncada et al., 1991). This newly formed NO is an uncharged molecule that can diffuse freely across membranes but has a very short half-life (2 to 30 seconds) (Lowenstein et al., 1994). The NO formed in the endothelial cells activates the vascular smooth muscle cyclic GMP and this accounts for the endothelial dependent vasodilation (Moncada et al., 1991).

1.1.3. Physiological and pharmacological properties of nitric oxide in blood vessels

There is substantial evidence that NO is an important modulator of circulatory control in intact animals (Moncada et al., 1991). More than a century ago nitroglycerin, the prototypical nitrovasodilator was synthesised (1846) and had its first clinical uses in 1879 (Rapoport & Murad, 1983). Other compounds such as nitroprusside, inorganic nitrate, glyceryl trinitrate, azide, hydroxilamine and nitroso compounds were shown to promote a direct relaxation of the vascular smooth muscle via stimulation of the GC and elevation of intracellular cyclic GMP levels (Ignarro, 1989). A first attempt to provide inhibitors for the NO actions was by the use of known inhibitors of the GC such as hemoglobin and methylene blue (Ignarro, 1989). Later it was demonstrated that there is an increase in arterial blood pressure after treatment with L-arginine analogues (Moncada et al., 1991). The first studies with inhibitors of the NOS used L-N^G-monomethyl arginine (L-NMMA) which promoted an increase in basal tone *in vivo* and *in vitro* that was

reversed by the actions of the enzyme substrate L-arginine. In search for more potent inhibitors of the synthesis of NOS, two novel compounds, L-N^G nitro methyl esther (L-NAME) and N-iminoethyl-L-ornithine (L-NIO), were found to be approximately 5 times more potent than the other analogues (Moncada et al., 1991). Differences in uptake, distribution, or metabolism may contribute to the different potencies observed with these compounds.

1.1.3.1. Mechanism of nitric oxide-induced vasodilation

At the cellular level, NO binds preferentially to iron-containing enzymes, either activating or inhibiting the enzyme. When bound to the iron in the heme group of GC, the enzyme is activated and a cellular cascade is initiated through the increase in cyclic GMP levels (Lowenstein, 1994). The observed NO-induced vasodilation is due to the activation of GC.

The NO-induced vasodilation occurs in response to variable stimuli in the vasculature, such as Ach, adenine nucleotides, thrombin, substance P, bradykinin, hypoxia and shear stress (Moncada et al., 1991). The dilatory effects produced by these stimuli are different in the arterial side compared to the venous side. Most of the arterial relaxants failed to relax the veins (Ignarro, 1989). However, the difference does not seem to lie within the vascular smooth muscle since EDRF seems to cause relaxation of the endothelium denuded vein (Ignarro, 1989).

1.1.3.2. Heterogeneity of nitric oxide-mediated vascular responses

1.1.3.2.1. Differences between and within species

Species Differences:

Endothelium-mediated responses seem to be universal in vertebrates (Shepherd, 1991). However, responses to NO-dependent dilators differ among species as illustrated by the vasodilatory effect of acetylcholine on the coronary artery of the dog but not that of the pig (Shepherd, 1991). It is important not only to be aware of the differences between the species but also between the experimental procedures that can lead to different results within species. For example, the use of acetylcholine-induced vasodilation as an index of NOS blockade shows inconsistant effects depending upon whether the tests are done *in vitro* versus *in vivo* and depending on the tissue tested (Aisaka et al., 1989; Gardiner et al., 1990; 1991).

Differences in Vascular Beds:

Heterogeneity exists in the endothelium-dependent relaxation among species, and the same pattern is shown within species. For example, a marked regional variation is observed to the effects of bradykinin and acetylcholine in the presence of the NOS inhibitor L-NAME. In the renal and hindquarters but not the mesenteric vascular bed, vasodilation to bradykinin is attenuated in the presence of L-NAME in the rat (Gardiner et al., 1991). In *in vitro* experiments with the porcine aorta, relaxation with acetylcholine is observed, however, the drug has no

effect on the coronary arteries (Shepherd, 1991). The endothelium dependent relaxation to serotonin is another example where coronary but not femoral or renal arteries show a relaxant effect to the drug (Shepherd, 1991). The reason for this heterogeneity is not clear. A differential can be present in the endothelial or the smooth muscle cells or both. Differences in the receptor population or the concentration of NOS could account for the differences in the endothelial cells. Smooth muscle deficiency in GC, cyclic GMP or alteration in the phosphodiesterases that regulate cyclic GMP levels may explain some heterogeneity shown in different vascular beds.

1.1.3.3. Vasodilator actions in intact animals

The pharmacological tools available nowadays to block NOS allows *in vivo* studies leading to comprehension of the relevance of NO in the different vascular beds. Early experiments using L-NMMA showed an increase in blood pressure in anesthetized rabbits and guinea pigs (Moncada et al., 1991). L-NMMA also showed an increase in blood pressure that was accompanied by an increase in vascular resistance in the renal, mesenteric, carotid, and hindquarters vascular beds of conscious rats (Gardiner et al., 1990). In dogs, L-NMMA induced an increase in resistance of the coronary circulation and a reduction in resting phasic coronary flow (Chu et al., 1991). Brachial artery and dorsal veins of the hand in humans showed a decreased vasodilation induced by acetylcholine or bradykinin in the presence of L-NMMA (Moncada et al., 1991). Greenblatt et al. (1993) showed a

reduction in blood flows, with a concomitant increase in resistance, to the cerebrum, heart, kidney, spleen, gastrointestinal tract, skin, ear, and white fat but not in the hepatic artery (HA) in the presence of L-NMMA.

1.1.3.4. Changes in shear stress

Blood flow in some regions of the vasculature seems to be regulated by NO. The primordial stimulus for the release of NO in response to blood flow through a vessel is shear-stress. With a decrease in diameter, at a constant arterial blood flow, shear stress increases because it is directly related to flow rate and inversely to the third power of the internal diameter. Coronary resistance vessels have shown flow-induced dilation, suggesting the envolvment of NO as the modulator (Kuo et al., 1990; 1991). More concise studies showed without doubt that shear stress promotes not only the release of NO with a subsquent increase in cyclic GMP and, therefore, vasodilation but shear stress also upregulates the expression of the NOS, hence the capacity for vasorelaxation (Buga et al., 1991; Lamontagne et al., 1992; Billiar, 1995).

Although several studies have been directed towards the understanding of the shear stress-dependent dilatory mechanism, researchers still feel a step away from its real pathophysiological and clinical implications.

1.1.4. Pathophysiological responses and clinical implications

NO plays a central role in regulating both blood pressure and flow. Hence, one can foresee implications in defects in the regulation of the endothelium cells and, therefore, in different types of pathologies. A loss of the NO-mediated vasodilation may be implicated in essential hypertension. This has been shown in hypertensive rats and seems to be related to an inability to release NO in response to shear stress (Koller & Huang, 1995). Endothelial dysfunctions are characteristic of other diseases such as diabetes and atherosclerosis (Lowenstein, 1994). A decrease is observed in endothelium-dependent vasorelaxation and in the release of EDRF in human and rabbit atherosclerotic vessels (Moncada, 1991). High resistance is seen in the mesenteric bed of diabetic rats with a dysfunctional endothelium that might account for the observed vascular dysfunctions (Taylor et al., 1994a,b). It may be beneficial to increase NO production in situations where vascular resistance is elevated in conjunction with reduced NO release. One successful therapy for pediatric and mild adult respiratory distress syndrome is the inhalation of low doses of the gas, NO, which appears to reduce pulmonary vascular resistance and reduce symptoms.

A group of compounds such as nitroglycerin and sodium nitroprusside have been in use for decades. However, as NO has been shown to be involved not only with the regulation of vascular pressure and flow but also in modulation of neuronal function and inflammatory process, NO-releasing drugs under development need to be highly specific to different molecular targets. Other

approaches to therapy in different tissues is to increase either the substrate or cofactors for NOS. Whether such an approach will be sufficiently beneficial awaits confirmation.

1.2 ADENOSINE EFFECT ON THE RESISTANCE VESSELS

1.2.1. Introduction

At the beginning of this century Drury and Szent-Gyorgyi extracted adenosine (ADO) for the first time from different tissues and observed its effects on cardiovascular functions (Drury, 1929). Nearly 30 years after, an expansion of interest on the vasodilator role of ADO started with Berne's proposal that ADO was the mediator of metabolically controlled coronary vasomotion (Berne et al., 1963). In the following years this hypothesis was broadened to other organs including kidney, skeletal muscle and brain (Cushing & Mustafa, 1991). Identification of specific receptors for adenine nucleotides and nucleosides by Burnstock was the next step in the ADO field (Burnstock, 1978). In the suggested terminology by Burnstock, now widely accepted, there are two types of receptors, the P1 and the P2 purinoceptors. The P1 purinergic receptors show greater affinity to ADO than the nucleotides. Two subtypes of P1 receptors are now recognized: A1 which inhibits, and A2 which activates adenylate cyclase (Calker, 1979). The P2 purinoceptor was characterized by a higher affinity for adenosine 5'-triphosphate (ATP) and diphosphate than for ADO (Cushing & Mustafa, 1991).

In vascular smooth muscle, a large emphasis has been placed on the A2 receptor activation. Activation of this receptor causes accumulation of intracellular cyclic AMP, decreasing vascular resistance by activating a cyclic AMP-dependent protein kinase (Cushing & Mustafa, 1991). Activation of other putative second messenger mechanisms such as activation of guanylate cyclase by ADO, was suggested by Kurtz (1987) and could also be associated with ADO-mediated vasodilation. Many subjacent mechanisms, such as inhibition of calcium influx and phosphatidylinositol-dependent kinase as well as hyperpolarization of vascular smooth muscle, also have been implicated in the ADO-mediated relaxation (Cushing & Mustafa, 1991).

It is important to emphasize that complex interrelations between second-messenger systems exist and consequently, under physiological stimulation, no single messenger may be responsible for the ADO action.

1.2.2. Synthesis and metabolism

ADO production and release is widely accepted to occur via two principal mechanisms, the trans-methylation pathway and through the actions of the enzyme 5'-nucleotidase (Collis, 1989). In the trans-methylation pathway, the non-reversible methyl donor S-adenosyl-L-methionine is converted to S-adenosyl-L-homocysteine which is continuously removed by the cytosolic S-adenosyl-L-homocysteine hydrolase to yield ADO and homocysteine (Schrader, 1991). This pathway appears to be oxygen insensitive. The ADO formed is then either salvaged by the ADO

kinase or further metabolized by ADO deaminase into inosine (Schrader, 1991). ATP breakdown, usually associated with increases in cellular ADO, functions as a second pathway for the formation of ADO, via the actions of the enzyme 5'-nucleotidase (Schrader, 1991). The present state of knowledge indicates a major role for the transmethylation pathway in ADO formation. Nucleotide breakdown is proposed to not contribute significantly to ADO production in the heart under normal function. Such a proposal is supported by the demonstration that inhibition of the ecto-5'-nucleotidase does not significantly change ADO release in the heart (Schrader, 1991).

Endothelial cells play a primordial role in ADO metabolism since the enzymes responsible for the ADO degradation to uric acid (adenosine deaminase, nucleoside phosphorylase and xanthine oxidase) are exclusively endothelial enzymes (Schrader, 1991). Suggestions have been made of an indirect effect on ADO levels at the tissues, not through enzymatic actions but by a washout mechanism of ADO from the respective tissue (Schrader, 1991; Lautt et al., 1985).

1.2.3. Physiological and pharmacological properties of adenosine in blood vessels

This purine has the capability of dilating many vascular beds. Targets for ADO-mediated vasodilation appear to be endothelial cells, smooth muscle cells and adrenergic terminal nerves, although the removal of the endothelium seems to not alter responses to ADO. However, some exceptions have been reported, and an

attenuation of the dilatory effects have been observed in the pig aorta, dog coronary and rabbit ear artery (Collis, 1989). The dilatory effects of ADO are endothelially mediated in the sense that the endothelium serves not only as a barrier for ADO diffusion but also serves as the site where the enzymes responsible for ADO metabolism are located (Schrader, 1991). In smooth muscle, the major site of action of the purine seems to be at a cell-surface receptor. This receptor has been implicated because compounds that block nucleoside transport into the cell, thereby increasing extracellular ADO, enhance its vasoactivity (Collis & Brown, 1983). According to some of the Burnstock criteria, this receptor can be classified as P1 because the alkylxanthines, such as theophylline and 8-phenyltheophylline antagonize the effects of ADO at the cell surface receptor (Collis, 1989). Based on the structure-activity relations for ADO analogs and their ability to displace radioactive ligands, both A1 and A2 subtype receptors of the P1 receptor were characterized (Collis, 1989). These studies showed that agonist analogs of ADO with the N^6-substituted bind with higher affinity to A1 receptors than the 2-chloroadenosine and 5′-substituted analogs (Collis, 1989). A reversed order of affinity is observed for binding to the A2 receptors, that is, the highest affinity was observed for the 5′-substituted analogs (Collis, 1989). More complex, due to a lack of confirmation in selectivity, seems to be the characterization of the receptors by the use of antagonists. However, 8-cyclopentyl-1,3,dipropylxanthine has the attribute of being a selective antagonist for A1 receptors. Selectivity was not confirmed for the presumed A2-selective antagonist triazoloquinazoline

CGS15943A (Collis, 1989). Since there is a consensus that activation of A2 receptors increases the levels of cyclic AMP, its measurement can be seen as a reflection of activation of this receptor and correlated to the vasodilatory effects of ADO.

The ADO evoked vasodilation can be also attributed to its inhibitory effects on the release of noradrenaline from adrenergic nerve terminals innervating the blood vessel walls. This apparent inhibitory effect of ADO seems to be more relevant for the contractions evoked by adrenergic nerve stimulation than for contractions evoked by exogenous norepinephrine in the dog isolated saphenous vein thus suggesting a direct action of ADO at a pre-junctional level (Collis, 1989). The underlying mechanism is still not clear and the definition of which receptor is involved in this process needs to be clarified.

1.2.3.2. Mechanisms that trigger the formation of adenosine

Both hypoxia and ischemia are potent stimuli that lead to the formation of ADO by promoting the degradation of adenine nucleotides (Schrader, 1991). In cardiac tissue a correlation has been shown between adrenergic stimulation in the presence of adequate oxygen supply and ADO formation, suggesting that ADO production is proportional to metabolic rate (Schrader, 1991). The triggering of ADO formation during hypoxia seems to be due to the activation of the 5'-nucleotidase since the transmethylation pathway is essentially oxygen insensitive. During hypoxia, degradation of adenine nucleotides is observed thereby increasing

the free concentration of AMP and consequently ADO, by the action of the 5'-nucleotidase. However, physiological increases in ADO are not always correlated with hypoxia. For example, in the feline intestine, ADO is not the mediator of hypoxia-induced vasodilation (Lautt, 1990).

1.2.3.3. Blood flow in vascular beds

Coronary Circulation:

When oxygen supply to the heart is inadequate, ADO is a good candidate in regulation of blood flow. Moreover, a change in ADO levels is also observed during the cardiac cycle, being increased during systole (Collis, 1989). These observations make the link between the purine and cardiac metabolism to coronary blood flow (Collis, 1989). Dipyridamol, an inhibitor of ADO uptake into different cell types such as red blood cells and endothelial cells, enhances the coronary vasodilatory effect of exogenous ADO. Such an effect may prove to be beneficial in certain diseases, e.g. coronary ischemia. However, dipyridamol does not seem to affect reactive hyperemia making it difficult to assess the overall beneficial effects of the drug in the heart (Collis, 1991). Another important approach to determine the relevance of ADO in the heart is by testing the effect of ADO deaminase, the enzyme responsible for ADO degradation. Conflicting results show an attenuation of the dilatory effects of ADO with no effects on resting coronary flow or the autoregulatory responses to reduction in perfusion pressure (Collis, 1991). A reduction but not an abolishment of reactive hyperemia is observed in

the presence of ADO deaminase (Collis, 1991). Studies using the ADO antagonists theophylline and 8-phenyltheophylline also revealed attenuation of the reactive coronary hyperemia (Collis, 1989). The precise physiological significance of these results is not clear and further studies to determine the exact proportion of ADO involvement in controlling coronary blood flow needs to be done.

Skeletal Muscle Circulation:

During exercise, skeletal muscle blood flow increases markedly simultaneously with the release of ADO (Collis, 1989). Release of a substance from myocytes results in exercise hyperemia, promoting an increase in flow up to tenfold (Gorman, 1991). ADO is a potential candidate as the mediator of exercise hyperemia. The proposed hypothesis is based on the observation that ADO formation is stimulated by a decrease in the O_2 supply/demand ratio observed during exercise. The trigger mechanism is still under investigation. An extreme difficulty is encountered in measuring ADO levels in skeletal muscle because it shows greater activity of ADO deaminase than other tissues (Collis, 1989). Even though an increase in venous ADO levels is seen during exercise, experiments in the presence of antagonists, metabolizers of ADO (adenosine deaminase) and potentiators of ADO actions (dipyridamole) are important to understand the role of ADO in exercise hyperemia. Studies with the antagonists, aminophylline and theophylline, were not conclusive and the results were dependent on the experimental conditions. ADO deaminase increased vascular resistance during

free-flow experiments in dog gracilis muscle, however, in moderate treadmill exercise in rats, the enzyme shows no effect on skeletal muscle flow (Gorman, 1991). Dipyridamole decreased vascular resistance during treadmill exercise in miniature swine (Gorman, 1991). These studies indicated a restricted involvement of ADO in voluntary exercise hyperemia, however ADO might contribute differently to exercise hyperemia depending if the exercise is intensive or not .

Cerebral Circulation:

ADO is one of the chemical mediators proposed to be involved in the metabolic adjustments of the cerebral circulation. Its effects are mediated by the interaction with the A2 ADO receptor. Studies with dipyridamol imply a role of ADO in the regulation of cerebral blood flow in the rabbit, however, ADO antagonists and inhibitors of ADO deaminase fail to significantly increase resting cerebral blood flow (Wylen, 1991). During a decrease in oxygen supply, ADO content increases and a reduction is observed in the hyperemic response to moderate hypoxia in the presence of the antagonist theophylline, providing evidence that ADO participates in the cerebrovascular adjustments to hypoxia (Wylen, 1991). Studies using either inhibitors of ADO deaminase or ADO uptake potentiated the cerebral blood flow response to hypoxic challenges (Phillis, 1985). Increases in ADO levels of almost 30-fold occur with an increase in blood flow of 3.5-fold during reactive hyperemia (Wylen, 1991). Beneficial effects of increases in ADO levels observed during ischemia are due not only to its effects on cerebral

blood flow but also because of its inhibitory effects on excitotoxic neurotransmitter release (Wylen, 1991). Since ADO actions in the brain are not limited to vascular effects it is relevant to be aware that pharmacological manipulations of ADO actions in the cerebral blood flow alter other cerebral functions and studies forseeing therapeutic uses of ADO can not be seen in isolation.

Mesenteric Circulation:

ADO causes dose-related increases in superior mesenteric arterial (SMA) blood flow (Proctor, 1991). In the SMA, ED_{50} for ADO is 0.034 ± 0.004 mg/kg (Lautt, 1990). Hypoxia-induced vasodilation in the feline intestine is not affected by ADO since the blockade of the vasodilating effects of exogenous, and presumably endogenous, ADO did not affect the decrease in resistance (Lockhart & Lautt, 1990). However, other studies suggested that, ADO favours the perfusion of more metabolically active intestine mucosa where local metabolism is dependent on blood flow and oxygen availability (Jacobson, 1994). During the locally mediated response of the gastrointestinal system after the ingestion of a meal there is a dramatic increase in gastric and intestinal blood flows. The postprandial hyperemia observed is increased by dipyridamol and inhibited by 8-phenyltheophylline (Jacobson, 1994). ADO appears not to be the sole substance to mediate hyperemic responses in the gut since supramaximal doses of either ADO deaminase or theophylline reduced but did not eliminate the hyperemia, suggesting that other local factors play a role (Proctor, 1991). The characteristic overshoot

in blood flow that follows arterial occlusion is known as reactive hyperemia. In several reports reactive hyperemia was inhibited considerably by theophylline, 8-phenyltheophylline, and ADO deaminase (Jacobson, 1994). Moreover, ADO levels sampled after the arterial occlusion increased by fourfold (Jacobson, 1994). Pressure-flow autoregulation, another intestinal autoregulatory phenomenon, which is the tendency to preserve blood flow with fluctuating perfusion pressure, is prevented by ADO receptor antagonists (Lautt, 1986). ADO seems however to be independent of other autoregulatory phenomena such as autoregulatory escape and post-stimulation hyperemia observed after cessation of adrenergic stimulation (Jacobson, 1994). A more relevant role is played by ADO during severe stress. ADO dramatically attenuates ischemia-reperfusion injury by different possible mechanisms including promoting direct vasodilation or indirectly by inhibiting sympathetic vasoconstrictor tone, interfering with granulocyte function or by restoring the ischemic purine deficit acting in this case as an endogenous anti-inflammatory autacoid (Proctor, 1991).

Liver Circulation:

The arterial but not the venous vasculature of the liver is affected by ADO (Lautt & Legare, 1986). ADO mediates the principal intrinsic regulation of the hepatic artery (HA) known as hepatic arterial buffer response (HABR), which is the inverse response of the hepatic arterial flow to changes in portal venous flow (Lautt et al., 1985). When portal flow increases or decreases, the HA flow will change

in the opposite direction to compensate for the portal flow change and thus to buffer the effect on total hepatic blood flow. The underlying mechanism for the HABR is explained on the basis of an ADO washout theory. The space of Mall is a small fluid space surrounding the terminal branches of the HA and portal vein (PV) and is restricted by a limiting plate of parenchymal cells to form a discreet fluid compartment. ADO concentration is dependent upon the rate of production and entry into the space of Mall and the rate of washout from the space of Mall. If portal blood flow decreases, less ADO is washed away and the accumulated ADO leads to arterial dilation. Conversely, increased portal blood flow leads to arterial constriction (Lautt et al., 1985). As in the gut, the HA autoregulation is dependent on the same ADO washout mechanism as the HABR. When arterial blood pressure is increased, the HA flow will increase and wash away more ADO from the space of Mall leading to a constriction of the HA (Ezzat & Lautt, 1987). The hyperdynamic circulation seen with portal hypertension is reversed by 8-phenyltheophylline thus suggesting a role for ADO during liver disease (Lee et al., 1992).

1.2.4. Pathophysiological responses and clinical implications

The clinical usefulness of ADO is becoming a reality with its application for the treatment of supraventricular tachyarrhythmia (Cushing & Mustafa, 1991). The usefulness of ADO to control hypotension during neurosurgery is under clinical trials (Cushing & Mustafa, 1991). Due to the evidence that ADO is involved with

hyperemia, ADO usefulness is based on its direct or indirect effects as an oxygen supplier. In the organs where there is evidence of a metabolic relation with ADO, its pharmacological manipulation could be used to obviate noxious decreases in blood flow. In organs such as the liver where hepatic metabolism is independent of ADO, the purine functions as an indirect oxygen deliverer by maintaining total blood flow. In the near future the development of therapeutic strategies to combat ADO-linked diseases will depend entirely on the development of drugs that will be capable of acting at specific ADO receptors or to modify ADO uptake or metabolism in specific target sites.

1.3 SPLANCHNIC CIRCULATION

1.3.1. Introduction

The regard by men of the viscera as a manifestation of the divine started as early as the Palaeolithic time (Granger et al., 1984). The Greeks, before a battle or passing judgments, would consult the entrails of sacrificed animals (Granger et al., 1984). The first medical texts with descriptions of the splanchnic circulation were written by Egyptians (Granger et al., 1984). For Greek "physiologists" the liver was considered the place of production of blood and the source of all veins, considered of more importance than the arteries. The PV was conceived as transporting food into the liver (Granger et al., 1984). The belief that blood vessels were gradually reduced in size until the point of not being able to admit more blood was advanced by Aristotle. It was only many centuries after, that Harvey

proposed, and Malpighi and Leeuwenhoek verified microscopically, that there is a continuity of the arteries and veins through capillaries (Granger et al., 1984). It was only in the nineteenth century that Poiseulle measured mesenteric venous pressure and directly related the venous pressure with arterial pressure (Granger et al., 1984). An interest not only for conceptual hemodynamics of the splanchnic circulation but also for the effects of splanchnic nerve stimulation on mesenteric blood flow arrived by the end of the last century (Granger et al., 1984). In this century, technical and conceptual advancements allowed for the understanding of the depth of this field, however, the contribution of the first discoveries should not be allowed to be forgotten as the foundations of today's research.

1.3.2. Mesenteric hemodynamics

1.3.2.1. Anatomy

The small and large intestine are perfused by branches of the celiac, the superior mesenteric and the inferior mesenteric arteries. The celiac artery, which arises from the abdominal aorta trifurcates into the splenic, common hepatic, and left gastric branches. The splenic branch of the celiac artery gives rise to numerous small vessels that supply the body and the tail of the pancreas (Granger et al., 1984). The common hepatic branch of the celiac artery gives rise to the gastroduodenal artery and the HA. The gastroduodenal in turn gives off the anterior and posterior superior pancreaticoduodenal arteries, providing a rich vascular network supplying the duodenum and pancreas. The inferior

pancreaticoduodenal vessels, which arise from the SMA, anastomoses with the SMA and supply the head and body of the pancreas. The HA contributes one third of the blood supplying the liver.

The SMA, distal to the celiac artery, supplies the distal small intestine and the proximal colon. Moreover, blood flow for the jejunum and the ileum is provided from mesenteric branches that arise from the SMA. Blood flow into the middle and right colic, the ileocolic is also provided from this large artery that supplies both the colon and the ileum (Granger et al., 1984).

The inferior mesenteric artery yields the left coelic artery, several sigmoidal branches and the superior hemorrhoidal branch supplying blood into the colon and rectum (Granger et al., 1984).

A significant feature of the gastrointestinal circulation is the anatomic presence of anastomotic connections at several levels of vessel branching, from the main celiac and mesenteric arteries to the extensive submucosal vascular plexus. Each of these anastomoses provides a potential conduit for collateral blood flow (Granger et al., 1984).

The venous drainage of both small bowel and colon is via the superior and inferior mesenteric veins. The venous arcades that join to form the superior mesenteric vein receive blood from the jejunum, ileum, and proximal colon. The descending colon, the sigmoid colon and the rectum are drained by the inferior mesenteric vein. Venous drainage from the duodenum is subserved by the inferior and superior pancreaticoduodenal veins that terminate at the superior mesenteric

vein. The final venous outflow into the portal vein (PV) is provided from the unification of the mesenteric veins with the splenic vein. Intestinal venous pressure is higher than in other beds since it shows the unique feature of representing the connection between two microcirculatory beds in sequence. The only venous drainage from the intestine that does not pass through the hepatic circulation is from the distal rectum that drains into the iliac veins and then into the systemic circulation.

1.3.2.2. Microvasculature

The deep submucosa, adjacent to the muscularis mucosa, contains the majority of the arterial vessels. After frequent anastomoses, blood is drained into vessels in the extramural arcading system of mesentric veins. Three layers of capillary network are present with the intestine-muscularis externa, in parallel with the two series-coupled networks of the submucosa and mucosa (Casley-Smith & Gannon, 1984).

The arterioles supplying the muscularis give off capillaries into the plane of the intermuscular septum that supplies the circular muscle. As the muscles contract, the microvascular network is distorted. The submucosa receives few capillaries except for the duodenum containing Brunner's glands (Casley-Smith & Gannon, 1984). It is, however, at the submucosa that the anastomotic plexus of distributing arteries and collecting veins is placed.

In the mucosa vascular bed, flow supplies each villus in a single arterial

branch travelling along the margin of the villus with flow radiating from the villus tip into a capillary net irrigating downward towards the base of the villus. The capillaries converge into minute venules and a single venule is formed and courses down to the submucosa draining into a submucosal vein (Casley-Smith & Gannon, 1984).

1.3.2.3. Mesenteric blood flow

Classically, two theories are proposed to explain regulation of the intestinal circulation, the myogenic and the metabolic. The myogenic response is based on the vessel property to constrict following a sustained or transient increase in transmural pressure (Johnson, 1984). The metabolic theory was first proposed to envisage a link between microvascular tone and intestinal oxygenation, thus arteriolar conductance and the number of perfused capillaries are inversely related to the tissue pO_2.

The myogenic theory is conciliatory with the control of transcapillary fluid filtration. During venous hypertension, a rise in arteriolar resistance returns the capillary pressure towards normal thus reducing the filtration area and stabilizing filtration rate (Johnson, 1984). According to the metabolic theory, intestinal vascular conductance and capillary exchange capacity rise during local arterial hypoxemia. Moreover, elevation in oxygen uptake promotes vasodilation of intestinal arterioles and precapillary sphincters (Shepherd & Granger, 1984).

A third theory based on a flow-dependent mechanism is now proposed. In

this theory flow acts either by a washout of vasodilators or by an indirect action liberating the vasodilators. This flow-dependent theory is discussed in more detail throughout the thesis.

Postprandial and Reactive Hyperemia:

After food ingestion, a transient intestinal hyperemia is observed in intestinal blood vessels. One of the potential mechanisms is the oxygen dependent hypothesis proposed by Shepherd & Granger (1984). If oxygen demand increases after food ingestion, tissue pO_2 falls and a local metabolic signal causes a reduction of arteriolar and precapillary sphincter tone (Shepherd & Granger, 1984). Consequently, an increase in blood flow increases oxygen delivery. Other recent mechanisms may supplement or alternatively contribute to the postprandial hyperemia (see section 1.2.3.3).

Three mechanisms could contribute to explain reactive hyperemia: a myogenic reaction, a metabolic vasodilation or a passive distension of the vasculature. ADO is however a strong candidate in the regulation of reactive hyperemia as was already discussed (see section 1.2.3.3).

Autoregulation:

The intestinal vasculature displays the basic mechanism of local control of blood flow known as autoregulation. This mechanism is characterized as the intrinsic ability of an organ to minimize changes in blood flow through a wide

range of perfusion pressures. As with the hyperemic responses, myogenic and metabolic theories are classically used to explain autoregulation. However, more recent work showed that a change in tissue oxygen tension is not essential for the autoregulation of mesenteric blood flow (Lang & Johnson, 1988). In contrast, the mechanism of autoregulation is clearly linked to ADO and it was suggested that ADO is produced at a constant rate and the local concentration in the area of the resistance vessels is determined by washout into the blood (Lautt, 1986). It was also argued that, since the response to ADO and autoregulation can be completely and selectively eliminated by ADO receptor blockade, that the myogenic hypothesis over physiologically relevant ranges of arterial and venous pressure is unlikely to account for autoregulation (Lautt, 1986; Lockhart & Lautt, 1990). Thus, a flow-dependent flow theory is proposed to account for the pressure-flow autoregulation (Lautt, 1986).

1.3.2.4. Pathophysiology

Mean arterial pressure is elevated in response to either increased total peripheral vascular resistance or increased cardiac output or both. Intestinal circulation contributes to peripheral vascular resistance and to venous return and cardiac output since the splanchnic organs are perfused with 25% of the cardiac output and the splanchnic veins contain 20% of total blood volume. Therefore, in volume-dependent hypertension, blood flow is increased in the intestinal circulation resulting in an increase in venous return and possibly in cardiac output (Overbeck,

1984). During the non-volume dependent type of hypertension, the essential hypertension that is frequently observed in humans, intestinal blood flow appears to be unchanged, however resistance increases to the same extent as total peripheral resistance. Thus, intestinal arterioles might contribute to the generalized vasoconstriction and consequently elevation of arterial pressure observed in this type of hypertension (Overbeck, 1984). The mechanism responsible for essential hypertension is still unknown and several theories have been suggested. The one suggested by Moncada, integrated in the context of this thesis, proposes a deficiency in NO release which accounts for the increased vascular resistance implicated in hypertensive states (Moncada, 1991).

Another important and life-threatening disorder of the splanchnic circulation is hepatic PV hypertension. Cirrhosis is the most common cause of portal hypertension and accounts for approximately 90% of all cases in developed countries (Bosch, 1989). In an advanced state of the disease, portal pressure is chronically increased concomitantly with hyperemic splanchnic circulation. The observed hyperemia results from a decrease in arterial resistance in the splanchnic vascular bed leading to an elevated portal flow. As a result of this hypertensive venous state, two major clinical sequela are developed: varix hemorrhage and ascites.

Another disease with microvascular consequences for the intestine is diabetes. Diabetes mellitus, in general, presents microangiopathy in advanced cases represented by gradual atrophy of the vessel wall and a loss of small

microvessels as well as impairment of large vessels by a form of atherosclerotic plaque formation. Even though the splanchnic circulation is affected in this pathology, the extent of its involvement is unknown (Bohlen, 1984).

The cardiovascular complications of portal hypertension and diabetes mellitus are far from being pharmacologically manipulated with a certitude of success, thus a rapid development in this area is obligatory.

1.3.3. Liver hemodynamics

1.3.3.1. Anatomy

From the 25% of the cardiac output that perfuses the liver, 20%-33% is derived from the HA. The common HA divides into the right and left HA supplying blood to the respective lobes. The other supplying vessel is the PV that carries blood flow from the spleen (10%), stomach (20%), pancreas (10%) and intestine (60%) into the liver, varying its contribution depending on the physiological state of the organism (Greenway & Lautt, 1989). The branches of the PV follow the liver disposition, eg. the right branch supplies the right side following closely the pattern of distribution of the HA. Both the PV and the HA pass to the liver, via the hilum, along with lymphatics and the hepatic nerve plexus. The blood vessels subdivide into fine branches that drain into the acinus (Greenway & Lautt, 1989). The blood bathing the parenchymal cells of the liver is mixed arterial and venous blood. The hepatic veins carry blood from the liver to the systemic circulation. As these veins emerge from the liver they drain directly into the inferior vena cava.

1.3.3.2. Microvasculature

The hepatic microcirculatory unit, known as the liver acinus, consists of the terminal hepatic arteriole, the portal venule, a bile ductule, lymphatics, nerves, sinusoids and terminal hepatic venules as described in 1954 by Rappaport (Rappaport, 1981). A portal triad, situated around a central axis of the acinus, incorporates the terminal portal venule, hepatic arteriole and bile ductule (Rappaport, 1981). As blood enters the acinus at the central region, or zone 1, it ensures a uniform blood supply to the highly oxygenated parenchyma cells. As blood flows through acinar zones, different microenvironmemts and gradients in enzymic and metabolic functions are observed. Zone 3 represents the microcirculatory periphery of the acinus close to the perivenular area with an increased concentration of the enzymes responsible for lipogenesis and the majority of drug metabolism (Rappaport, 1981). Sulfation, however, occurs preferentially in the periportal zone (Jungermann, 1988). The periportal area or zone 1 is competent for glycogen and protein synthesis (Rappaport, 1981). Pericentral regions are responsible for nitrogen and ammonia metabolism as well as glutamine formation (Rappaport, 1981).

The sinusoids are specialized capillaries that connect the inflow vessels to the hepatic venules. They consist of a lining of endothelial cells perforated by fenestrae. These sieve plates, as proposed by Wisse, allow communication between the sinusoidal lumen and the perisinusoidal space of Disse where the microvilli of

the hepatocytes provide an amplified surface area for uptake processes (Wisse et al, 1985). Blood passing the full length of a sinusoid is sequentially distributed to approximately 20 hepatocytes (Greenway & Lautt, 1989). Within the sinusoids other cells such as macrophagic Kupffer cells and the fat storing lipocytes, Ito cells, are established.

1.3.3.3. Hepatic blood flow

Of the 25% of cardiac output that perfuses the liver, corresponding to a total blood flow of 100-130 $ml \cdot min^{-1} \cdot 100g^{-1}$ liver in the dog, cat and human, the PV contributes two-thirds of the total (Greenway & Lautt, 1989). The arterial and the venous extrahepatic pressures are approximately 100 mm Hg and 7-10 mm Hg respectively. When the intrahepatic vascular resistance is raised, pressure increases by 2-3 times without altering portal blood flow. Portal flow depends on the arteriolar resistance in the splanchnic organs that drain into the PV (Greenway & Lautt, 1989). Because of the lack of control by the liver over the PV side, the only control of flow within the liver is via the HA. However, the PV is able to regulate total blood flow by regulating HA blood flow, as will be further discussed.

Extrinsic and Intrinsic Hepatic Arterial Flow Regulation:

The nervous control of the HA flow will be further discussed in the next chapter.

Representing an exception when compared to the majority of the arteries,

the HA is not metabolically regulated, being therefore independent of the hepatic parenchymal cell metabolism (Greenway & Lautt, 1989). Therefore, the intrinsic regulation of the HA is essentially based on the hepatic arterial buffer response (HABR) and the autoregulatory capacity of this artery.

Hepatic Arterial Buffer Response:

Even though there is no metabolic direct control of the HA, ADO production within the liver is the responsible substance for the HABR. The first report of a reciprocal relationship between the flows of the HA and the PV was attributed by Child (1954) to Betz (1863) and Gad (1873). It was only one century after that Lautt et al. (1985) performed experiments that elucidated this mechanism. Although a reciprocal mechanism had been suggested, in fact, changes in portal flow produce inverse changes in HA flow or resistance but changes in HA flow do not produce changes in portal flow or resistance (Lautt, 1981). The hypothesis proposed is based on an ADO washout from the space that surrounds the HA resistance vessels and the portal venules, the space of Mall. A limiting plate separates the fluids contained in the space of Mall from the surrounding environment. This hypothesis states that ADO, produced at a constant rate, is washed away by blood flowing from the PV and HA, thus regulating its final concentration. As portal flow decreases, less ADO is washed away and therefore this increase in ADO concentration promotes dilation of the HA (Lautt, 1985). The inverse mechanism also occurs whereby increases in portal flow lead to a

constriction of the HA. This was the first blood flow-dependent mechanism ever described. Several criteria had to be fulfilled to support this washout theory. First ADO was proved to dilate the HA. Secondly, portal flow has to have access to the HA resistance vessels to be able to wash away ADO from the space where arterial resistance vessels are placed. ADO and other vasoactive substances, when infused directly into the PV, were shown to alter HA conductance (Lautt, 1985). Third, potentiators of the exogenous ADO effects, such as dipyridamol, potentiate the magnitude of the HABR. And finally, but not less important, pharmacological antagonists of ADO, such as 8-phenyltheophylline, also produce competitive blockade of the HABR (Lautt & Legare, 1985).

Autoregulation:

The autoregulatory mechanism is characterized as the intrinsic ability of an organ to minimize blood flow changes over a wide range of perfusion pressures. In the HA, autoregulation is based on an ADO washout mechanism similar to the HABR. An increase in blood pressure leads to an increase in blood flow and an augmentation in ADO washout which leads to a constriction of the HA (Ezzat & Lautt, 1987). This mechanism was proven by the use of the selective antagonist of ADO, 8-phenyltheophylline, to produce a parallel antagonism of the buffer response and autoregulation (Ezzat & Lautt, 1987).

1.3.3.4. Pathophysiology

The mechanisms of HABR and autoregulation are relevant to maintain hepatic clearance and total hepatic blood flow. The clearance of blood born compounds such as hormones is blood flow dependent (Greenway & Lautt, 1989). Adjustment of increased hormonal output by glandular sources is dependent on the rapid turnover or metabolism by the liver maintaining hormonal clearance as constant as possible.

The maintenance of hepatic blood volume is of primordial importance in overall cardiovascular homeostasis. Because of the known high capacitance of the liver, active and passive changes in liver volume can mobilize a large amount of blood volume into the systemic circulation with effects on venous return and cardiac output. By maintaining hepatic blood flow, and therefore portal and intrahepatic pressures constant, passive alterations in hepatic blood volume are minimized, thus stabilizing venous return (Greenway & Lautt, 1989).

Indirectly, the HABR is also responsible for the constancy of oxygenation of the liver. For example, during the response to hemorrhage, the decrease in portal blood flow is proportionally higher than the decrease in HA flow, which may actually increase with the result of supplying highly oxygenated flow to the liver during mild or moderate hemorrhage (Lautt & McQuaker, 1989).

It seems that the HABR is depressed or absent in severe liver disease and when present appears to be a potent prognostic indicator for the establishment of a portacaval shunt used for reducing portal hypertension (Burchell, 1976). The

HABR, shown to occur in the human liver, also has been suggested to be used as an index of viability after liver transplantation (Henderson, 1992).

The use of ADO or ADO analogues to produce HA dilation is problematic since its dilatory effect on the mesenteric arteries will promote an increase of PV flow and therefore a decrease in HA flow (Lautt et al., 1988). To increase highly oxygenated blood flow into the liver a selective mesenteric arterial vasoconstrictor might be useful since the decreased PV blood flow will result in an increase in HA blood flow.

1.4 SPLANCHNIC CONTROL BY SYMPATHETIC NERVES

1.4.1. Mesenteric innervation

1.4.1.1. Anatomy

Mesenteric blood vessels are innervated by sympathetic fibers from the cervical region of the spinal cord. Preganglionic fibers join to form the splanchnic nerves from which arise the ganglia responsible for the mesenteric innervation, the celiac, the superior mesenteric, and the inferior mesenteric ganglia (Kreulen & Keef, 1989). It is, however, important to mention that the SMA also receives sympathetic fibers directly from the lumbar region at least in the dog (Greenway, 1984). Thus, section of the splanchnic nerves does not completely denervate the splanchnic circulation. The mesenteric nerve fibers enter the gastrointestinal tract along with the blood vessels. The fibers lying along, within and outside the adventitia are known as perivascular nerves (Kreulen & Keef, 1989). The

perivascular nerves give off branches that enter the adventitia of the arteries and veins, subdividing into the terminal ground plexus. In coexistence with the terminal plexus, the perivascular nerves are found in the adventitia and in the surface of the smooth muscle (Kreulen & Keef, 1989).

The density of the innervation seems to correlate well with the magnitude of the response to contraction to nerve stimulation. Although adrenergic innervation is most dense at the advential-medial border in all blood vessels, some fibers penetrate into the media of mesenteric arteries of different species such as the dog, rat, and guinea pig (Kreulen & Keef, 1989). The mesenteric arteries appear to be unique in the sense that the junctional cleft width, this is the distance between the varicosities and the muscle cell, is approximately four times smaller when compared to the carotid and renal arteries (Kreulen & Keef, 1989). Sympathetic nerve stimulation causes vasoconstriction by mainly releasing the neurotransmitter, norepinephrine, from its adrenergic nerves. Its postsynaptic effects are mainly through alpha-1 and alpha-2 receptors.

The vagus gives rise to extrinsic cholinergic nerves that innervate the intestine but they do not affect vascular responses. Thus, vagotomy has no effect on the SMA flow. However, vagal stimulation causes acid secretion accompanied by gastric vasodilation. It remains unknown if this observed dilation is due to direct vasodilator fibers or indirectly to the release of vasodilatory substances (Greenway, 1984).

1.4.1.2. Vascular responses

The failure of arterial smooth muscle to maintain a contraction in the presence of continued nerve stimulation is known as autoregulatory escape. Contrary to studies done in the skeletal muscle, the intestinal vascular bed, after electrical stimulation of the splanchnic nerves, shows a frequency-dependent vasoconstriction followed by a recovery within 2 min of the flow towards control levels in spite of maintained nerve stimulation (Greenway, 1984). Autoregulatory escape to reflex activation, or during direct stimulation of sympathetic nerves, occurs in the arterial but not in the venous smooth muscle. When the arterial resistance decreases towards control levels, the venous response is maintained. Escape occurs either at a constant pressure or constant flow perfusion of the vascular bed (Greenway, 1984). After cessation of the stimulus, reactive hyperemia is observed and its intensity seems to be proportional to the intensity of the stimulation (Greenway, 1984).

Autoregulatory escape to sympathetic nerve stimulation is observed in the small intestine of dogs, cats, and humans (Greenway, 1984). Escape during norepinephrine infusion has been confirmed in cats and dogs (Greenway, 1984). The hypothesis that autoregulatory escape is due to a redistribution of the blood flow has been refuted based on microspheres studies (Greenway, 1984). Thus, two hypothesis might be considered to explain autoregulatory escape: a direct or indirect release of a specific substance, or a failure of arteriolar smooth muscle to maintain contraction. A case has been made for the failure in arteriolar smooth

muscle maintenance of contractions based on accumulation of intracellular sodium, however this does not seem universal for different experimental procedures (Greenway, 1984). Thus the autoregulatory escape seems to be dependent on the production of a vasodilatory substance. ADO, considered a good candidate, seems not to be involved either in the autoregulatory escape or post-stimulation hyperemia (Jacobson, 1994). An alternative hyothesis to explain escape was made by Chen & Shepherd (1991) who suggested that hydrogen ion formation during nerve stimulation inhibits postjunctional alpha-2 adrenoceptor-induced constriction in the intestine.

During the steady-state phase of nerve-induced vasoconstriction a moderate decrease in blood flow is observed. The arterial response to the sympathetic stimulation is almost completely abolished by alpha receptor-adrenergic antagonists (Jodal & Lundgren, 1989). The effect of sympathetic stimulation in blood flow distribution within the mucosa of the small intestine is however controversial. From different studies it has been suggested that constriction occurs almost exclusively in the crypts, whereas villus blood flow is not influenced when compared to control (Jodal & Lundgren, 1989). Venous constriction induced by sympathetic nerves expels blood from the intestine with a maximal effect of 40% in cats and 60% in dogs (Jodal & Lundgren, 1989).

1.4.1.3. Neurotransmitters and neuromodulation

The classic view that each neuron releases only one transmitter substance is becoming obsolete. Histochemical studies revealed that two or more putative neurotransmitters may coexist in the same neuron. Neuropeptide Y is an example, it coexists with norepinephrine in many adrenergic fibers innervating vascular smooth muscle (Jodal & Lundgren, 1989). The vascular innervation of neuropeptide Y appears to be identical, in its organization, to the adrenergic innervation as already described for norepinephrine (Jodal & Lundgren, 1989). In the vascular innervation of the gastrointestinal tract, neuropeptide Y is a cotransmitter released with norepinephrine. In the colon, neuropeptide Y-induced vasoconstriction was estimated to be approximately 25 times more potent than norepinephrine when compared at the same molar concentrations (Jodal & Lundgren, 1989). Neuropeptide Y release seems to be dependent on the frequency of nerve stimulation, low frequencies preferentially release norepinephrine and high frequencies release neuropeptide Y. Its *in vivo* effects appear to be greater for smaller vessels and more potent for the arterial side (Jodal & Lundgren, 1989).

Vasoactive intestinal polypeptide is a vasodilator in the gastrointestinal circulation (Chou et al, 1984). All the vessels of the gastrointestinal tract show vasoactive intestinal polypeptide innervation with the veins being less innervated (Jodal & Lundgren, 1989). Vasodilation suppression by blockade of nerve conduction, using tetrodotoxin, also inhibits vasoactive intestinal polypeptide release, imputing a relevant role to vasoactive intestinal polypeptide in the

intestinal vasodilatory mechanisms (Jodal & Lundgren, 1989). Substance P, a polypeptide composed of 11 amino acid residues, has dilatory effects on the gastrointestinal vascular smooth muscle. Because nerve conduction blocking agents and various neurotransmitter receptor blockers did not alter substance P-induced vasodilation, its effects appear to be directly on the vascular smooth muscle (Jodal & Lundgren, 1989). However, the physiological relevance of substance P in the control of gastrointestinal blood flow remains veiled.

Other substances, such as cholecystokinin and gastrin, are able to increase blood flow with the vasodilatory effects being less potent for gastrin (Chou et al, 1984). Gastrin vascular effects seem, however, to be secondary to effects on intestinal functions; only the vasodilatory effect of cholecystokinin in the duodenum and jejunum could be considered metabolically independent (Chou et al, 1984).

1.4.2. Hepatic innervation

1.4.2.1. Anatomy

The extrinsic innervation of the efferent hepatic nerves affects the hepatic vasculature and metabolism. Associated with blood vessels and bile ducts, branches of the vagus, the splanchnic and occasionally phrenic nerves enter the liver. The HA is surrounded by the anterior plexus, composed of fibers from the celiac ganglia and the anterior vagus (Friedman, 1988). The posterior plexus, composed of fibers from the celiac ganglia and posterior vagus, surrounds the PV

and bile duct and communicates with the anterior plexus (Friedman, 1988). Although sympathetic and parasympathetic nerves co-exist in the liver, blood vessels appear to receive only sympathetic nerves (Rapapport, 1975). Suggestions were made that intrahepatic branches of the HA, PV and hepatic veins in the human liver receive cholinergic nerve fibers (Amenta et al., 1981). The role of parasympathetic nerves in the hepatic vascular response is unclear and controversial. Sinusoidal dilation was reported by Koo & Liang (1979) in response to vagal stimulation and intraportal acetylcholine infusion. However, others did not observe this effect after parasympathetic nerve stimulation. Moreover, after topical acetylcholine application, constriction was observed (Greenway & Stark, 1971).

Most intrinsic nerves are associated with the vascular system. Even though there is considerable interspecies variation, nerve fibers appear to be associated with the adventitia of the arterioles and venules (Friedman, 1988). Smooth muscle and endothelial cells also show extended fibers (Friedman, 1988). Neurons also terminate in Kupffer cells and fat storing cells of Ito (Lautt, 1983). There is little evidence for extensive cholinergic innervation of the liver. In contrast, hepatic blood vessels such as the PV, branches of the HA, and sinusoids are richly innervated by adrenergic nerves (Friedman, 1988). HA sympathetic tone is not well defined. However, some studies have shown that in anesthetized cats and dogs acute denervation showed insignificant hemodynamic effects (Greenway & Lautt, 1989).

1.4.2.2. Vascular responses

After electrical stimulation of the hepatic nerves a marked reduction in arterial blood flow was observed for the first time in 1910 (Burton-Opitz, 1910). A decrease in arterial flow in a frequency dependent manner is observed after nerve stimulation with the mean frequency required to produce 50% of the maximal conductance response being 2.4 $\pm$ 0.9 Hz in cats (Legare and Lautt, unpublished observation). Responses to maintained electrical stimulation, known as vascular escape, is species dependent. As discussed later, in the cat but not dog, HA flow and conductance both return towards control levels despite continued nerve stimulation (Greenway & Lautt, 1989). As with vascular escape, the mechanism responsible for the observed post-stimulatory hyperaemia, seen in cats and dogs after cessation of nerve stimulation, is not clear (Greenway & Lautt, 1989). As mentioned before, PV flow is not regulated by the liver and stimulation of the hepatic nerves only has effects on PV pressure (Greenway & Lautt, 1989). An increase in total resistance is observed with the site of resistance usually being undetermined. In the cat and dog, a distinction between pre- and postsinusoidal resistance was made by Lautt's group (Lautt et al., 1986; 1987; Lautt & Legare, 1987; Legare & Lautt, 1987). Experimentally, venous pressure is measured by placing a retrograde catheter into the hepatic veins until the pressure measured is similar to PV pressure. Using this catheter, a pressure gradient from the intrahepatic site (lobar venous pressure) to the post-hepatic venous site is observed and represents the postsinusoidal resistance (Lautt et al., 1986; 1987; Lautt &

Legare, 1987; Legare & Lautt, 1987). The gradient observed between portal pressure and lobar pressure reflects the presinusoidal (plus sinusoidal) resistance. By the use of this methodology, when a rise in PV pressure is similar to the rise in lobar venous pressure the response can be inferred to be due to a postsinusoidal constriction (Lautt et al., 1986; 1987; Lautt & Legare, 1987; Legare & Lautt, 1987). This is observed at 2 Hz; presinusoidal constriction only becomes significant at 4 Hz (Lautt et al., 1987). Postsinusoidal constriction is maximal at 4 Hz but presinusoidal constriction continues to rise until it accounts for approximately 40% of the total resistance change at 10 Hz (Lautt et al., 1987). Increases in resistance promoted by intraportal norepinephrine infusions (0.25 to 1.25 $\mu g \cdot kg^{-1} \cdot min^{-1}$) in contrast to nerve stimulation, seem to be due to an increase in postsinusoidal constriction accounting for approximately 45% of the total rise in portal pressure (Lautt et al., 1987). Consequently, a rise in portal pressure is not a good index of intrahepatic pressure when presinusoidal constriction occurs.

Vascular escape is a phenomenon that is not exclusive to cat livers and was observed in other species and other organs in response to nerve stimulation and norepinephrine infusion (Greenway & Lautt, 1989). The HA shows mean vascular escape of 87% within two minutes. Escape also occurs to intra-arterial or intra-portal infusion of norepinephrine in cats, excluding as a possible mechanism for vascular escape a depression in transmitter release (Greenway & Lautt, 1989). In an attempt to suppress escape, several different substances were tried, and from those, only glucagon was able to suppress vascular escape of the cat HA but not

the SMA (d'Almeida & Lautt, 1989). The hypothesis in which formation of hydrogen ions during nerve stimulation inhibits post-junctional alpha-2 adrenoceptor-induced constriction in the intestine has been recently proposed by Chen and needs to be tested in the liver (Chen & Shepherd, 1991). Even though there is some controversy, portal pressure appeared to not show escape. When present it was suggested to be only at the presinusoidal site (Lautt & Legare, 1992). Some research has been done in this field, however further work in this area is clearly merited since the mechanism of vascular escape from neurogenic constriction is still unknown.

1.4.2.3. Neurotransmitters and neuromodulation

HA and portal venous response to nerve stimulation is mediated by alpha receptors, however, a small beta adrenergic vasodilation can be observed and blocked by propranolol (Greenway & Lawson, 1968).

Mainly localized in arterial vessels, imunoreactivity studies for neuron-specific enolase show the presence of neuropeptide Y, substance P and vasoactive polypeptide. However, the involvement of neurotransmitters other than norepinephrine still remains obscure. HA constriction to nerve stimulation and norepinephrine infusion is inhibited by ADO in *in vivo* studies thus suggesting an ADO post-junctional antagonism (Lautt & Legare, 1986).

In the extrahepatic PV, ADO modulation of noradrenergic neurotransmission is pre-junctional (Kennedy & Burnstock, 1984). However, ADO is without effect

on the PV resistance vessels and does not modulate vasoconstriction in the PV in contrast to the post-junctional antagonism of a variety of constrictors in the HA (Lautt & Legare, 1986). Alpha 1 and 2 appear to control portal pressure but the contribution of each one for pre and post-sinusoidal sites remains unknown (Greenway & Lautt, 1989). Eicosanoid inhibitors decrease vasoconstriction induced by nerve stimulation and norepinephrine infusion (Iwai & Jungermann, 1987). Hypoxia appears to also be a modulator of the constrictor effects of sympathetic nerves and norepinephrine infusion and a suggestion was made that this modulation is mediated by eicosanoids (Becker et al., 1990). Studies performed regarding total hepatic blood volume show that sympathetic nerve stimulation is mediated by alpha 2 adrenoceptors (Greenway, 1979). Because hepatic nerves play a role in the maintenance of blood volume and therefore cardiac output, further knowledge on hepatic nerve function is eminent.

1.5. THEORETICAL ASPECTS OF QUANTITATION

1.5.1. Calculations of vascular resistance and conductance

An intricate part in the development of this thesis work was to assure that the expression of results were appropriate. Changes in vascular tone, expressed as vascular conductance (flow/pressure gradient), represents the more physiological state where the primary change that occurs is in blood flow (Lautt, 1989). Traditionally, vascular responses are expressed as vascular resistance (pressure gradient/flow), which is inversely and, therefore, non- linearly, related to blood

flow at a constant perfusion pressure (Lautt, 1989). Thus, conductance is preferable to resistance as an *in vivo* index of vascular tone. As flow is dramatically reduced during intense vasoconstriction, calculated resistance would approach infinity, a value that is not mathematically useful and difficult to visualize in physiological terms. Calculated conductance in this situation, unlike resistance, parallels the changes in blood flow resulting from changes in vascular tone. In this case even if blood flow is reduced to zero, calculated conductance is zero, a value mathematically usable.

It is important to note that conductance is not always the best method to express vascular tone. All the reasons given for the use of conductance are now applicable for resistance if changes in vascular tone produce primarily changes in pressure, for example under conditions of constant flow (Lautt, 1989). Therefore, the appropriate index that reflects changes in vascular tone should be selected on the basis that the parameter that undergoes the primary change, flow or pressure, must be in the numerator (Lautt, 1989).

In this investigation, experiments were performed by using an external perfusion circuit in which blood flow arriving to the vessel under study was controlled by the use of a pump incorporated in the circuit. This experimental procedure allows changes in vascular tone to be reflected by changes in either blood flow or perfusion pressure. Experiments performed at a constant flow should have their results expressed as resistance. Conversely, the constant pressure experiments should be expressed as conductance. Because of the complexity of the

experimental protocol, with the need to compare the two types of protocols, I made the decision to express the results as absolute changes either in pressure or blood flow. Since one of the variables is always constant, the use of absolute change does not incur errors.

1.5.2. Constant flow and constant pressure preparations

In the studies originated in this thesis, the control of either blood flow or perfusion pressure was a key point since vascular tone changed in the *in vivo* preparations between the control state and after the infusion of the NOS inhibitor L-NAME. In order to control one or the other key factor, the experiments were performed with the use of the external circuit allowing the study of varying shear stress and the effects of the vasodilatory substance, ADO, at both controlled pressure and flow.

Shear Stress Experiments:

A critical aspect of these studies regards the use of a pump-controlled long-circuit which allowed vasoconstriction to be induced in either a constant flow or a constant pressure situation. Vasoconstriction during constant flow will increase shear stress, as illustrated by the following relationships.

The shear rate (γ) at the inner surface of the vessel wall is given by

$$\gamma = (m + 2)Q/(\pi r^3) \qquad \text{(Kamika \& Togawa, 1980)}$$

and the wall shear stress (τ) by

$$\tau = \eta \gamma$$

where Q is the total blood flow, r is the internal radius of the vessel, and η is the blood viscosity. The value of m depends on the flow condition; with laminar flow $m=2$ and with turbulent flow $m>2$. The value of m in our preparation was assumed to remain constant during all phases of the experiment. In the case of constant flow under sympathetic stimulation, the only factor that varies is the internal radius of the vessel which will decrease due to sympathetic stimulation. Thus, shear rate (γ) and shear stress (τ) will increase. If turbulence did increase at the constricted site, shear stress would further increase. Resistance has been shown to be related to r^3 in a complex vascular network (Mayrovitz & Roy, 1983). When vasoconstriction is induced under constant pressure perfusion, flow was reduced using the perfusion pump such that the ratio of flow to the radius of the vessels was maintained constant, as defined by this cubic relationship. Therefore, shear rate would not be altered and consequently the wall shear stress should be maintained constant throughout this experimental period. Thus a primary assumption is that shear stress, at the site of constriction, is not altered if perfusion pressure is held constant.

Quantitation of shear stress was a limitation of this preparation however, it was possible to quantitate the vascular responses to sympathetic nerve stimulation or norepinephrine infusion in the presence and absence of change in shear stress. Increases in shear stress were achieved by maintaining the flow delivered to the

vessel under study constant when vasoconstriction was induced, either by stimulation of the sympathetic nerves or by infusion of norepinephrine. If the arterial pressure in the vessel is maintained constant by decreasing blood flow delivered to the artery during vasoconstriction, shear stress remains constant. Thus, during increases in shear stress the response was assessed as an increase in perfusion pressure. Conversely, when perfusion pressure and shear stress were maintained constant, the responses were assessed as a decrease in blood flow.

Adenosine Studies:

In these studies the vasodilatory effect of ADO was determined before and after the increase in basal tone by L-NAME. Because of the change in baseline, studies were performed at different baselines. There was therefore the need to assess the responses to ADO at the same perfusion pressure after L-NAME as in the control and at the same blood flow before and after the rise of basal tone with L-NAME. The dilatory response to ADO was assessed as a decrease in absolute change in perfusion pressure.

1.5.3. Autoregulation

Autoregulation curves can be performed as full curves with decreases in perfusion pressure of small steps averaging 10 mm Hg where a "shoulder" separates the autoregulatory range from the non-autoregulatory range. The "shoulder" is assessed by plotting pressure versus resistance from a full pressure-

flow curve, where the shoulder is identified as the pressure at which resistance is the lowest. Knowing the shoulder for a specific vessel, two step autoregulation curves can be performed in which the first step is in the autoregulatory range, reducing pressure from a maximal physiological pressure to the point of the shoulder. The second step is performed in the non-autoregulatory range, from the shoulder to a lower pressure.

Classically, autoregulation was quantitated as change in calculated vascular resistance. A disadvantage of using resistance is that the calculated resistance rises to infinity at low pressures (see section 1.5.1). Recently, more accurate indices have been used to measure autoregulation.

In this thesis, autoregulation is assessed by the use of two autoregulatory indices:

1) Autoregulation index, ARI: This index calculates the proportion of flow change expressed relative to the proportion of pressure change (Semple & DeWardner, 1959).

$$ARI = 1 - [SMAF_c - SMAF_s / SMAF_c] / [(SMAP_c - SMAP_s) / SMAP_c]$$

where SMAF refers to superior mesenteric arterial flow, SMAP superior mesenteric arterial pressure and the subscripts C and S refer to control levels and values measured at the shoulder respectively.

2) Autoregulation is assessed as well by a second index, the slope index (Ezzat & Lautt, 1987). To calculate this index, full pressure-flow curves need to be performed with the intent of determining the lowest resistance point which is

considered to be the "shoulder" that separates the autoregulatory range from the non-autoregulatory range. The slope index is an index of linearity of the pressure-flow curve calculated by dividing the slope of the best fitting linear regression analysis above "the shoulder" by the slope "below the shoulder" expressed as percent. A perfectly straight pressure-flow curve would give a slope index of 100%. The two step method can also be used if the shoulder is known and the slope of the pressure-flow relationship above the shoulder is compared to that below the shoulder.

1.6. OBJECTIVES OF THE THESIS

Only by proceeding along the path of objective investigation can we, step by step, reach the understandable. My first insight to science was to understand that a philosophical approach to the objective problem should be taken. I felt grateful that in this program a student is not enrolled in a doctorate in medicine but in philosophy. Because living creatures are a complex organization of multiple organs and tissues interacting in intricate ways, I was, since the beginning, more attracted to *in vivo* studies than to the study of the isolated cell.

In the present *in vivo* studies, I examined the effects of ADO and NO on the regulation of splanchnic vascular resistance. The following hypotheses led to the development of the work presented in this thesis. The objective of the thesis studies was to test these hypotheses.

1. Increases in shear stress in the resistance vessels promote NO release from the endothelium and the released NO modulates vasoconstriction both in the intestine and the liver.
2. Autoregulation, which is mediated by ADO in the intestine and liver, is antagonized by NO.
3. NO potentiates the vasodilatory properties of ADO.

The results of the research projects contained in this thesis are presented in the following four chapters. The second chapter discusses methodologies applied to these studies. Chapter three consists of studies of NO release triggered by shear stress in the intestine and liver. In chapter four, studies related to the NO capacity to antagonize autoregulation are presented. Finally, in chapter 5, results of the potentiation of ADO vasodilatory effects are presented and discussed. In chapter three, four, and five, an introduction is given, followed by materials and methods utilized in each investigation, as well as results and discussion of the results.

2. MATERIALS AND METHODS

2.1. PRE-SURGICAL SUPPORT

The ultimate goal of these studies is to extrapolate findings obtained in experimental models to physiological states in humans. Some procedures are imposed by the experimental setting, and may result in modified splanchnic hemodynamics and oxygenation. During these experiments, an attempt to minimize these problems was made. All the animals were treated according to the guidelines of the Canadian Council on Animal Care and all protocols were approved by the Animal Use Committee of the University of Manitoba.

It is well known that anesthesia can alter splanchnic hemodynamics through a direct vascular effect or indirectly through an effect on the heart by increasing cardiac output and/or on neurohumoral mechanisms (Kvietys et al., 1981). In our experiments, fasted cats of either sex (3.3 $\pm$ 0.2 kg) were anesthetized with sodium pentobarbital (32.5 mg/kg) administered intraperitoneally. Sodium pentobarbital is a commonly used anesthetic for studies on the splanchnic bed. At the doses used, it is described that this anesthetic causes an initial transient increase in intestinal blood flow and cardiac output, which disappears within 30-60 minutes (Kawaue & Iriuchijima, 1984). Normally, from the time of anesthesia to the experimental procedure an interval of at least 2.5 to 3 hours is attained; consequently vascular alterations due to anesthesia are not expected. During and after the surgical procedure anesthesia was maintained through a drip bag (390 mg sodium pentobarbital/500 ml dextran). This small and constant dosage of the given

anesthetic obviates the problems of giving higher doses of anesthetic during the experiment, therefore the use of constant and diluted doses of anesthetic minimizes any secondary effects on the splanchnic vasculature. To certify that the animal was in deep anesthesia, limb reflexes, ear or eye movement, were frequently checked.

It is also imperative to monitor and control both body and ambient temperatures during experiments in anesthetized animals. A decline in temperature would lead to an increased redistribution of blood flow to the splanchnic vascular; the converse occurs when core temperature is increased (Hardy & Bard, 1974). Recently, a study indicated that a change in ambiental temperature between 31^0-40^0 C including the temperature at which intestinal preparations have been maintained (35^0-39^0 C) does not alter intestinal blood flow (Kvietys et al., 1985). In our experiments, body temperature was maintained at 37.5°C by the use of a rectal probe and a thermally-controlled unit (Yellow Springs Instruments model 72) operating heating rods in the table.

A third important step during the pre-surgical manipulations was to heparinize all the cannulas used during the surgical procedure. Because the use of external vascular circuits increased the probability of formation of clots, there was a need to heparinize all the cats (3000 IU) before establishing the vascular circuits. The vascular circuit consisted of two cannulas that were inserted in the arteries (femoral arteries) from which blood was pumped by a variable speed pump to control the blood flow rate through the circuit. The pump was followed by a silk filter that removes blood clots and a windkessel chamber to trap air bubbles

as well as to buffer pressure fluctuations generated by the pump. After the windkessel chamber a multi-line infusion cannula was placed. A Y branch was placed in the circuit with an electrical ground for the flow probe in one branch and the electromagnetic flow probe in the other branch. This Y branch was necessary to allow blood flow to be diverted around the flow probe to obtain a blood flow zero baseline. A cannula for monitoring the circuit pressure and a cannula to return the blood into the vessel under study were also incorporated in the circuit. This external vascular circuit was sterilized with formaldehyde 10%, recirculated for 2 hours. After rinsing with 1 litre of distilled water, a 3-5% ammonia solution was passed through and followed by a flush of 6 litres of distilled water. Before the surgical procedure the circuit was filled with saline.

2.2. SURGICAL METHODOLOGY

All blood pressures were monitored via catheters using Gould and Statham pressure transducers, and all parameters were recorded on a Sensor Medics R611 Dynograph. Transducers were set to zero reference level relative to the midpoint of the inferior vena cava at the hepatic outlet. The zero reference was checked for drifting before and after every experimental manoeuvre. Systemic arterial pressure and central venous pressure were monitored via a right carotid arterial catheter and a left femoral venous catheter, respectively. The trachea was cannulated.

Deficient ventilation has important implications in the splanchnic hemodynamics. Unventilated animals have a tendency towards hypoxia,

hypercapnia and acidosis, resulting in a decrease in vascular resistance (Svanvik et al., 1968). In our experiments, ventilation was controlled and provided by use of a respirator before laparotomy.

In the normal course of abdominal surgery, laparotomy, traction and viscera manipulation are inevitable. Over the time period necessary to open the abdomen and isolate mesenteric arteries from the surrounding tissue, intestinal vascular resistance increases dramatically (McNeill & Pang, 1982). It is only 60 minutes after completion of surgery that resistance tends to return towards presurgical levels; the observed increased resistance is suggested to be due to an activation of the angiotensin and vasopressin systems (McNeill & Pang, 1982). During the surgical procedure, an abdominal incision was made along the midline (6-8 cm long) and, when necessary, along the left subcostal margin (3-4 cm). The cutaneous and muscle layers along the incision were tied separately. Great care was necessary at this stage to avoid blood loss throughout the experiment. Because of the effects of the trauma of surgery on the splanchnic hemodynamics, a recovery time of 45 to 60 minutes was always taken.

Superior Mesenteric Artery Surgical Protocol:

After laparotomy, the inferior mesenteric artery was ligated, the SMA was isolated, and its periarterial nerve bundle was gently separated, ligated and cut. Both femoral arteries were isolated, cannulated and connected to the SMA through a pump-controlled long circuit as previously described. Briefly, the blood passed

sequentially through a rotary pump, a silk filter, a windkessel bubble trap and an electromagnetic flow probe (Carolina Medical Electronics EP 408). As already mentioned this circuit allowed control and measurement of SMA blood flow and pressure. SMA pressure was monitored from a catheter in the circuit. An infusion line in the circuit allowed intra-arterial administration of drugs.

Hepatic Artery Surgical Protocol:

Via laparotomy, the inferior mesenteric artery was ligated and the spleen was removed. The periarterial nerve bundle of the SMA was gently separated, ligated and cut. An electromagnetic flow probe and a micrometer controlled screw clamp were placed on the SMA (Carolina Medical Electronics EP 408). Anastomotic connections to the SMA provided adequate blood supply to areas normally supplied by the ligated vessels. This methodology assures that all blood supplying the PV derives from the SMA, thus SMA flow was synonymous with PV blood flow, as previously described (Lautt et al., 1985). The HA was isolated and the gastroduodenal and celiac arteries were isolated and ligated. After the nerve bundle around the HA was gently separated, ligated and cut, a vascular long circuit identical to that described in the previous protocol was connected between the femoral arteries and the HA. HA pressure was monitored from a catheter incorporated into the circuit, and drugs were administered through a separate infusion line.

2.3. NON-SURGICAL METHODOLOGY

All intra-arterial infusions were accomplished by the use of a cannula in the circuit. Infusions were made with an infusion pump (Harvard apparatus, model 901). Intravenous infusions were made directly into the inferior vena cava through a cannula placed in the femoral vein.

All drugs, ADO, isoproterenol, L-NAME, L-arginine and norepinephrine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Isoproterenol, L-NAME, and L-arginine were dissolved in saline. ADO was dissolved in warm saline and norepinephrine was dissolved in saline and diluted in a 5.6 pH ascorbic acid solution. All drugs, were dissolved accordingly with the necessary dose adjusted for the weight of the animal.

Calibration of all pressure transducers was verified regularly using a mercury and water manometer for the arterial and venous transducers respectively. Both SMA and HA pressures were calibrated in situ at the end of each experiment to account for resistance to flow in the circuit. A graph of flow against pressure was plotted and a linear regression where the intercept was forced to pass through zero allowed the calculation of the slope which was used to correct circuit pressures. The transducers and recorder were found to be extremely stable. By using the variable speed pump, flowmeters were calibrated at the end of each experiment by measuring the time taken to fill a fixed volume in a reservoir at different blood flows. Zero flow was also checked by either clamping the side of the Y branch in the external circuit that contains the flow probe and allowing the blood to pass

through the other side, or if applicable, clamping the SMA distal to the flow probe.

Statistical data analysis was performed and the results were expressed as mean $\pm$ S.E. Statistical analysis between groups was made using paired t-test or, when applicable, Analysis of Variance (ANOVA) for multiple comparisons followed by Tukey's HSD (honestly significant difference). Data compared followed normal distribution. Differences were accepted as statistically significant at $P \leq 0.05$.

3. SHEAR-INDUCED MODULATION BY NITRIC OXIDE OF SYMPATHETIC NERVES IN THE SMA AND HA

3.1. INTRODUCTION

The ability of peripheral vascular beds to regulate blood flow is important for the regulation of arterial blood pressure and cardiac output as well as to subserve local tissue requirements. NO has been implicated in the regulation of blood pressure due to its potent vasodilator properties (Moncada and Higgs, 1993; Rees et al., 1989; Vane et al., 1990). NO release is both blood flow and endothelium-dependent (Kelm et al., 1991; Kuo et al., 1990; Kuo et al., 1991). The production of NO, due to changes in blood flow, is mediated by shear stress which is the mechanism for the flow-induced dilation (Buga et al., 1991; Lamontagne et al., 1992; Reinhart, 1994; Smiesko et al., 1989). Shear stress is caused by the movement of fluid past the endothelial cells. A decrease in diameter at a constant arterial flow augments shear stress because of its direct relation to flow and an inverse relation to the third power of the internal diameter of the blood vessel (Kamika & Togawa, 1980). Recently, NO has been found to modulate vascular responses to nerve stimulation in an endothelium-dependent manner (Cohen & Weisbrod, 1988; Greenberg et al., 1989; Macedo & Lautt, 1994; Najafipour & Ferrel, 1993; Tesfamariam & Cohen, 1988; Thorin & Atkinson, 1994; Yasuhiro et al., 1994). However, a number of observations appear to be incompatible with a neuromodulatory role for NO (Hynes et al., 1988; Thatikunta et al., 1993; Thorin & Atkinson, 1994; Wennmalm & Benthin, 1991). This controversy may be related

to the lack of consideration of the fact that the modulatory effect of NO on sympathetic nerves is promoted by shear stress, so that in its absence no modulation occurs or that variable changes in shear stress will lead to variable modulation.

Thus, it is important to elucidate the regulatory mechanisms of NO on sympathetic stimulation as well as the underlying mechanism that promotes NO production. To achieve this objective, an in situ preparation of blood perfused feline intestine or liver was used as a model with a perfusing circuit to obtain situations of varying shear stress. Our results are consistent with the hypothesis that an increase in shear stress in the resistance vessels promotes NO release from the endothelium and that this NO suppresses sympathetic nerve activity, possibly by a prejunctional effect of NO on nerves in the intestine but a postjunctional effect in the liver.

3.2. METHODS

Superior Mesenteric Arterial Surgical Protocol:

As described in Chapter 2, laparotomy, splenectomy, and ligation of the inferior mesenteric artery were performed. The SMA was isolated and its peri-arterial nerve bundle was separated, ligated, cut, and the peripheral end placed in a circular bipolar stimulating electrode. Both femoral arteries were isolated, cannulated, and connected to the SMA through a pump-controlled long-circuit as shown in Figure 1 and previously described (Lockhart & Lautt, 1990). SMA

EFFECTS OF VASOCONSTRICTION ON SHEAR STRESS

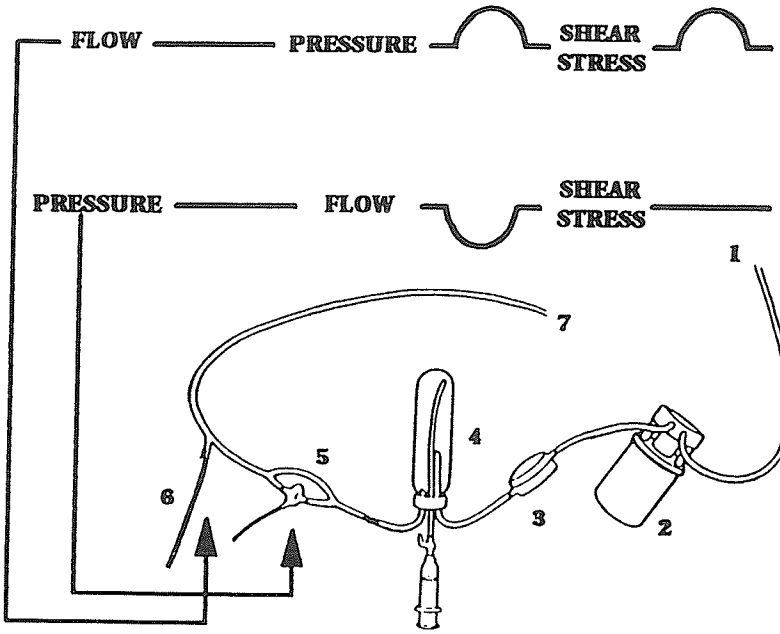

FIGURE 1: Schematic diagram of the arterial long-circuit including explanation of the experimental protocol. Blood from the animal originated from both femoral arteries (1), passed through the arterial long-circuit, and was returned back into the superior mesenteric artery (7). Blood in the circuit passed though a roller pump (2), which allowed the regulation of blood flow, then passed through a filter (3) and a windkessel chamber-bubble trap (4) and a flow probe (5). Circuit pressure is measured via a cannula (6). The illustration above represents two experimental situations: one where shear stress is increased and a second one where it is maintained constant during vasoconstriction. The arrows indicate the variable (flow or pressure) that changes.

pressure was monitored from a catheter incorporated into the circuit.

Liver Surgical Protocol:

As previously described in Chapter 2, after laparotomy, the inferior mesenteric artery and the celiac artery were ligated and the spleen was removed. The periarterial nerve bundle of the SMA was gently separated ligated, cut and an electromagnetic flow probe and a micrometer controlled screw clamp were placed on the artery (Carolina Medical Electronics EP 408). The HA was isolated and the gastroduodenal artery was isolated and ligated. The nerve bundle around the HA was gently separated, ligated and cut and the peripheral end placed in a circular bipolar stimulating electrode. A vascular long circuit identical to that described in the previous protocol was used and made the connection between the femoral arteries and the HA. HA pressure was monitored by a catheter incorporated into the circuit, and drugs were administered through a separate infusion line.

Two series of experiments were performed: studies of shear stress in the SMA and studies in the liver (HA and PV). The circuit used in both protocols allowed control and measurement of blood flow by the use of an electromagnetic flow probe (Carolina Medical Electronics EP408). Shear stress was increased or maintained constant by controlling the circuit blood flow. The increase in shear stress was achieved by maintaining the flow delivered to either the SMA or HA constant when vasoconstriction was induced, either by stimulation of the sympathetic nerves or by infusion of norepinephrine. If the SMA or HA pressure

is maintained constant by decreasing blood flow delivered to the artery during vasoconstriction, shear stress remains constant (see Discussion for explanation).

Shear stress studies on the PV were possible by the manipulation of the screw clamp placed on the SMA for the liver series, since SMA blood flow corresponds to PV blood flow with this surgical preparation (see Chapter 2 for explanation). Increase in shear stress was achieved by maintaining the flow delivered to the PV constant when vasoconstriction was induced by stimulation of the sympathetic nerves or by infusion of norepinephrine. If PV pressure is maintained constant by decreasing blood flow delivered to the vein during vasoconstriction, shear stress remains constant.

Both SMA and HA pressures were calibrated in situ at the end of each experiment to account for resistance to flow in the circuit. All reported pressures were corrected for circuit resistance.

Protocol 1: Sympathetic nerve stimulation (2 Hz and 10 Hz) for SMA and (2-6 Hz) for HA and PV (15 V square pulse, 1 ms duration) was performed under conditions of constant flow or constant pressure achieved by adjusting the perfusing circuit. Responses to nerve stimulation were determined in a control condition, following intravenous injection of L-NAME (2.5 $mg \cdot kg^{-1}$), and after an intravenous injection of L-arginine (75 $mg \cdot kg^{-1}$) to reverse the action of L-NAME. Preliminary studies showed that this dose of L-NAME produced significant vascular effects. The L-arginine dosage was based on in vivo reversal of L-NAME-induced hypertension (Rees et al., 1990). In the SMA studies we attempted to evaluate NO

synthase blockade by the degree of suppression of acetylcholine-induced vasodilation but we were unable to demonstrate any significant or consistent suppression of acetylcholine-induced vasodilation by L-NAME at any tested dose.

Protocol 2: In separate sets of experiments, a similar approach to protocol 1 was used but sympathetic nerve stimulation was replaced by norepinephrine infusion (0.50 μg/kg/min) into the SMA or into the PV for the liver studies.

3.3. RESULTS

3.3.1. Superior mesenteric arterial studies

Sympathetic Nerve Stimulation: Mean ($\pm$ SE) control carotid arterial blood pressure was 102.4 $\pm$ 12.1 mmHg and 100.5 $\pm$ 9.8 mmHg respectively at the beginning of the 2 Hz and the 10 Hz nerve stimulation experiments. SMA pressure measured from the pump-controlled long-circuit was 82.4 $\pm$ 12.4 and 91.4 $\pm$ 9.5 mmHg; PV pressure, 7.0 $\pm$ 0.8 and 6.5 $\pm$ 1.1 mmHg; and SMA flows were 18.5 $\pm$ 5.8 and 16.4 $\pm$ 5.0 ($ml \cdot kg^{-1} \cdot min^{-1}$) (measured in 6 cats for the 2 Hz experiments and 4 cats for the 10 Hz experiments). A maximum effect of L-NAME on basal systemic arterial pressure and SMA pressure appeared around 10 to 20 min after the injection of the drug with an increase of 14.6 $\pm$ 3.3 and 30.5 $\pm$ 6.4 mmHg, respectively. After this peak, the pressures decreased approaching control values by 60 min. L-arginine caused a decrease in perfusion pressure of 40.8 $\pm$ 8.9 mmHg. Nerve stimulation and norepinephrine infusion were delivered in random order after basal pressure had stabilized following L-NAME and L-arginine.

Two frequencies of nerve stimulation were selected, one to produce a mild but significant constriction (2 Hz) and a second to produce a near maximal or maximal degree of constriction (10 Hz). It was previously shown that maximal constriction occurs at 10 Hz and half maximal constriction occurs at 2 Hz (Lockhart et al., 1988). A typical trace of control responses to nerve stimulation (2 Hz) in the control state and in the presence of L-NAME under constant flow (increased shear stress) as well as constant pressure (maintained shear stress) are shown in Figure 2. Under the constant flow conditions, the change in perfusion pressure was used as the index of vasoconstriction whereas under constant pressure conditions, constriction was assessed from the decrease in flow. For this one experiment, 2 Hz nerve stimulation under conditions of constant flow caused perfusion pressure to rise by 41.4 mmHg in the control state and by 136.1 mmHg in the presence of L-NAME. Under conditions of constant pressure, vasoconstriction resulted in reductions of flow of 3.7 and 3.7 ($ml \cdot kg^{-1} \cdot min^{-1}$) for the control response and after L-NAME, respectively.

Figure 3 represents the mean response of 6 cats to the 2 Hz stimulation under the state of constant flow (increased shear stress). Nerve stimulation in the control state caused the perfusion pressure to rise by 24.8 $\pm$ 4.7 mmHg. In the presence of L-NAME, the pressure rose by 74.0 $\pm$ 21.6 mmHg which was greater than the control response ($p < 0.006$). The observed potentiation was able to be reversed by L-arginine as the increase in pressure in response to nerve stimulation was only 9.2 $\pm$ 2.7 mmHg ($p < 0.006$). Figure 4 represents the same experiment

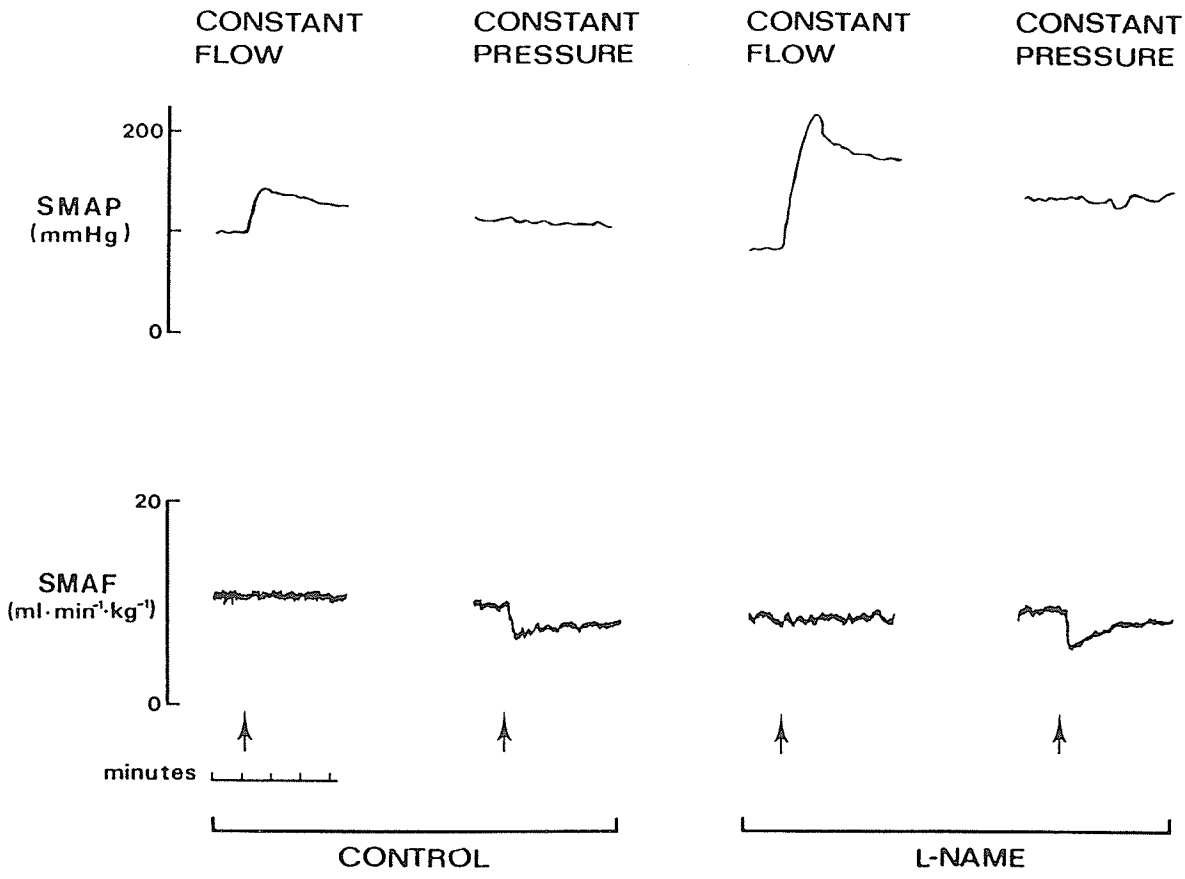

FIGURE 2: Representative figure of responses to sympathetic nerve stimulation (arrows indicate the point at which nerve stimulation 2 Hz started) in the superior mesenteric artery where both pressure and blood flow were measured. Under conditions of constant flow there was a potentiation of peak constriction in the presence of L-NAME compared to control state. Potentiation was not observed if pressure was held constant.

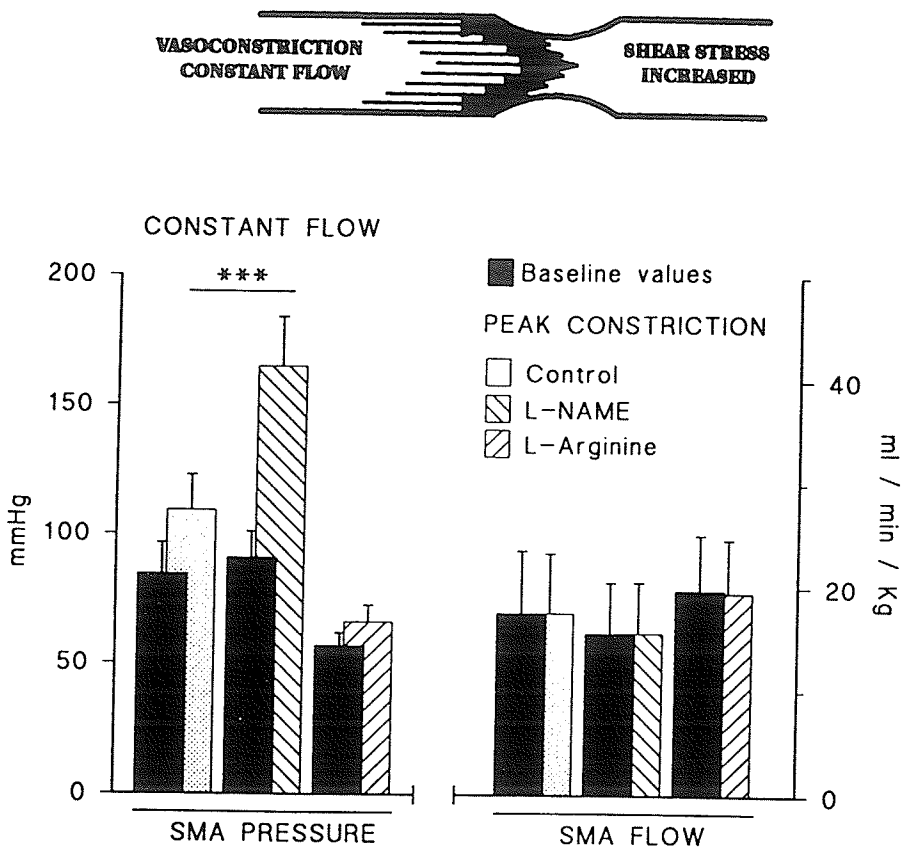

FIGURE 3: Superior mesenteric arterial (SMA) response to sympathetic nerve stimulation (2 Hz) under constant flow such that shear stress was allowed to increase. The response is the difference between baseline and peak constriction assessed from the rise in pressure during control and after L-NAME and L-arginine. Values are mean $\pm$ SE of $n=6$ and *** $p<0.001$ by Analysis of Variance (ANOVA) for multiple comparisons followed by Tukey's HSD (honestly significant difference).

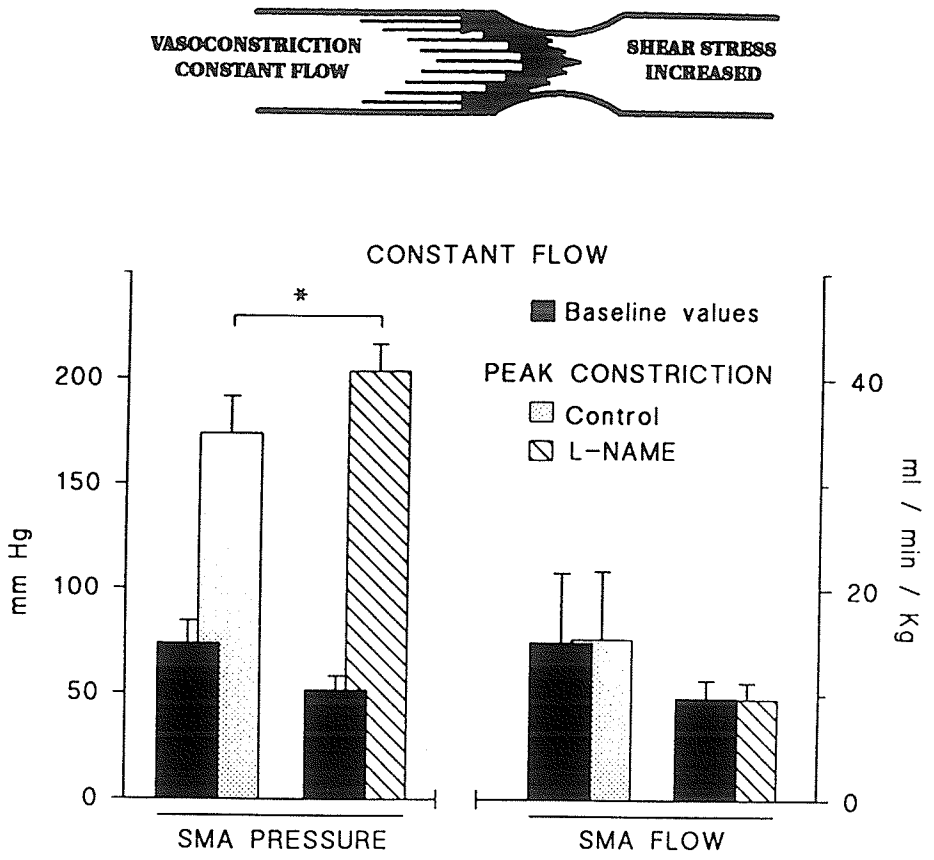

FIGURE 4: Superior mesenteric arterial (SMA) response to sympathetic nerve stimulation (10 Hz) under constant flow such that shear stress was allowed to be increased. The response is the difference between baseline and peak constriction assessed from the rise in pressure during control and after L-NAME. Values are mean $\pm$ SE of $n=4$ and $*p<0.05$ by paired t-test.

repeated with 10 Hz nerve stimulation (n=4) instead of 2 Hz. The pressure increment in response to control nerve stimulation at constant flow was 100.0 $\pm$ 15.4 mmHg and 151.9 $\pm$ 12.2 mmHg in the presence of L-NAME ($p<0.03$).

To determine if the peak constriction was increased in a situation where the shear stress was maintained constant, the sympathetic nerves were stimulated under constant pressure responses so that constriction resulted in reduced blood flow. The calculated mean values for the decrease in flow during 2 Hz nerve stimulation in control state (5.5 $\pm$ 2.1 ml$\cdot$ kg$^{-1}\cdot$ min^{-1}), after L-NAME (5.2 $\pm$ 1.7 ml$\cdot$ kg$^{-1}\cdot$ min^{-1}), and after L-arginine (3.2 $\pm$ 1.6 ml$\cdot$ kg$^{-1}\cdot$ min^{-1}) were similar (Fig. 5). The 10 Hz stimuli caused a similar degree of constriction in the control state (13.1 $\pm$ 3.5 ml$\cdot$ kg$^{-1}\cdot$ min^{-1}) and after L-NAME (12.2 $\pm$ 4.4 ml$\cdot$ kg$^{-1}\cdot$ min^{-1}) as measured by a similar decrease in blood flow (Fig. 6).

Norepinephrine Infusion Experiments: Control carotid arterial blood pressure was 118.9 $\pm$ 15.9 mmHg; SMA pressure measured downstream of the flow probe in the circuit was 90.3 $\pm$ 8.9 mmHg; PV pressure was 7.6 $\pm$ 0.5; and SMA flow was 9.5 $\pm$ 0.8 ml$\cdot$ kg$^{-1}\cdot$ min^{-1} (n=5). Responses measured before and after L-NAME at a constant flow (increased shear stress) are shown in Figure 7. Flow did not change significantly from the baseline to the peak constriction for control (0.1 $\pm$ 0.1 ml$\cdot$ kg$^{-1}\cdot$ min^{-1}) or after L-NAME (0.0 $\pm$ 0.1 ml$\cdot$ kg$^{-1}\cdot$ min^{-1}) ($p<0.42$). The index of vasoconstriction, represented by the increase in perfusion circuit pressure, due to norepinephrine infusion was 59.1 $\pm$ 5.6 mmHg for the control and 59.6 $\pm$ 8.1 mmHg for L-NAME ($p>0.90$).

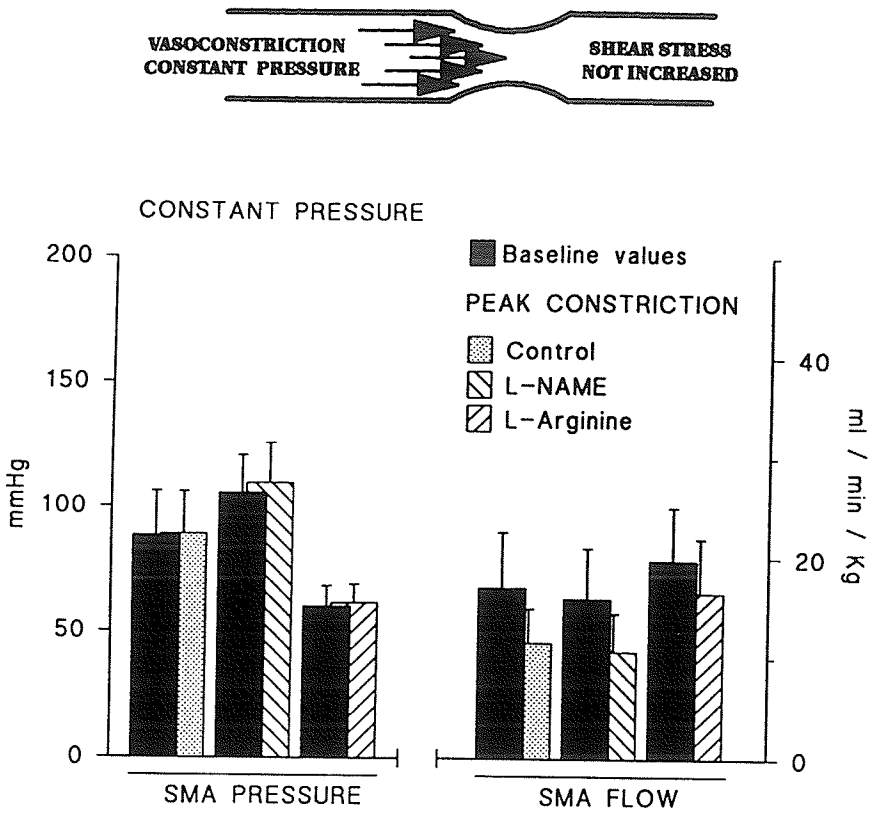

FIGURE 5: Superior mesenteric arterial (SMA) response to sympathetic nerve stimulation (2 Hz) under constant pressure such that shear stress was maintained constant. The response is the difference between baseline and peak constriction assessed from the decrease in flow during control and after L-NAME and L-arginine. Values are mean $\pm$ SE of n=6. No significant difference exists between responses by Analysis of Variance (ANOVA) for multiple comparisons followed by Tukey's HSD (honestly significant difference).

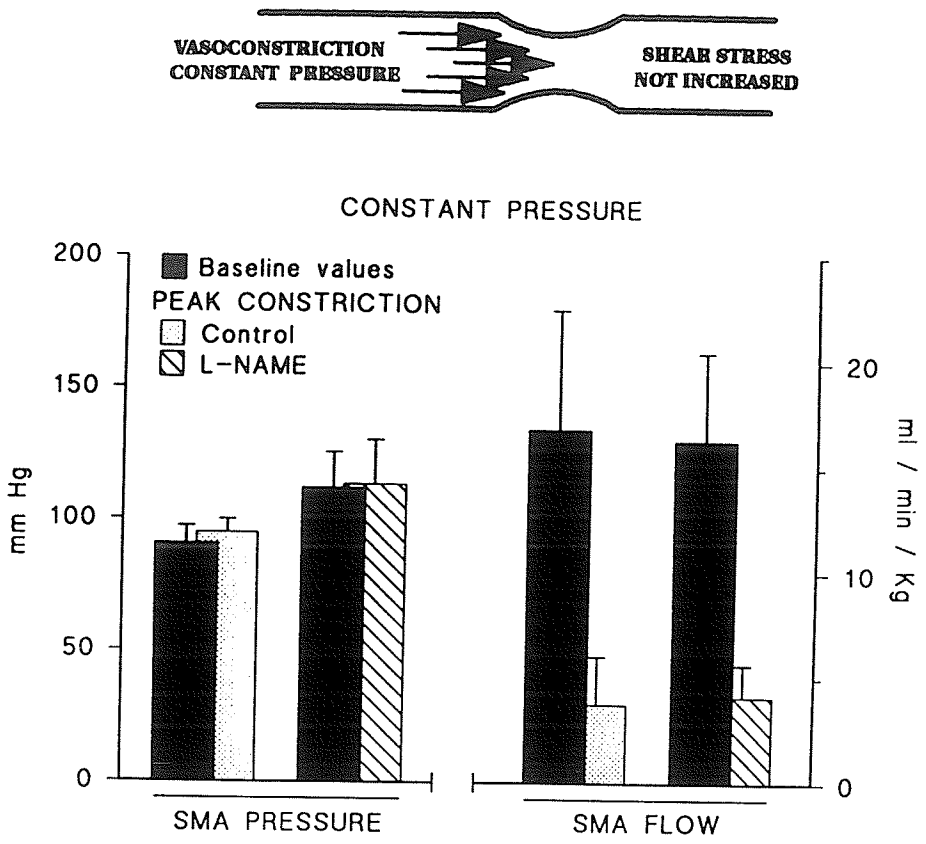

FIGURE 6: Superior mesenteric arterial (SMA) response to sympathetic nerve stimulation (10 Hz) under constant pressure such that shear stress was maintained constant. The response is the difference between baseline and peak constriction assessed from the decrease in flow during control and after L-NAME. Values are mean $\pm$ SE of n=4. No significant difference exists between responses by paired t-test.

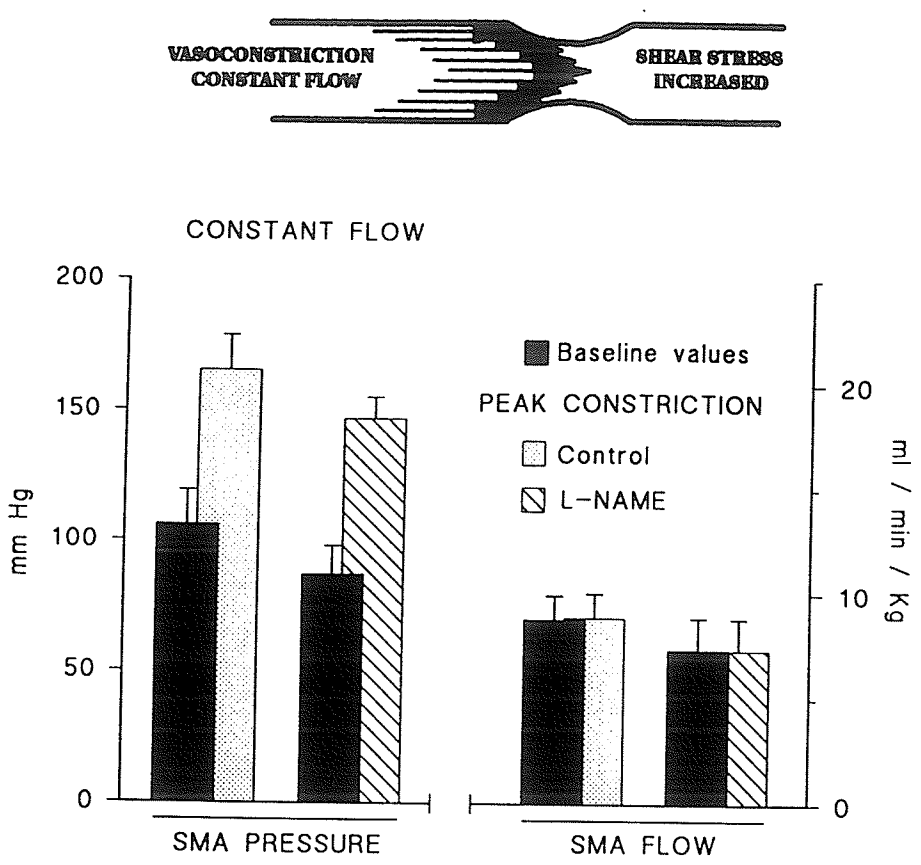

FIGURE 7: Superior mesenteric arterial (SMA) response to norepinephrine infusion (0.50 μg/kg/min) under constant flow such that shear stress was allowed to increase. The response is the difference between baseline and peak constriction assessed from the increase in pressure during control and after L-NAME. Values are mean $\pm$ SE of n=5. No significant difference exists between responses by paired t-test.

The reduction in flow (Fig. 8), as the index of vasoconstriction, observed during the effect of norepinephrine infusion at a constant pressure (shear stress not increased) was 4.9 $\pm$ 1.2 ml· kg^{-1}· min^{-1} and 4.4 $\pm$ 1.5 ml· kg^{-1}· min^{-1} for control and after L-NAME, respectively ($p > 0.42$). As an index of the effectiveness of manual adjustment of the perfusion circuit to maintain pressure constant during vasoconstriction, pressures at the end of peak constriction did not change significantly from baseline values with the mean change being 2.0 $\pm$ 1.4 mmHg for control and 3.5 $\pm$ 1.2 mmHg after L-NAME.

3.3.2. Liver studies

Mean arterial pressure, as measured from the carotid artery, was 103.8 $\pm$ 7.6 mm Hg before and 144.3 $\pm$ 9.9 mm Hg after the influence of L-NAME ($p < 0.001$, $n = 12$). HA pressure was not altered by L-NAME (108.5 $\pm$ 6.6 and 112.7 $\pm$ 6.8 mm Hg during the control state and L-NAME respectively). Basal HA flow was maintained constant in all the experiments (9.4 $\pm$ 0.9 and 9.8 $\pm$ 1.2 ml. kg^{-1}. min^{-1} before and after L-NAME). The drug had no significant effect on central venous pressure or PV pressure.

Sympathetic Nerve Stimulation:

HA responses: Figure 9 represents the mean response of 6 cats to the nerve stimulation under the state of constant flow. Nerve stimulation in the control state caused the perfusion pressure to rise by 28.8 $\pm$ 6.5 mmHg. In the presence of L-

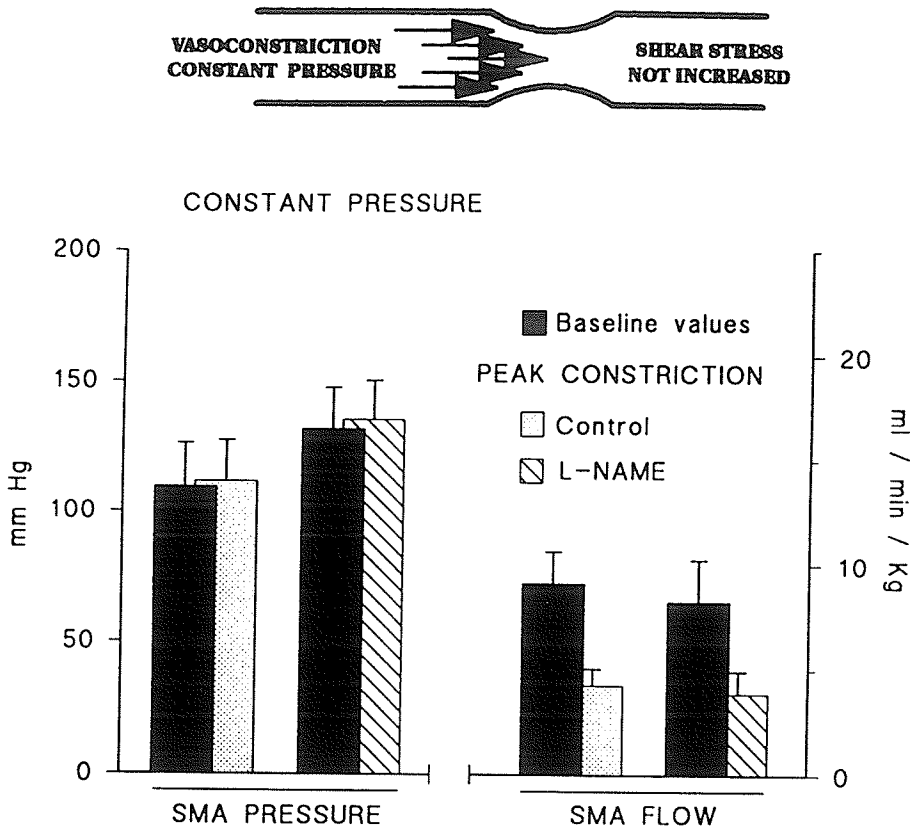

FIGURE 8: Superior mesenteric arterial (SMA) response to norepinephrine infusion (0.50 μg/kg/min) under constant pressure such that shear stress was maintained constant. The responses were quantified by the difference between baseline and peak constriction assessed from the decrease in flow during control and after L-NAME. Values are mean $\pm$ SE of n=5. No significant difference exists between responses by paired t-test.

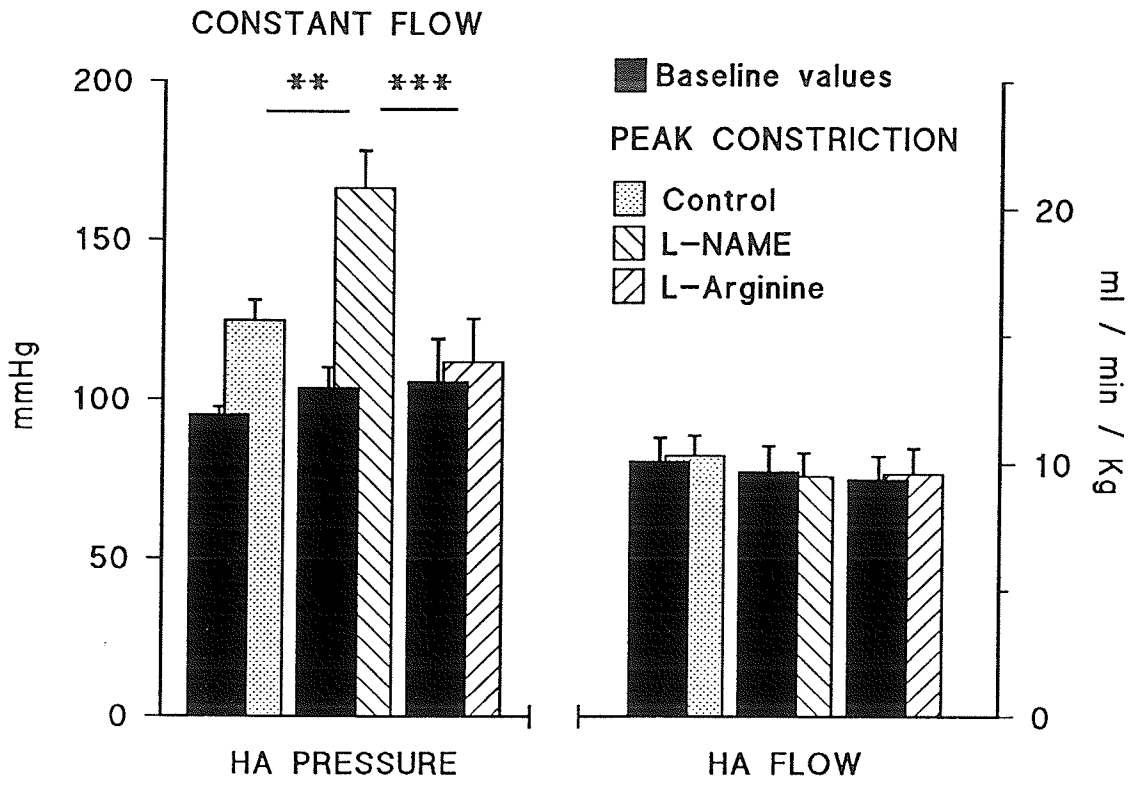

FIGURE 9: Hepatic arterial (HA) response to sympathetic nerve stimulation (2-6 Hz) under constant flow such that shear stress was allowed to increase. The response is the difference between baseline and peak constriction assessed from the rise in pressure during control and after L-NAME and L-arginine. Values are mean $\pm$ SE of $n=6$ and $^{**}p<0.01$, $^{***}p<0.001$ by Analysis of Variance (ANOVA) for multiple comparisons followed by Tukey's HSD (honestly significant difference).

NAME, the pressure rose by 62.7 $\pm$ 14.6 mmHg which was greater than the control response ($p < 0.01$). The observed potentiation was reversed by L-arginine as the increase in pressure in response to nerve stimulation was only 6.3 $\pm$ 3.0 mmHg ($p < 0.001$).

To determine if the constriction was increased in a situation where the shear stress was maintained constant, the sympathetic nerves were stimulated under constant pressure responses so that constriction resulted in reduced blood flow. The calculated mean values for the decrease in flow during nerve stimulation in control state (2.5 $\pm$ 0.8 ml$\cdot$ kg$^{-1}\cdot$ min^{-1}), after L-NAME (3.8 $\pm$ 0.9 ml$\cdot$ kg$^{-1}\cdot$ min^{-1}), and after L-arginine (1.2 $\pm$ 1.1 ml$\cdot$ kg$^{-1}\cdot$ min^{-1}) were similar (Fig.10).

PV responses: Figure 11 represents the mean responses to nerve stimulation under constant flow ($n = 6$). The pressure increment in response to control nerve stimulation at constant flow was 1.5 $\pm$ 0.5 mmHg and 3.3 $\pm$ 0.5 mmHg in the presence of L-NAME ($p < 0.01$). The increase due to the effect of L-NAME was reversed by L-arginine 1.2 $\pm$ 0.5 mm Hg ($p < 0.01$).

The responses to nerve stimuli under constant pressure caused a similar degree of PV constriction in the control state (15.7 $\pm$ 6.1 ml$\cdot$ kg$^{-1}\cdot$ min^{-1}), after L-NAME (12.3 $\pm$ 3.5 ml$\cdot$ kg$^{-1}\cdot$ min^{-1}) and after L-arginine (7.7 $\pm$ 1.8 ml$\cdot$ kg$^{-1}\cdot$ min^{-1}) as measured by a similar decrease in blood flow (Fig. 12).

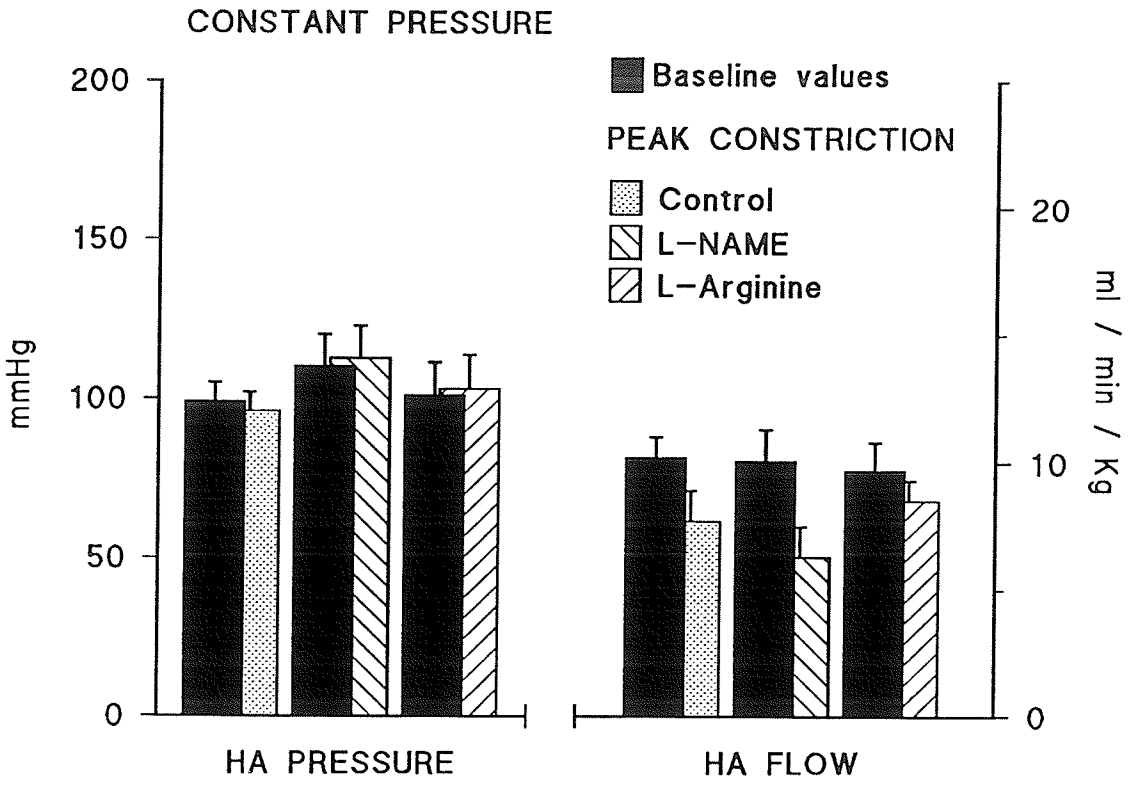

FIGURE 10: Hepatic arterial (HA) response to sympathetic nerve stimulation (2-6 Hz) under constant pressure such that shear stress was maintained constant. The response is the difference between baseline and peak constriction assessed from the decrease in flow during control and after L-NAME and L-arginine. Values are mean $\pm$ SE of n=6. No significant difference exists between responses by Analysis of Variance (ANOVA) for multiple comparisons followed by Tukey's HSD (honestly significant difference).

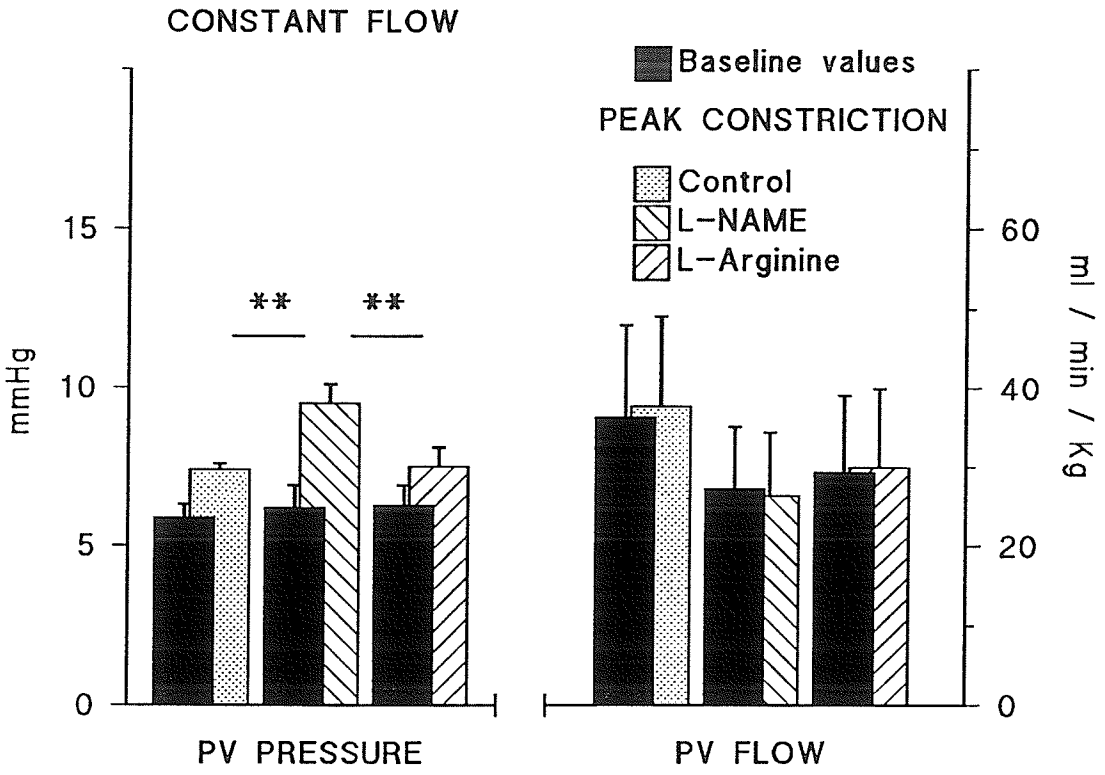

FIGURE 11: Portal vein (PV) response to sympathetic nerve stimulation (2-6 Hz) under constant flow such that shear stress was allowed to be increased. The response is the difference between baseline and peak constriction assessed from the rise in pressure during control and after L-NAME and L-arginine. Values are mean $\pm$ SE of $n=6$ and $**p<0.01$ by Analysis of Variance (ANOVA) for multiple comparisons followed by Tukey's HSD (honestly significant difference).

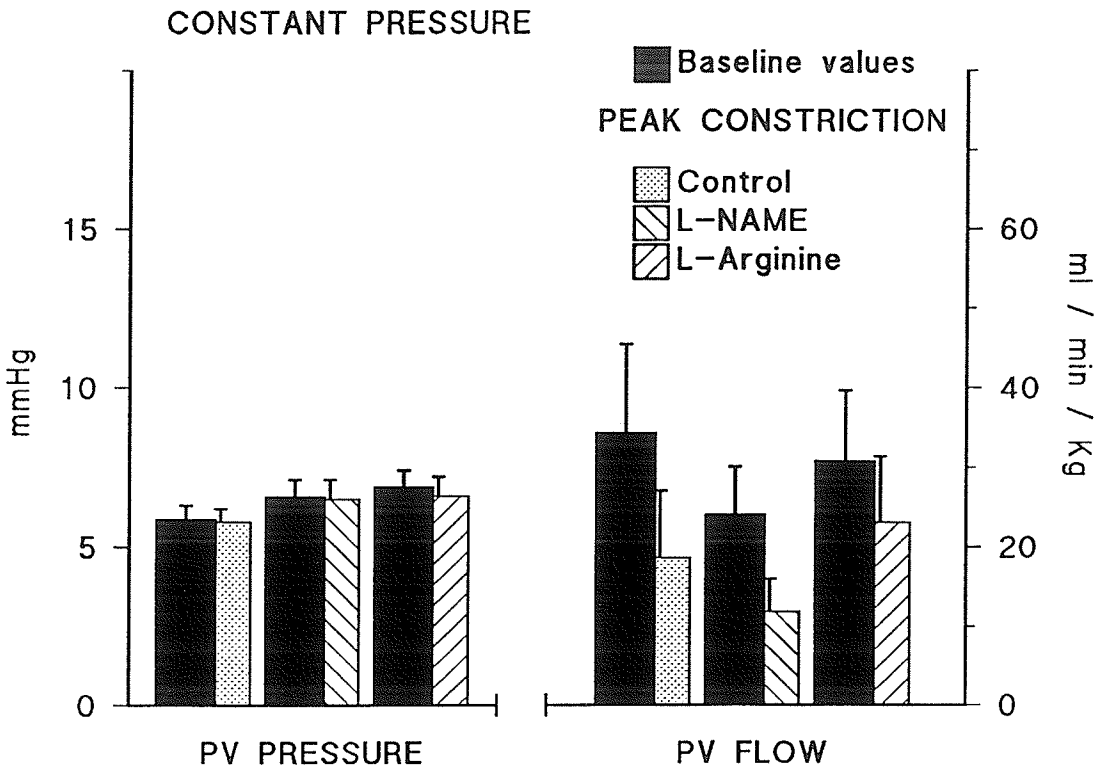

FIGURE 12: Portal vein (PV) response to sympathetic nerve stimulation (2-6 Hz) under constant pressure such that shear stress was maintained constant. The response is the difference between baseline and peak constriction assessed from the decrease in flow during control and after L-NAME and L-arginine. Values are mean $\pm$ SE of n=6. No significant difference exists between responses by Analysis of Variance (ANOVA) for multiple comparisons followed by Tukey's HSD (honestly significant difference).

Norepinephrine Infusion Experiments

HA responses: Responses to norepinephrine, expressed as increased pressure, measured before (32.4 $\pm$ 9.0 mm Hg), after L-NAME (60.3 $\pm$ 8.0 mm Hg) and after L-arginine (23.8 $\pm$ 9.1 mmHg) at a constant flow (increased shear stress) are shown in Figure 13 ($p < 0.01$).

The reduction in flow, as the index of vasoconstriction, observed during the effect of norepinephrine infusion at a constant pressure (shear stress not increased) was 3.2 $\pm$ 2.6 $ml \cdot kg^{-1} \cdot min^{-1}$ and 4.1 $\pm$ 0.6 $ml \cdot kg^{-1} \cdot min^{-1}$ for control and after L-NAME, and 2.17 $\pm$ 0.3 after L-arginine as represented in Figure 14 ($p < 0.01$).

PV responses: The index of vasoconstriction, represented by the increase in perfusion circuit pressure (shear stress increased), due to norepinephrine infusion was 1.25 $\pm$ 0.3 mmHg for the control, 3.4 $\pm$ 0.7 mmHg for L-NAME and 1.4 $\pm$ 0.2 for L-arginine ($p > 0.05$) (Fig.15).

The calculated mean values for the decrease in PV flow during norepinephrine infusion under constant pressure were of 32.9 $\pm$ 9.4 $ml \cdot kg^{-1} \cdot min^{-1}$ in control state, 28.5 $\pm$ 6.3 $ml \cdot kg^{-1} \cdot min^{-1}$ after L-NAME, and 21.6 $\pm$ 10.4 $ml \cdot kg^{-1} \cdot min^{-1}$ after L-arginine and there was no statistical difference between the three responses (Fig.16).

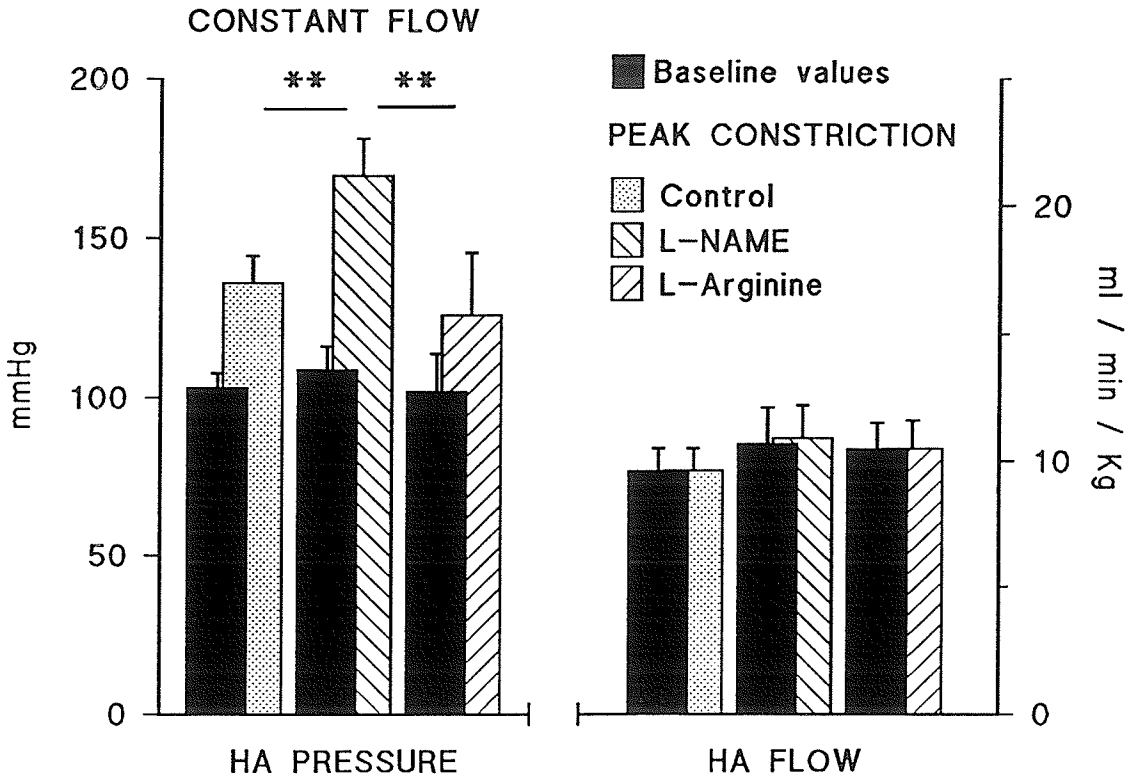

FIGURE 13: Hepatic arterial (HA) response to norepinephrine infusion (0.50 μg/kg/min) under constant flow such that shear stress was allowed to increase. The response is the difference between baseline and peak constriction assessed from the increase in pressure during control and after L-NAME and L-arginine. Values are mean $\pm$ SE of $n=6$ and $^{**}p<0.01$ by Analysis of Variance (ANOVA) for multiple comparisons followed by Tukey's HSD (honestly significant difference).

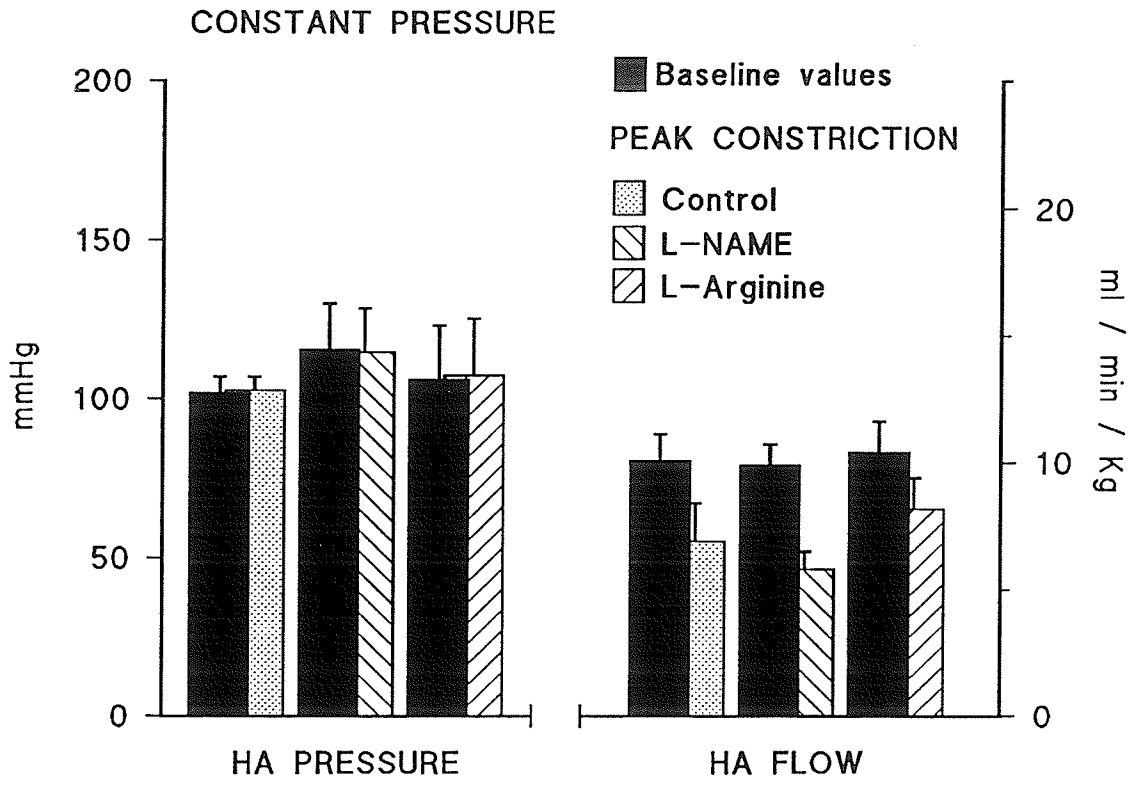

FIGURE 14: Hepatic arterial (HA) response to norepinephrine infusion (0.50 μg/kg/min) under constant pressure such that shear stress was maintained constant. The responses were quantified by the difference between baseline and peak constriction assessed from the decrease in flow during control and after L-NAME and L-arginine. Values are mean $\pm$ SE of n=6. No significant difference exists between responses by Analysis of Variance (ANOVA) for multiple comparisons followed by Tukey's HSD (honestly significant difference).

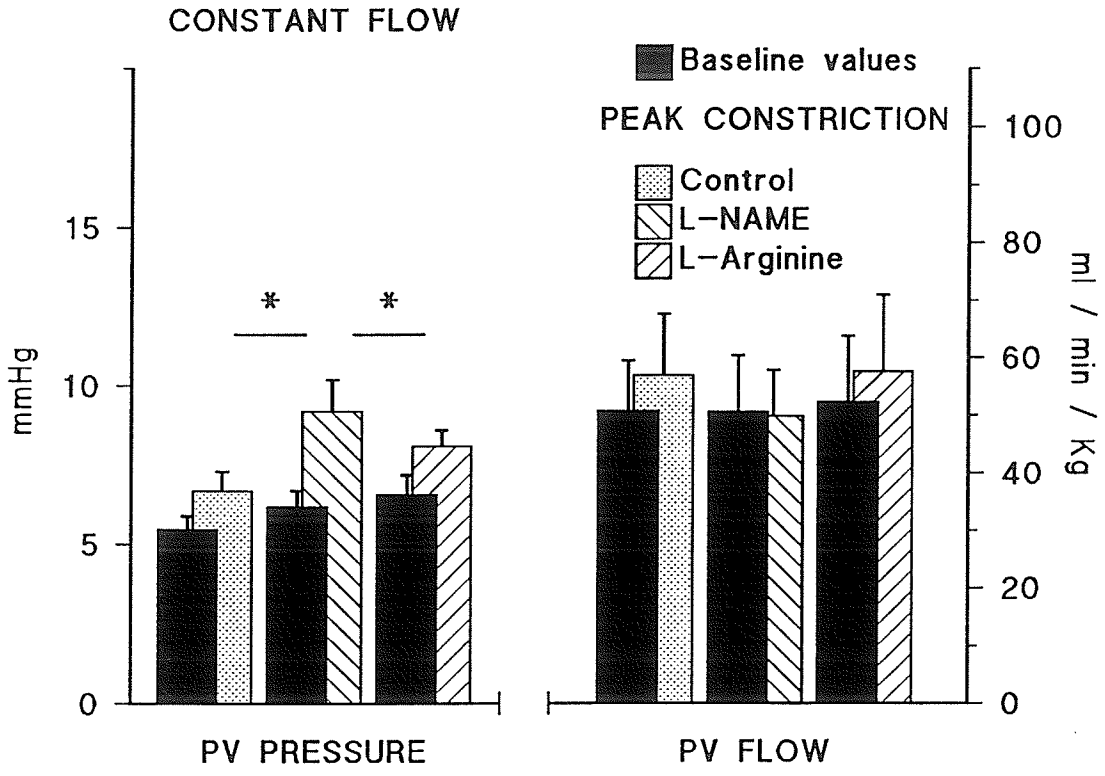

FIGURE 15: Portal vein (PV) response to norepinephrine infusion (0.50 μg/kg/min) under constant flow such that shear stress was allowed to increase. The response is the difference between baseline and peak constriction assessed from the increase in pressure during control and after L-NAME and L-arginine. Values are mean $\pm$ SE of $n=6$ and $*p<0.05$ by Analysis of Variance (ANOVA) for multiple comparisons followed by Tukey's HSD (honestly significant difference).

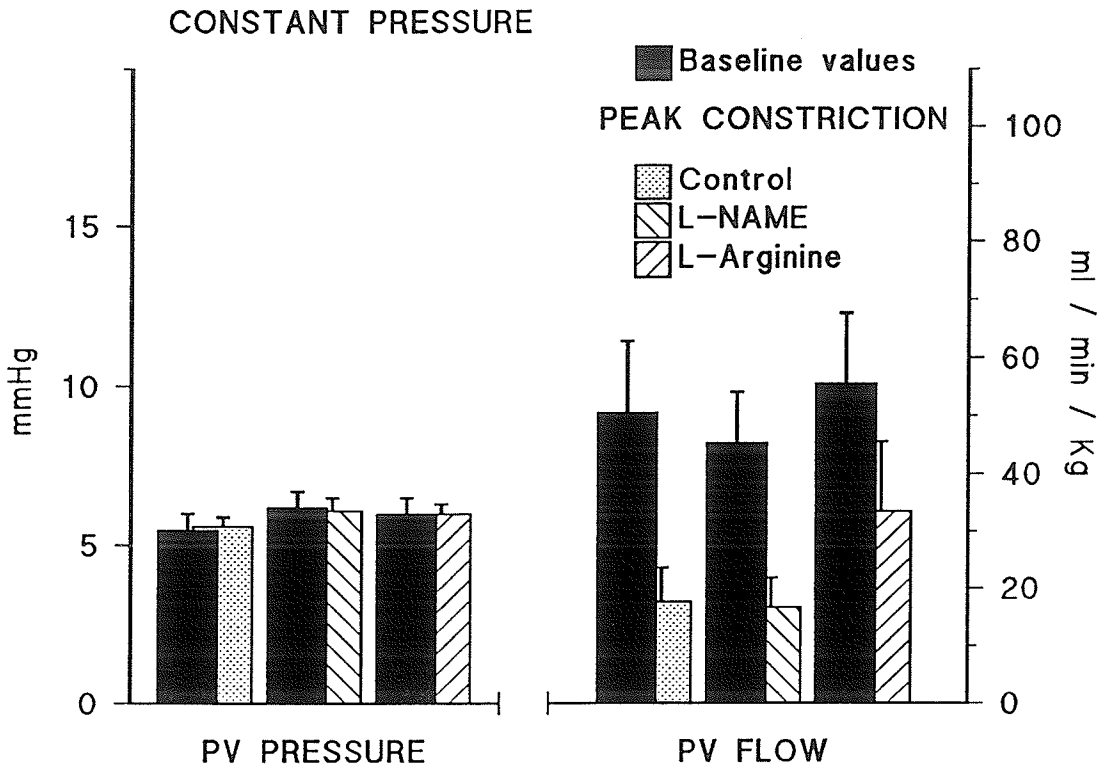

FIGURE 16: Portal vein (PV) response to norepinephrine infusion (0.50 μg/kg/min) under constant pressure such that shear stress was maintained constant. The responses were quantified by the difference between baseline and peak constriction assessed from the decrease in flow during control and after L-NAME and L-arginine. Values are mean $\pm$ SE of n=6. No significant difference exists between responses by Analysis of Variance (ANOVA) for multiple comparisons followed by Tukey's HSD (honestly significant difference).

3.4 DISCUSSION

L-NAME inhibits NO synthase and effects reported are based on the assumption that NO release due to shear stress is suppressed. This interpretation is supported by the reversal of L-NAME-induced responses by provision of the NO synthase substrate L-arginine. L-NAME affected arterial basal tone in the intestine but not in the liver. These studies provide evidence that NO plays an important role in modulating the sympathetic nervous system when sympathetic nerve activation results in increased vascular shear stress in an organ specific manner. Vasoconstriction due to exogenous norepinephrine was not modulated by NO, suggesting a pre-junctional action of NO in the intestine. The increased NO production occurred only under conditions where shear stress was increased. The data suggest that when nerve-induced vasoconstriction causes elevated shear stress at the site of the constriction, NO is released which then leads to suppression of norepinephrine release from the nerves. The action of NO does not seem to be directly on the smooth muscle at the constricted site in the intestine based on the observation that the norepinephrine-induced constriction was not modulated by shear stress. In contrast, in the liver, NO released due to shear stress modulates vasoconstriction promoted by both the sympathetic nervous system and exogenous norepinephrine. Because the amplitude of the potentiation is of the same extent for sympathetic nerves and norepinephrine it is suggested that in the liver NO acts post-junctionally.

An aspect to consider related to the intestine study is that the baseline SMA pressure after L-NAME in the 10 Hz and norepinephrine experiments had to be lowered by regulating the perfusion pump to decrease the blood flow. This procedure was necessary to prevent the pressure inside the circuit during the potentiated constriction from rising to levels that would compromise the integrity of the circuit. This precaution was not necessary for the 2 Hz nerve stimulation. The results from the 2 and 10 Hz studies are compatible.

Another methodological aspect to consider deals with the inability of NOS blockade to suppress acetylcholine-induced vasodilation in the SMA. The use of acetylcholine-induced vasodilation as an index of NOS blockade shows inconsistent effects depending upon whether the tests were done in vivo or in vitro and depending upon the tissue tested (Aisaka et al., 1989; Gardiner et al., 1990; Gardiner et al., 1991). We confirmed the observation by Gardiner et al., (1991) that acetylcholine-induced dilation of the SMA is not altered by NOS blockade.

Related Studies: With perfused pulmonary vessels, isolated segments of both rabbit carotid artery and rat tail artery, the responses to nerve stimulation depend on the presence or absence of the endothelial cells, with vasoconstriction being enhanced when the endothelium was removed (Cohen and Weisbrod, 1988; Greenberg et al., 1989; Thorin and Atkinson, 1994). These groups also showed that there was an increased release of norepinephrine in the vessels when the endothelium was removed compared to the intact vessels. In the pulmonary vessels this effect could be attenuated if the vessels without endothelium were perfused

with effluent from the intact vessels (Greenberg et al., 1989). Contrary observations to the previous studies were reported by Wennmalm et al., (1991) and Thatikunta et al., (1993) in the coronary artery and external anal sphincter respectively. However, direct comparison is difficult because of lack of control of shear stress and the possibility of organ-specificity of this mechanism. Hynes et al., (1988) found an enhanced contractile response to adrenergic nerve stimulation in the absence of endothelium in the rat caudal artery although they concluded that the mechanism was dependent on a desensitization of the smooth muscle due to the release of EDRF from the endothelial cells. Shear-dependent inhibition of nerve-induced constriction was shown in isolated carotid arteries but it was not confirmed that the effect was selective for nerve-induced responses (Tesfamariam and Cohen, 1988).

Other studies have suggested as well that NOS inhibitors increase sympathetic neural activity in the kidney (Sakuma et al., 1992) and that their hypertensive effect is due to an increase in sympathetic neurogenic drive rather than simply to a blockade of the direct NO-induced relaxation of the vascular smooth muscle (Lacolley et al., 1991). Yasuhiro et al., (1994) have also shown that increments in renal sympathetic nerve activity as well as norepinephrine release occurs in the presence of L-NAME. It is possible that elevations of intraneuronal cGMP contribute to the NO-induced suppression of the sympathetic stimuli. This effect might be due to the inhibitory modulation of calcium and depolarization-dependent norepinephrine release from sympathetic nerves by cGMP (Greenberg

et al., 1990).

Thus many, but not all, of the studies with isolated vessels suggest a role for NO suppression of norepinephrine release that is uncovered by blockade of NOS. This demonstration, combined with the in vitro (Buga et al., 1991; Kelm et al., 1991; Kuo et al., 1990; Kuo et al., 1991) and in vivo (Bigoud and Vatner, 1991; Miller and Burnett, 1992; Yasuhiro et al., 1994) demonstration of shear stress-dependent release of NO, makes the interpretation of contradictory results difficult. Although tissue selectively clearly exists, based on the different results in the HA and SMA, our present study may explain some of the discrepancy in the literature in that we show that NO-dependent inhibition of nerve-induced vasoconstriction occurs only in conditions where shear stress is increased and in many previous studies shear stress cannot be evaluated.

Influence of L-NAME and L-arginine on the SMA and HA:

L-NAME was chosen as being one of the more potent stereospecific inhibitors of NO biosynthesis (Rees et al., 1990). Although the effect of the same dose of L-NAME on the two vascular beds was dramatically different, adequacy of the dose was supported by the effect on systemic arterial pressure. The effect of L-NAME to increase the basal tone in the SMA and its reversal by L-arginine was confirmed. The effect of L-NAME on the HA basal tone was insignificant and confirms results obtained by others (Mathie et al., 1991a; Browser et al., 1994; Greenblatt et al., 1993). Greenblatt et al. (1993) observed a decrease in HA

resistance and an increase in HA blood flow as PV blood flow decreased in conscious rats. The response of the HA was likely a consequence of the HA buffer response (HABR), whereby a decrease in PV flow produces an increase in HA blood flow via an ADO-mediated mechanism previously described (Lautt et al., 1985). HA vascular tone did not change in response to L-NAME. This lack of effect could be because: either the HA exhibits less synthesis or basal release of NO, or there is a lack of the specific NOS enzyme responsible for the basel tone, or the HA has less smooth muscle guanylate cyclase (Ignarro, 1989).

3.4.1. Superior Mesenteric Arterial Studies

Effects of L-NAME and L-Arginine on Sympathetic Nerve-Induced Constriction: In the situation where vasoconstriction was induced by either 2 Hz or 10 Hz stimulation, the response in the control state was substantially less than after NO synthase had been blocked by L-NAME and this effect was reversed by administration of L-arginine at constant flow (shear stress increased). Such effects were not seen when shear stress was prevented from increasing during the vasoconstriction. This observation could be explained either by a shear-dependent NO release acting to decrease norepinephrine release from the nerves or by a direct action of NO on the constricted vascular smooth muscle. The effects seen with direct infusion of norepinephrine clarified these alternatives.

Effects of L-NAME on Norepinephrine-Induced Constriction: The vasoconstriction induced by intra-arterial infusion of norepinephrine was not

altered by L-NAME administration regardless of whether shear stress was allowed to rise (constant flow) or held steady (constant pressure) as shown in Figures 7 and 8. We conclude that the NO effect was not directly on the contracted smooth muscle because of the lack of modulation of the constriction produced by direct norepinephrine administration. These data are consistent with the hypothesis that L-NAME caused a potentiated constriction induced by sympathetic nerve stimulation as a result of inhibition of the release of NO. Thus, the implication is that NO released by the increased shear stress occurs in a region where NO exerts no action on the smooth muscle. The site of nerve-induced constriction and NO release is unknown, but reports in the literature suggest that the smooth muscle of the smallest resistance vessels are the least sensitive to NO (Selke et al., 1990). The nerve-induced constriction may be occurring in these NO-resistant vessel segments so that an increased shear stress results in NO release acting indirectly secondary to inhibition of neural activity.

These observations are compatible with the following proposed mechanism (Fig. 17). NO could serve a protective role to prevent shear stress-induced damage to the small resistance vessels that are constricted by sympathetic nerves. If the nerve-induced constriction occurs locally, the net effect on systemic blood pressure will be minimal and the constriction will result in decreased flow with no rise in shear stress. In contrast, if a generalized sympathetic nerve-induced constriction occurs at multiple organs, the increase in total peripheral resistance will cause pressure to rise and flow reduction will be minimized, thus increasing shear stress.

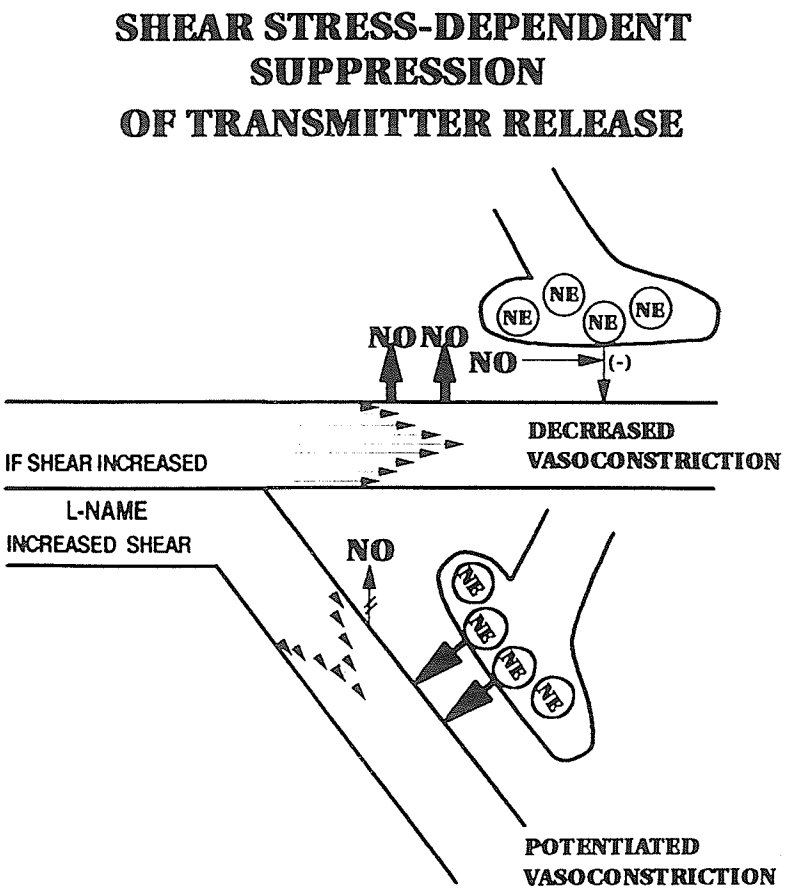

FIGURE 17: Illustration of the hypothesis to explain nitric oxide (NO) suppression of sympathetic nerve activity. The upper part of the diagram represents a situation during which adrenergic nerves are activated resulting in an increase in shear stress triggering the release of NO which inhibits norepinephrine (NE) release. The end result is a decreased vasoconstriction. The lower part of the diagram is representative of the situation in which adrenergic nerves are activated under constant flow where shear stress increases but, due to the presence of L-NAME, NO is not released. Therefore, norepinephrine release is not suppressed, resulting in potentiated vasoconstriction.

If shear stress is increased, the resultant release of NO from the endothelial cells will inhibit norepinephrine release, perhaps by modulating cGMP production at the sympathetic nerves, thereby inhibiting vasoconstriction and reducing shear stress on endothelial cells.

3.4.2. Liver studies

Effects of L-NAME and L-Arginine on Sympathetic Nerve-Induced Constriction: Vasoconstriction due to nerve stimulation was potentiated when NO release, in a shear dependent manner, was inhibited in both PV and HA (Figs. 9,11). This observation could be explained either by a shear-dependent NO release acting directly on the nerves or by the action of NO on the constricted vascular smooth muscle.

Effects of L-NAME on Norepinephrine-Induced Constriction: The vasoconstriction induced by intra-arterial infusion of norepinephrine was also potentiated after the NOS had been blocked by L-NAME in the HA and PV. Such effects were observed only when shear stress was increased (Figs. 13,15). We concluded that the NO effect in these vessels was directly on the contracted smooth muscle because the extent of potentiation was similar in both nerve stimulation and norepinephrine infusion.

A difference exists between the intestine and liver results. As was discussed before, in the liver both HA and PV appear to behave in a different fashion than other vessels in relation to the involvment of NO-regulation of basal tone. In this

study it was observed that NO is involved in a shear dependent manner in the regulation of vasoconstriction but not in the basal tone in the HA and PV. We therefore propose independent mechanisms for the regulation of the basal tone and vasoconstriction. In the SMA, both exist and NO, produced by shear stress, modulates nerve stimulation and not norepinephrine infusion because it is released at the smallest resistance vessels that are least sensitive to NO (Selke et al., 1990). In the liver, because of its unique anatomy, NO is released to modulate vasoconstriction but the enzyme is not preponderantly present in the small vessels but at larger vessels and NO therefore regulates vasoconstriction at the smooth muscle preferentially. The liver per se does not control PV flow. The HA blood flow is modulated locally through the PV blood flow by an ADO-dependent mechanism, the HABR. In this case, if the HA basal tone was dependent on NO levels, the HABR would become not only dependent on the PV blood flow but as well on the basal levels of NO. The lack of NO effect on the HA would appear to uniquely allow the HA to be under local regulation that has evolved to maintain a total hepatic flow as constant as possible. However, vasoconstriction needs to be locally regulated both in the intestine and liver by NO as suggested in these studies.

We believe this to be the first report to encompass both the NO release dependence on shear stress and the selective NO modulation of vasoconstriction. In conclusion, our studies suggest that the observed potentiation during sympathetic nerve stimulation in the presence of L-NAME in the SMA is due to the elimination of an NO-induced modulation of sympathetic nerves supplying the

artery. We provide further evidence that this mechanism is shear stress-dependent. Moreover, since during norepinephrine infusion, L-NAME did not potentiate the vasoconstrictor response, the regulation of sympathetic nerve stimulation by NO is suggested to be a pre-junctional effect leading to suppression of neurotransmitter release. However, other hypotheses accounting for the neuromodulation cannot be excluded since NO modulation can occur either by a direct or indirect effect on sympathetic stimulation. NO production could result in the release of a second factor that suppresses transmitter release. Since nerve stimulation may also release other vasoconstrictor co-transmitters, such as neuropeptide Y and ATP (Kugelgen and Starke, 1991; Lundberg et al., 1989; Potter, 1988; Taborsky et al., 1994), NO may modulate any or all co-released factors. Resolution of these alternate hypotheses requires further study.

In the liver, the release of NO might be not at the smallest vessels, which are less sensitive to NO and where NO could have a closer contact with the nerve endings, but at larger vessels. Therefore the regulation of local vasoconstriction by NO is suggested to be a post-junctional effect leading to inhibition of norepinephrine effects at the smooth muscle in a shear stress dependent manner. Thus, shear stress in the PV modulates constriction of the portal vessels and shear stress in the HA modulates constriction of the HA in a post-junctional manner. From our study we cannot assess whether shear stress in one vessel is capable of modulating effects in the other vessel. The significance of these observations is that during pathological conditions, such as hypertension, the impairment of the

endothelial cells would result in reduced ability of the vessels to defend themselves against increased shear stress, thereby leading to endothelial damage.

4. EFFECT OF NITRIC OXIDE ON THE AUTOREGULATORY CAPACITY IN THE SUPERIOR MESENTERIC AND HEPATIC ARTERIES

4.1. INTRODUCTION

The intestinal vasculature displays the basic mechanism of local control of blood flow known as autoregulation. This mechanism is characterized as the intrinsic ability of an organ to minimize changes in blood flow through a wide range of perfusion pressures (Johnson, 1986; Lang & Johnson, 1988). Three alternate mechanisms have been suggested to account for autoregulation; the myogenic, the metabolic and ADO washout theories. Shepherd and Granger (1973) first proposed the metabolic theory in which autoregulatory changes in vascular tone are dependent on tissue levels of oxygen and are mediated via a vasodilator metabolite released from the parenchymal cells. However, more recent work showed that a change in tissue oxygen tension is not essential for the autoregulation of splanchnic blood flow (Lang & Johnson, 1988). In contrast, the mechanism of autoregulation is clearly linked to ADO and it was suggested that ADO is produced at a constant rate and the local concentration in the area of the resistance vessels is determined by washout into the blood (Lautt, 1986). It was also argued that, since the response to ADO and autoregulation can be completely and selectively eliminated by ADO receptor blockade, that the myogenic hypothesis over physiologically relevant ranges of arterial and venous pressure is unlikely to account for autoregulation (Lautt, 1986; Lockhart & Lautt, 1990). Thus ADO was the first flow-dependent flow regulator proposed (Ezzat & Lautt, 1987; Lautt, 1986).

It is now clear that the vascular endothelium plays an important role in modulation of vascular tone. Endothelium dependent relaxing factors, generally recognized as being NO, have been shown to contribute to the regulation of vascular tone in a variety of organs and systems including the mesenteric bed (Macedo & Lautt, 1994; Moncada & Burnett, 1993). NO has been implicated as an antagonist of autoregulation in the coronary vasculature (Pohl et al., 1994; Ueeda et al., 1992). One of the mechanisms of release of NO is blood flow dependent (Johnson, 1986; Kuo et al., 1991). Increased flow has been shown to cause increased shear stress with consequent increased NO release and vasodilation in the SMA as well as other tissues (Kuo et al., 1991; Macedo & Lautt, 1994).

The present investigation was carried out to evaluate the possible role of NO in altering the autoregulatory response in the SMA and the HA. We tested the hypothesis that inhibition of NO synthesis potentiates the autoregulatory responses.

4.2. METHODS

Surgical Preparation: Fasted cats of either gender were anesthetized with sodium pentobarbital (32.5 mg/kg) administered intraperitoneally. Anesthesia was maintained through a drip bag (390 mg sodium pentobarbital/500 ml dextran). Body temperature was maintained at 37.5°C by the use of a rectal probe and a thermal control unit (Yellow Springs Instruments, model 72) operating heating rods in the table. All blood pressures were monitored via catheters using Gould and Statham pressure transducers, and all parameters were recorded on a Sensor

Medics R611 Dynograph. Transducers were set to zero reference level relative to the midpoint of the inferior vena cava at the hepatic outlet. The zero reference was checked for drifting before and after every experimental manoeuvre. Cats were heparinized (3000 IU) before establishing the vascular circuits to prevent clotting in the external vascular circuits. Controlled ventilation was provided by use of a respirator before laparotomy.

Systemic arterial pressure and central venous pressure were monitored via a right carotid arterial catheter and a left femoral venous catheter, respectively. Laparotomy, and ligation of the inferior mesenteric artery were subsequently performed.

Superior mesenteric artery surgical protocol:

The SMA was isolated, and its periarterial nerve bundle was gently separated, ligated and cut. Both femoral arteries were isolated, cannulated and connected to the SMA through a pump-controlled long circuit as previously described (see chapter 2). This circuit allowed control of blood flow and its measurement was obtained by the incorporation of an electromagnetic flow probe (Carolina Medical Electronics EP 408) into the circuit. SMA pressure was monitored from a catheter incorporated into the circuit.

Hepatic artery surgical protocol:

As previously described in chapter 2 briefly, after laparotomy, the inferior mesenteric artery and the celiac artery were ligated and the spleen was removed. The periarterial nerve bundle of the SMA was gently separated ligated, cut and an electromagnetic flow probe and a micrometer controlled screw clamp were placed on the artery (Carolina Medical Electronics EP 408). The HA was isolated and the gastroduodenal artery was isolated and ligated. After the nerve bundle around the HA was gently separated, ligated and cut, a vascular long circuit identical to that described in the previous protocol was connected between the femoral arteries and the HA. HA pressure was monitored by a catheter incorporated into the circuit, and drugs were administered through a separate infusion line.

Both SMA and HA pressures were calibrated in situ at the end of each experiment to account for resistance to flow in the circuit. All reported pressures were corrected for circuit resistance. Pressure-flow curves were performed by controlling the circuit pump.

Autoregulation Curves: After allowing the animal to recover from surgery for at least 45 minutes, pressure-flow autoregulation curves were performed.

A full pressure-flow curve was performed to determine the lowest resistance point considered to be the "shoulder" that separates the autoregulatory range from the non-autoregulatory range. Each curve had a minimum of 8 points over 150 mmHg to 40 mmHg with each stepwise decrease in perfusion pressure being 10 to 20 mmHg. Each pressure level was maintained for two minutes which was

sufficient to reach both a pressure and flow steady-state. This experimental protocol was repeated after the blockade of NOS by using the competitive inhibitor of the NOS, NG-nitro-L-arginine methyl ester (L-NAME) (2.5 mg/kg iv bolus).

Two step autoregulation curves were performed in which the first step was in the autoregulatory range, reducing pressure from approximately 140 mmHg to 70 mmHg. The second step was in the non-autoregulatory range from 70 mmHg to 40 mmHg. This protocol was repeated in the presence of L-NAME (2.5 mg/kg, iv bolus) and after injection of L-arginine, a NOS substrate, (75 mg/kg, iv bolus) to reverse the action of L-NAME. Preliminary studies showed that this dose of L-NAME was adequate to produce significant vascular effects. The L-arginine dosage was based on *in vivo* reversal of L-NAME-induced hypertension (Rees et al., 1990).

Similar two step autoregulation curves were executed in the control and in the presence of norepinephrine (0.50 μg/kg/min).

Calculations: The determination of the "shoulder" between the autoregulatory range and the non-autoregulatory range was assessed by plotting pressure versus resistance from a full pressure-flow curve, where the shoulder is identified as the pressure at which resistance is the lowest.

Autoregulation was assessed by the use of two autoregulatory indices:

1) Autoregulation index, ARI: This index calculates the proportion of flow change expressed relative to the proportion of pressure change (Semple & DeWardener, 1959).

$$ARI = 1 - [SMAF_c - SMAF_s / SMAF_c] / [(SMAP_c - SMAP_s) / SMAP_c]$$

where SMAF refers to superior mesenteric arterial flow, SMAP superior mesenteric arterial pressure and the subscripts C and S refer to control levels and values measured at the shoulder respectively. When applicable, SMAF was substituted by hepatic arterial flow (HAF) and SMAP by hepatic arterial pressure (HAP).

2) Autoregulation was assessed as well by a second index, the slope index (Ezzat & Lautt, 1987) which is an index of linearity of the pressure-flow curve calculated by dividing the slope of the best fitting linear regression analysis above the shoulder by the slope below the shoulder expressed as percent. A perfectly straight pressure-flow curve would give a slope index of 100%.

4.3. RESULTS

4.3.1. Effects of nitric oxide on the superior mesenteric artery autoregulatory capacity

Effects of L-NAME and L-Arginine on Basal Pressures: L-NAME had a peak effect between 15 and 20 min which increased SMA pressure from 95.9 $\pm$ 3.1 mm Hg (control) to 169.9 $\pm$ 9.9 mm Hg (L-NAME). The SMA flow (15.2 $\pm$ 2.6; 15.1 $\pm$ 2.6 ml/kg/min) was held constant by use of the pump-controlled long circuit in all experiments (n=7 $\pm$ SE). L-arginine decreased SMA pressure (100.0 $\pm$ 3.3 to 63.6 $\pm$ 7.4 mm Hg) at constant SMA flow (10.4 $\pm$ 2.3; 10.7 $\pm$ 2.3 $ml \cdot kg^{-1} \cdot min^{-1}$) (n=7 $\pm$ SE). L-NAME and L-arginine had no effect on the central venous pressure or portal pressure.

Shoulder Assessment: A stepwise pressure-flow autoregulation curve is shown in Figure 18 in which ARI over the autoregulatory range for control and L-NAME respectively in this example was -0.09 and 0.38 demonstrating potentiation of autoregulation after L-NAME. ARI was not significantly altered over the non-autoregulatory range (-0.29 and -0.31) for control and L-NAME. The slope index in the control was 94.1 and in the presence of L-NAME was 30.1. The point at which the artery showed the lowest resistance in the presence of L-NAME in this example occurred at the pressure of 67.3 mmHg. This pressure is representative of the shoulder; pressures above it were in the autoregulatory range and pressures below it were in the non-autoregulatory range. Experiments with stepwise pressure-flow curves were performed to assess the lowest resistance point, which occurred at a pressure of 74.1 $\pm$ 5.3 for control and 70.5 $\pm$ 3.7 mmHg for L-NAME (not significantly different).

Effects of Norepinephrine on Autoregulation: Difference in the autoregulatory capacity between control and during norepinephrine infusion in two step curves were performed and the respective slope index values were calculated. In these experiments there was no significant difference nor any tendency for a difference between the two slope indices of 75.6 $\pm$ 3.9 and 76.5 $\pm$ 8.7 respectively for control and norepinephrine (n=3).

Effects of L-NAME and L-Arginine on Autoregulation: In a representative example shown in Figure 19, the flow change (6.8, 7.0, and 6.1 ml/min/kg) relative to the change in pressure (37.3, 32.0, 35.6 mmHg) was similar in the control, after

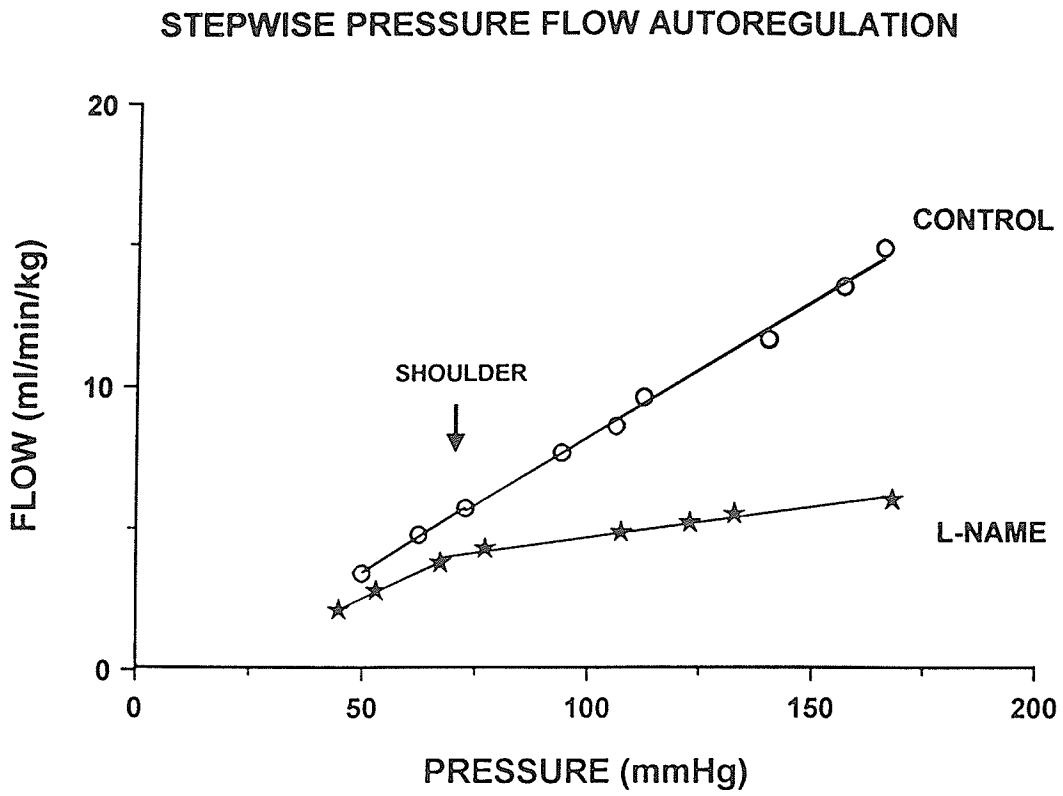

FIGURE 18: Stepwise pressure-flow autoregulation curve in the superior mesenteric artery for control and after L-NAME. The shoulder is calculated based on the lowest resistance, with pressures above the shoulder representative of the autoregulatory range and pressures below it the non-autoregulatory range. L-NAME potentiated autoregulation (data reported in text).

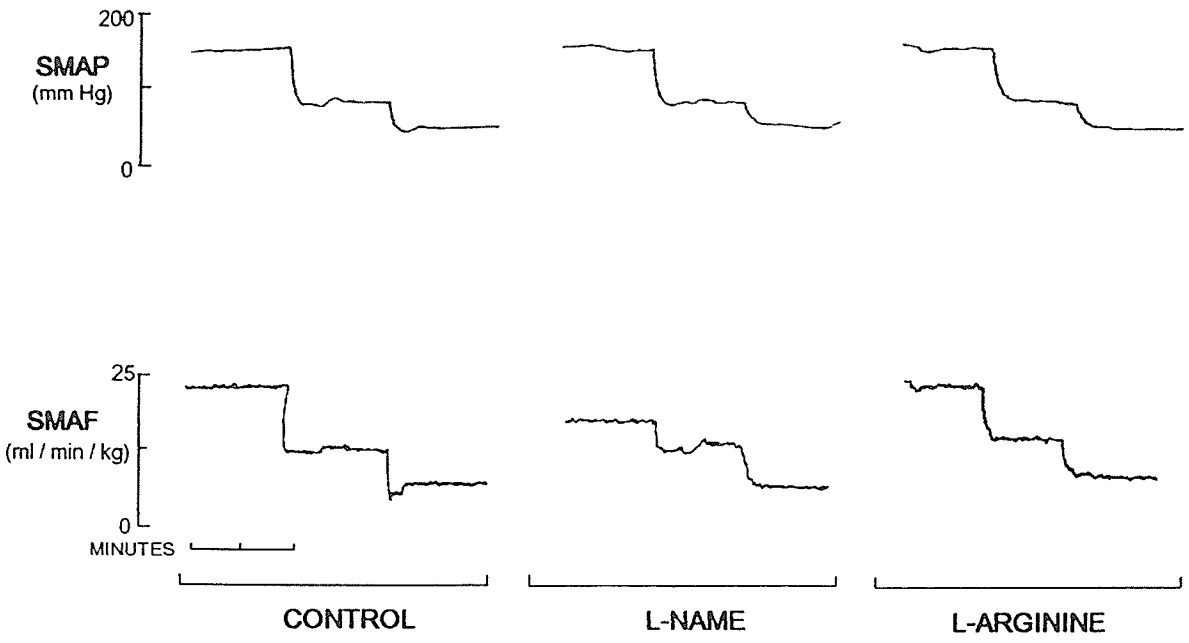

FIGURE 19: Effect of autoregulation two step curve in the superior mesenteric artery. In the non-autoregulatory range 70-40 mmHg the proportion of flow change relative to the change in pressure was similar in the control, after L-NAME and L-arginine. The autoregulatory range showed a lesser decrease in flow per unit decrease in pressure after L-NAME, which was reversed by L-arginine (data in text).

L-NAME and L-arginine for the non-autoregulatory range. The autoregulatory range showed a lesser decrease in flow per unit decrease in pressure after L-NAME; a decrease of flow of 8.1 ml/min/kg resulted from a decrease in pressure of 70.9 mmHg in the control compared to 3.1 ml/min/kg for a decrease in pressure of 66.0 mmHg in the presence of L-NAME. L-arginine reversed this effect in which a decrease in pressure of 68.6 mmHg resulted in a 7.7 ml/min/kg flow decrease (Fig 19).

Figure 20 shows pooled pressure-flow curves from 7 animals in which a decrease in pressure from control to the shoulder (autoregulatory range) was 65.2 $\pm$ 1.5 mmHg resulting in a decrease in flow of 10.9 $\pm$ 0.7 ml/min/kg. The same corresponding decreases in pressures and flows for L-NAME were 69.8 $\pm$ 1.7 mmHg and 4.8 $\pm$ 0.8 ml/min/kg. After L-arginine, a decrease in pressure of 66.3 $\pm$ 1.0 mmHg resulted in a flow decrease of 8.3 $\pm$ 0.7 ml/min/kg ($p < 0.001$ comparing L-NAME blood flow changes to control and L-arginine). Below the shoulder (non-autoregulatory range) the decreases in pressures were 35.8 $\pm$ 1.2, 34.4 $\pm$ 1.2, and 36.4 $\pm$ 1.3 mmHg for control, L-NAME and L-arginine with the respective decreases in flow of 7.6 $\pm$ 0.7, 5.1 $\pm$ 0.8 and 6.2 $\pm$ 0.4 ml/min/kg.

Figure 21 represents autoregulation assessed by two indices, the autoregulatory index (ARI) and the slope index. Over the autoregulatory range (70-140 mmHg) L-NAME significantly increased autoregulation from an ARI of 0.06 $\pm$ 0.05 to 0.28 $\pm$ 0.09 ($P < 0.003$) and L-arginine reversed ARI to 0.12 $\pm$ 0.07. ARI in the non-autoregulatory range (40-70 mmHg) was not different among control,

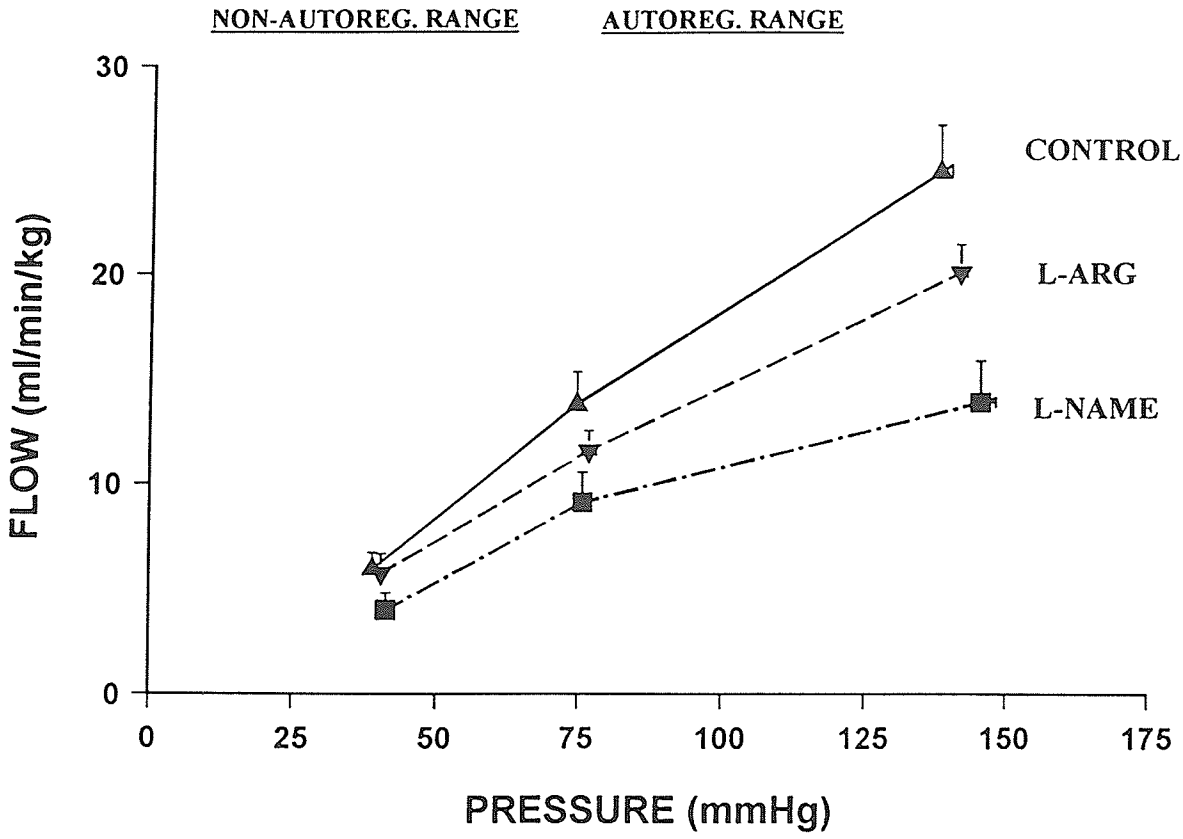

FIGURE 20: Autoregulatory responses (n = 7): The decrease in flow for the same decrease in pressure below the shoulder (non-autoregulatory range) was not different for control, L-NAME and L-arginine. The decrease in flows for the same decrease in pressure in the autoregulatory range was significantly different between control and L-NAME: L-arginine significantly reversed the effect of L-NAME. Results are means $\pm$ SE (data in text) and significant difference was accepted for $p < 0.05$ (repeated measures ANOVA followed by Tukey HSD test).

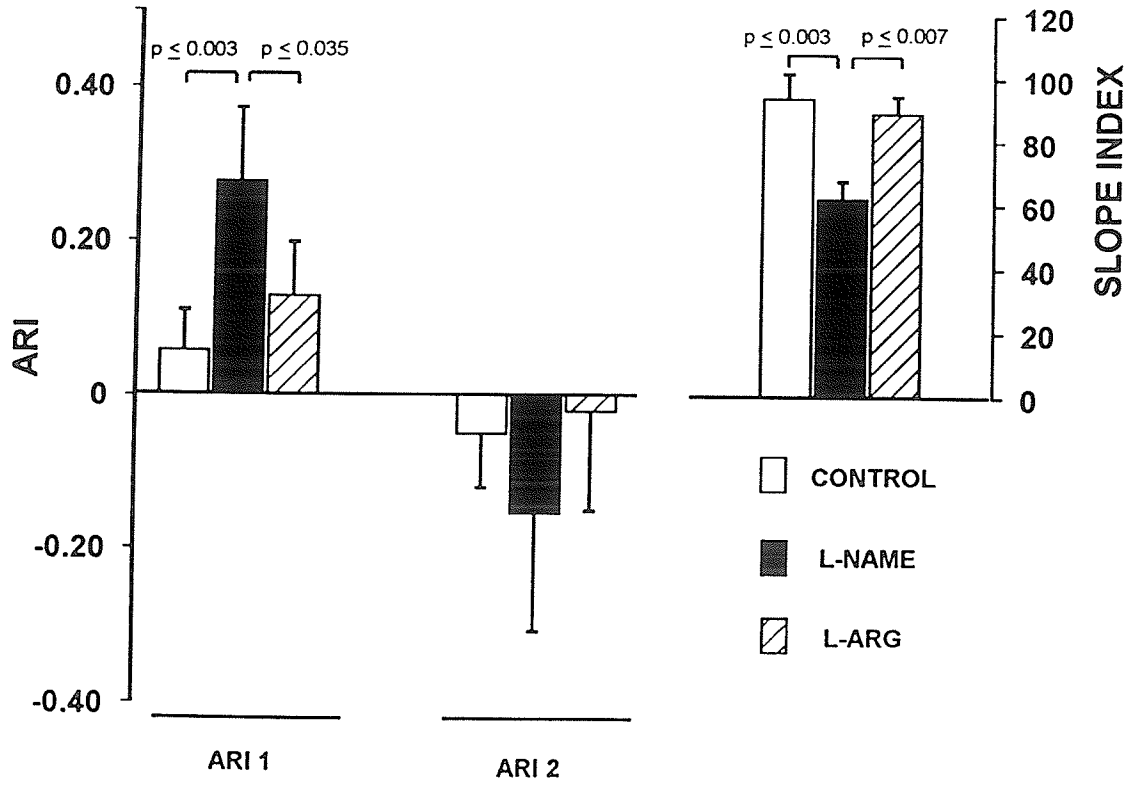

FIGURE 21: Autoregulation expressed as two indices, the autoregulatory index-ARI and the slope index. ARI 1 represents the autoregulatory index over the autoregulatory range and ARI 2 the non-autoregulatory range. The slope index is assessed as well; the lower the index the higher the autoregulation. Results are means $\pm$ SE and significant difference was accepted for $p < 0.05$ (repeated measures ANOVA followed by Tukey HSD test).

L-NAME and L-arginine groups (-0.05, -0.1, -0.02). The slope index in the presence of L-NAME (61.8 $\pm$ 5.6) was significantly different from control 93.4 $\pm$ 7.9 ($p < 0.003$) and this index was reversed by L-arginine to 88.9 $\pm$ 5.5 which was significantly different from the slope determined after L-NAME ($p < 0.007$).

4.3.2. Effects of nitric oxide on the hepatic artery autoregulatory capacity

Effects of norepinephrine on the Autoregulation: Autoregulatory capacity in control and during norepinephrine infusion in two step curves were determined and the respective slope index values were calculated. In these experiments there was no significant difference nor any tendency for a difference between the two slope indices of 81.8 $\pm$ 3.4 and 77.9 $\pm$ 4.7 respectively for control and norepinephrine ($n = 4$).

Effects of L-NAME on Autoregulation: Autoregulation was assessed by two indices, the autoregulatory index (ARI) and the slope index. Over the autoregulatory range L-NAME did not affect the ARI (-0.11 $\pm$ 0.05 for control and -0.07 $\pm$ 0.07. ARI in the non-autoregulatory range was not different between control and L-NAME (-0.31 $\pm$ 0.1, -0.29 $\pm$ 0.1). The slope index in the presence of L-NAME (83.9 $\pm$ 1.9) was insignificantly different from control (86.3 $\pm$ 5.4).

4.4. DISCUSSION

The salient finding of this study is that in the SMA of the cat, autoregulatory responses were augmented over the autoregulatory range after bolus infusion of the

competitive inhibitor of NOS, L-NAME, and this effect was reversed by supplying L-arginine as a substrate for NOS. These results are in accordance with the hypothesis that NO is involved in the inhibitory control of autoregulation, suggesting that, whereas ADO is a flow-dependent flow regulator leading to constriction with elevated flows, NO is a flow-dependent flow regulator that counteracts this effect and leads to dilation in response to higher flows.

Methodological Considerations: The present results confirm that the SMA shows a classic pressure-flow autoregulation where a curve of absolute blood pressure against blood flow intercepts the pressure axis at a positive pressure. An external perfusion circuit was chosen for the study of autoregulation in the SMA and HA in order to allow pressures to be attained over a representative autoregulatory range regardless of systemic arterial pressure of the animal. The use of the arterial circuit affords the advantage of allowing greater ranges of blood pressure to be obtained but it has the clear disadvantage of resulting in reduced vascular reactivity by unknown mechanisms. This observation has previously been made for the vascular bed of skeletal muscle (Folkow, 1953), the intestine (Dresel & Wallentin, 1966; Johnson, 1960), and the liver (Greenway et al., 1967). This limitation is problematic for studies aimed at determining the mechanism of autoregulation but it was not a limitation for the present study since potentiation of autoregulation was able to be clearly and consistently shown by blocking NO production, an effect that was reversed by L-arginine. The weak autoregulation observed in the control curves made assessment of the mean shoulder position

difficult, however previous work showed that the shoulder occurs at approximately 70 mmHg both in the SMA and in the HA (Ezzat & Lautt, 1987; Lautt, 1986). Where a shoulder from the control curve was difficult to determine, the shoulder seen after L-NAME was used as the relevant point for the other curves.

Because L-NAME results in an increase in basal vascular tone, we evaluated the possibility that the changes in autoregulation were an artifact of altered basal tone by raising vascular tone using intravenous norepinephrine on several occasions. The slope index showed no tendency to alter.

We attempted a series of experiments in which pressure-flow autoregulatory curves were performed in the presence of both the NOS blocker (L-NAME) and the ADO receptor blocker (8-phenyltheophylline). The doses of 8-phenyltheophylline needed to block the responses to ADO infusion into the SMA after administration of L-NAME led to a rapid deterioration of the preparation.

Previous Studies and Discussion of Results: Autoregulation is well known to occur in the SMA and HA (Burrows & Johnson, 1983; Ezzat & Lautt, 1987; Johnson & Hanson, 1962; Lautt, 1986). A modification of the metabolic theory of the mechanism of autoregulation has been proposed. In the classical metabolic theory, oxygen tension reductions or altered substrate delivery that occur upon decreased blood flow lead to production of vasodilators. Tissue oxygen tension, however, does not affect autoregulation of the SMA (Kontos et al., 1978; Lang & Johnson, 1988; Shepherd & Granger, 1973; Sullivan & Johnson, 1981). Lautt has previously shown (Ezzat & Lautt, 1987; Lautt, 1986) that autoregulation in the

feline SMA and HA is ADO-mediated and can be completely eliminated by ADO receptor antagonists. It is suggested that ADO is produced at a constant rate, independent of local oxygen tension and that the concentration in the area of the resistance vessels is dependent upon washout into the blood. ADO production may occur by pathways both dependent on and independent of the metabolic status of the intestinal parenchyma. Reduced oxygen tension does not lead to ADO mediated dilation (Lockhart & Lautt, 1990) but postprandial hyperemia is ADO-mediated (Sawmiller & Chou, 1988; 1991; 1992) and fed dogs show greater autoregulation than do fasted dogs (Norris et al., 1979). ADO has been proposed to be a flow-dependent flow regulator according to a washout mechanism for both the SMA and the HA (Ezzat & Lautt, 1987; Lautt, 1985; 1986).

The involvement of NO in autoregulation appears to be organ specific. The autoregulation of cerebral blood flow is probably not critically regulated by NO (Buchanan & Phillis, 1993; Wang et al., 1992). The situation in the kidney is less clear with reports indicating that NOS antagonism impairs autoregulation (Kiyomoto et al., 1992) or has no effect (Beierwaltes et al., 1992; Majid & Navar, 1992). In contrast, in the coronary arteries, NO appears to be a relevant component of the autoregulation and its release is suggested to be dependent upon shear stress induced by blood flow producing distortion of endothelial cells (Pohl et al., 1994; Ueeda et al., 1992).

Our experiments demonstrated that autoregulatory responses in the SMA were augmented after treatment with L-NAME, and that this effect was reversed

by L-arginine. This action was selective over the autoregulatory range. This suggests that NO is involved in the inhibitory control of autoregulation. Endothelial cells release NO when the shear stress is increased (Smiesko et al., 1989). Since shear stress is linearly related to flow rate and inversely dependent on the radius, the dependence on the flow-induced NO release mechanism seems to be more prominent at higher flow rates (Kamika & Togawa, 1980). Considering that the small vessels are more sensitive to the autoregulatory mechanism in the SMA and that the smaller the radius of the artery the more sensitive the artery is to shear stress, we suggest that the release of NO is shear stress dependent (Johnson & Hanson, 1962; Macedo & Lautt, 1994). An increase in blood flow induces shear stress, thus increasing NO formation and vasodilation resulting in a less autoregulatory system. Blockade of NO release thus enhances autoregulation. The observation that the NO effect is seen only over the autoregulatory range could indicate that the shear stress becomes significant only at higher flow rates or it could simply be that at flows below the autoregulatory range the blood vessels are fully dilated. We have not attempted to evaluate these alternatives.

The liver does not control PV blood flow. The HA blood flow is modulated locally through the PV blood flow by an ADO-dependent mechanism (the HABR). In this case, if the HA basal tone and the ADO hypotensive effects were dependent on NO levels, the HABR would become not only dependent on the PV blood flow but as well on the basal levels of NO. The lack of NO effect on the HA would appear to uniquely allow the HA to be under local regulation that has evolved to

maintain a total hepatic flow as constant as possible. Thus the observation that NO does not attenuate the autoregulatory capacity in the HA is in accordance with the HABR theory where ADO is the only substance responsible for the HA basal tone.

In conclusion, we propose that NO is implicated in antagonizing the autoregulatory mechanism over the autoregulatory range through a shear stress-dependent mechanism resulting in a self-regulated protective mechanism for the endothelial cells in the SMA but not in the HA. In the intestine, ADO and NO act in a "yin-yang" fashion to minimize flow increment in response to elevated perfusion pressure while preventing shear stress-induced disruption of endothelial cells. Impairment of the release of NO might promote the injury of the endothelial cells due to an excess of shear stress resulting in pathological conditions such as hypertension.

5. NITRIC OXIDE-ADENOSINE INTERACTION IN THE SPLANCHNIC BED

5.1. INTRODUCTION

The splanchnic bed is endowed with an intrinsic capacity to regulate its blood flow when neural and hormonal influences are eliminated. There is increasing evidence for a role for both ADO and NO in the local regulation of blood flow (Macedo & Lautt, 1995; 1996; Jacobson & Pawlik, 1994; Mathie et al., 1991a; Greenblatt et al., 1993). ADO is involved with regulation of postprandial hyperemia, reactive hyperemia and pressure-flow autoregulation in the SMA (Jacobson & Pawlik, 1994; Macedo & Lautt, 1995; Lautt, 1986). In the liver ADO is responsible for both the autoregulation of the HA and the HABR (Ezzat & Lautt, 1987; Lautt et al., 1985). ADO vasodilation is through A2 receptors in the SMA and HA vascular smooth muscle cells mediated by an enhancement of cyclic AMP (Jacobson & Pawlik, 1994; Mathie et al., 1991b). NO has been shown to contribute to the regulation of the vascular tone in a variety of organs and systems including the SMA (Palmer & Moncada, 1987; Pohl et al., 1994; Ueeda et al., 1992). NO, released by shear stress on the endothelial cells at the site of constriction, causes suppression of sympathetic nerve activity in the intestinal circulation (chapter 3). NO also antagonizes ADO-mediated pressure-flow autoregulation in the SMA (chapter 4). In contrast, the effect of inhibitors of the NOS on the HA basal tone has been shown to be insignificant (Mathie et al., 1991b; Browser et al., 1994; Greenblatt et al., 1993).

The observation that NO produced physiological antagonism of ADO-mediated autoregulation in the intestine (chapter 4) led to the hypothesis that NO modulates the vasodilatory properties of ADO. The purpose of this study was to elucidate the contribution of NO in the regulation of the vasoactive effects of ADO. For a better understanding of the possible mechanism of NO-ADO interactions we compared the responses to ADO with the vasodilatory effects of isoproterenol acting on beta-2 receptors with a subsequent activation of the nucleotide cAMP (Harrison et al., 1988; Taira et al., 1977). In the present investigation, we tested the hypothesis that inhibition of NO synthesis potentiates the vasodilatory effects of ADO in an organ-specific manner, by carrying out studies in both the intestine and the liver.

5.2. METHODS

Superior mesenteric artery surgical protocol:

After laparotomy, the inferior mesenteric artery was ligated, the SMA was isolated, and its periarterial nerve bundle was gently separated, ligated and cut. Both femoral arteries were isolated, cannulated and connected to the SMA through a pump-controlled long circuit as previously described (chapter 2). This circuit allowed control and measurement of SMA blood flow and pressure. SMA pressure was monitored from a catheter in the circuit. An infusion line in the circuit allowed intra-arterial administration of drugs.

Hepatic artery surgical protocol:

As previously described (chapter 2), after laparotomy, the inferior mesenteric artery and the celiac artery were ligated and the spleen was removed. The periarterial nerve bundle of the SMA was gently separated ligated, cut and an electromagnetic flow probe and a micrometer-controlled screw clamp were placed on the artery (Carolina Medical Electronics EP 408). HA was isolated and the gastroduodenal artery was isolated and ligated. After the nerve bundle around the HA was gently separated, ligated and cut, a vascular long circuit identical to that described in the previous protocol was connected between the femoral arteries and the HA. HA pressure was monitored by a catheter incorporated into the circuit, and drugs were administered through a separate infusion line.

Both SMA and HA pressures were calibrated in situ at the end of each experiment to account for resistance to flow in the circuit. All reported pressures were corrected for circuit resistance.

Dose-response studies: Preparations were allowed to equilibrate for 45 minutes before any protocol was started. The pump-controlled long circuit was used to maintain the flow constant during drug-induced responses. Responses to intra-SMA ADO infusion (0.1,0.2 and 0.4 $mg \cdot Kg^{-1} \cdot min^{-1}$ were determined at two different pressures, 100 and 150 mm Hg, attained by adjusting the flow into the SMA. These doses were selected because they showed maximal dilation effects at all doses and because the protocol required the use of different flow rates. The testing of all doses at each of the different flows removed the possibility that drug

concentration became a confounding aspect. Bolus infusion of L-NAME (2.5 mg/kg, i.v.), caused vasoconstriction thus shifting the baseline. To account for the possibility that a baseline shift would alter the response to ADO, the responses were tested at different baselines of pressure and flow matched to what occurred in the control state. Two sets of responses to ADO were obtained, one at the same flow that had produced the pressure of 100 mm Hg at the control state and a second at a flow that was estimated to produce a pressure of 100 mm Hg under the effect of L-NAME. Following the bolus infusion of L-arginine (75 $mg \cdot kg^{-1}$, i.v.), responses to ADO were again repeated as described for the L-NAME experiments.

Responses to intra-arterial isoproterenol (ISO) (0.05, 0.1, 0.2, 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) were obtained and a dose that produced a maximum response was chosen to reproduce the protocol used for ADO.

Identical protocols (ADO and ISO) were followed for studies in the HA, with the exception that all studies were carried out only at the same flow because the lack of vasoconstriction produced by L-NAME on the HA precluded the need for the separate pressure controls.

5.3. RESULTS

Influence of L-NAME on the vasodilatory effects of ADO (n=7) and ISO (n=5) in the SMA: Mean ($\pm$ SE) carotid arterial blood pressure was 111.6 $\pm$ 7.6, 140.0 $\pm$ 7.5 and 102.0 $\pm$ 7.7 mm Hg respectively before and after L-NAME and L-arginine ($p < 0.001$ between control and L-NAME as well as between L-NAME and

L-arginine) (n = 12). SMA pressure measured from the circuit was 95.9 $\pm$ 5.9 mm Hg at the control state and 160.2 $\pm$ 8.9 at the peak effect of L-NAME ($p < 0.001$). L-arginine reversed the effect of L-NAME, bringing the SMA pressure to 72.3 $\pm$ 12.0 mm Hg ($p < 0.001$). The effect of L-NAME and L-arginine were observed at a constant SMA flow of 12.7 $\pm$ 1.9 ml $\cdot$ kg^{-1} $\cdot$ min^{-1} at the control state, 12.7 $\pm$ 2.1 and 12.6 $\pm$ 2.2 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}, after the administration of these drugs. Central venous pressure and portal venous pressure did not change throughout the experiment.

Three doses of ADO were selected to produce a maximal vasodilation of the SMA. Since flow was held steady, the vasodilation to ADO was seen as a decrease in perfusion pressure. A typical trace is shown (Fig. 22) of control responses to ADO producing a maximal decrease in pressure of 49.6 mm Hg at a blood flow of 15.7 ml $\cdot$ kg^{-1} $\cdot$ min^{-1} and a decrease of 98.4 mm Hg at a flow of 19.0 ml $\cdot$ kg^{-1} $\cdot$ min^{-1} (0.4 mg $\cdot$ kg body wt^{-1} $\cdot$ min^{-1} ADO). Under the effect of L-NAME, the maximal decrease in pressure determined at the control blood flow (15.5 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}) was 144.2 mm Hg. The circuit was then adjusted to bring the basal pressure to the control pressure and the ADO infusion was repeated. To attain the same control pressure under the effect of L-NAME basal flow was adjusted to 6.6 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}. ADO caused a decrease in perfusion pressure of 68.3 mm Hg. After the bolus infusion of L-arginine, the maximal decrease in pressure produced by ADO at the control blood flow of 15.8 ml $\cdot$ kg^{-1} $\cdot$ min^{-1} was 25.4 mm Hg and 49.4 mm Hg from the control pressure of approximately 100 mm Hg. Thus the maximal vasodilation

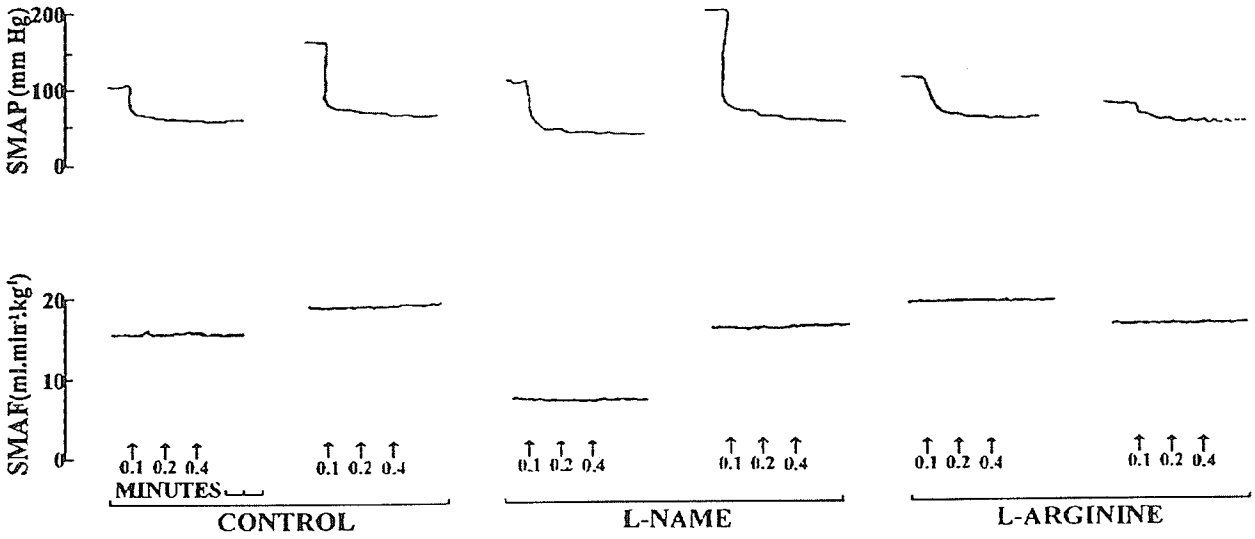

FIGURE 22: Representative figure of the effects of adenosine (ADO) on the superior mesenteric artery (SMA) at the control state in the presence of the nitric oxide synthase inhibitor L-NAME and in the presence of the nitric oxide synthase substrate L-arginine. SMAP represents the superior mesenteric arterial pressure and SMAF the superior mesenteric arterial flow. Potentiation of adenosine is seen after L-NAME with tests done at basal (pre-L-NAME) pressure or flow with results expressed as absolute or % change (see text).

of the SMA induced by ADO was potentiated by L-NAME and reversed by L-arginine when tested at either control pressure or flow.

Figure 23 represents the mean maximal decrease in absolute pressure and Figure 24 shows the % decrease in pressure induced by ADO on the SMA ($n=7$). L-NAME potentiated and L-arginine reversed the potentiation of SMA responses to ADO measured at control pressures or flow with the responses expressed either as absolute changes or % changes.

The same pattern of results appear from the calculations of absolute or % changes for the doses of 0.1 and 0.2 mg· kg body wt^{-1}· min^{-1} of ADO ($p<0.05$ between control - L-NAME and L-NAME - L-arginine).

ISO-induced dilation of the SMA was also potentiated in the presence of L-NAME both during the constant pressure protocol and the constant flow protocol and the potentiation was reversed by L-arginine. The effects are shown with vasodilation expressed either as absolute or % decrease in pressure (Fig. 25,26).

Influence of L-NAME on the vasodilatory effects of ADO and ISO in the HA ($n=6$): Mean arterial pressure, as measured from the carotid artery, was 103.8 $\pm$ 7.6 mm Hg before and 144.3 $\pm$ 9.9 mm Hg after the influence of L-NAME ($p<0.001$). HA pressure was not altered by L-NAME (108.5 $\pm$ 6.6 and 112.7 $\pm$ 6.8 mm Hg during the control state and L-NAME respectively). Because of a lack of effect by L-NAME on basal pressure, all studies were carried out only at a constant flow (9.4 $\pm$ 0.9 and 9.8 $\pm$ 1.2 ml· kg^{-1} · min^{-1}) held at the control level. L-NAME had no effect on central venous pressure or portal venous pressure.

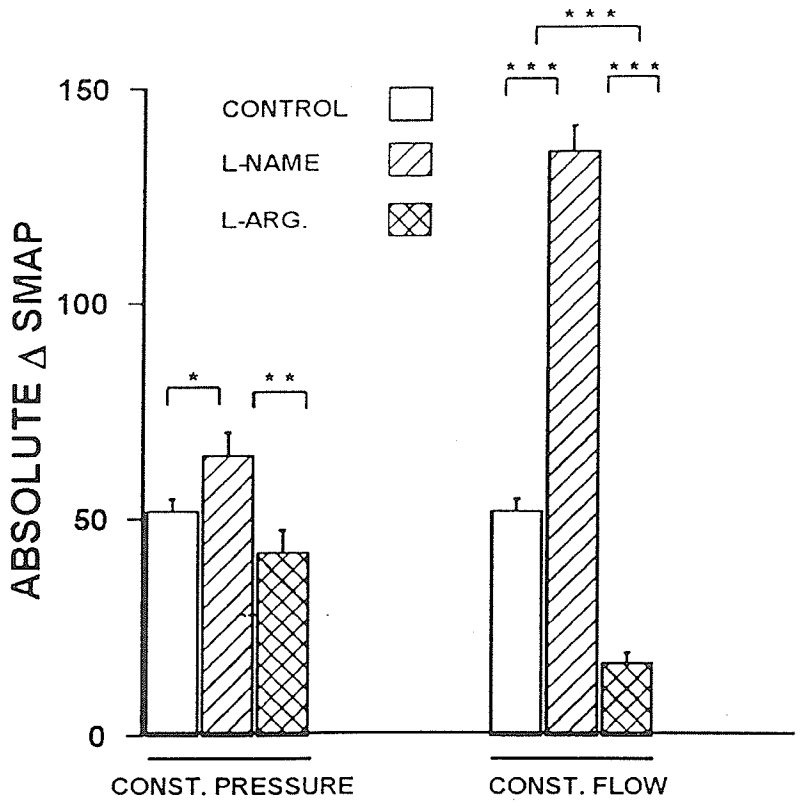

FIGURE 23: The effect of adenosine expressed as absolute changes in superior mesenteric arterial perfusion pressure. The decrease in pressure in response to adenosine measured from the same basal pressure was 51.7 $\pm$ 2.9 mm Hg, 64.7 $\pm$ 5.3 mm Hg and 42.2 $\pm$ 5.2 mm Hg respectively for control, L-NAME and L-arginine. From the same basal flow adenosine decreased SMAP by 51.7 $\pm$ 2.9 mm Hg, 135.2 $\pm$ 6.1 mmHg and 16.7 $\pm$ 2.4 mm Hg respectively for control, L-NAME and L-arginine. *$p<0.05$, **$p<0.01$, ***$p<0.001$ by Analysis of Variance (ANOVA) for multiple comparisons followed by Tukey's HSD (honestly significant difference)($n=7$).

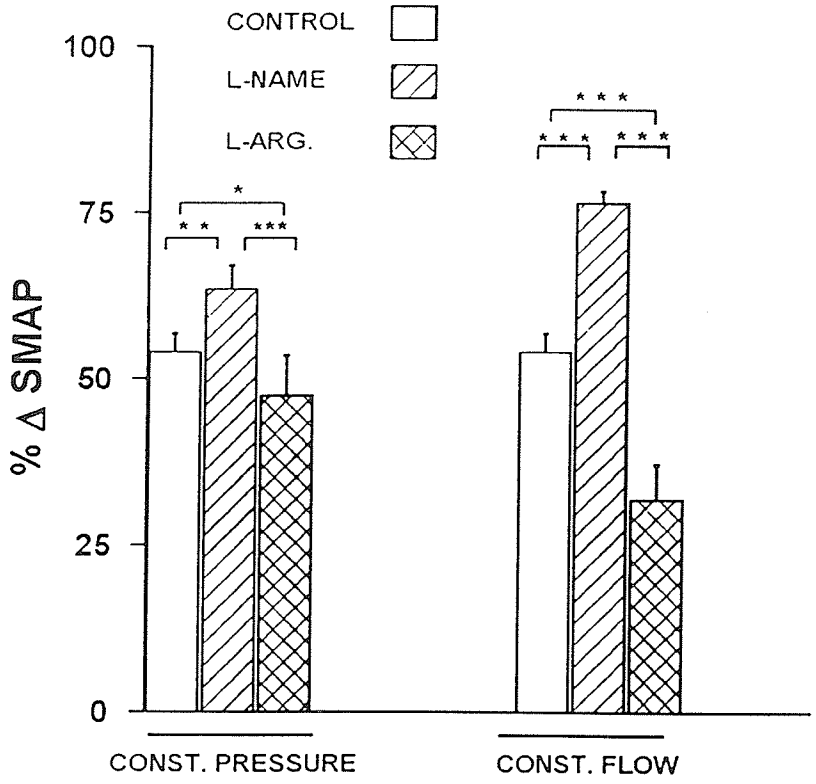

FIGURE 24: The effect of adenosine expressed as % changes in superior mesenteric arterial perfusion pressure. The decrease in pressure in response to adenosine measured from the same basal pressure was 54.0 $\pm$ 2.8%, 63.4 $\pm$ 3.6% and 47.3 $\pm$ 6.1% respectively for control, L-NAME and L-arginine. From the same basal flow adenosine decreased pressure by 54.0 $\pm$ 2.8%, 76.4 $\pm$ 1.9% and 31.9 $\pm$ 5.3% respectively for control, L-NAME and L-arginine. *$p<0.05$, **$p<0.01$, ***$p<0.001$ by Analysis of Variance (ANOVA) for multiple comparisons followed by Tukey's HSD (honestly significant difference)($n=7$).

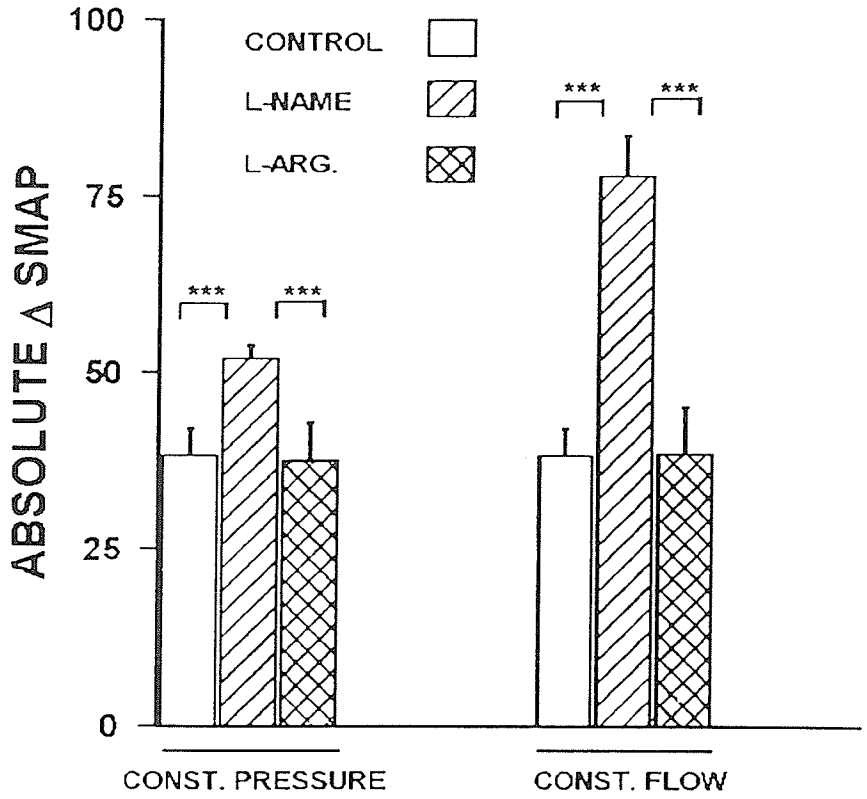

FIGURE 25: The effect of isoproterenol expressed as absolute changes in superior mesenteric arterial perfusion pressure. The decrease in pressure in response to isoproterenol measured from the same basal pressure was of 38.2 $\pm$ 3.9 mm Hg, 52.0 $\pm$ 1.8 mm Hg and 37.4 $\pm$ 5.4 mm Hg respectively for control, L-NAME and L-arginine. From the same basal flow the vasodilatory effects of isoproterenol decreased SMAP by 38.2 $\pm$ 3.9 mm Hg, 77.9 $\pm$ 5.8 mmHg and 38.4 $\pm$ 6.7 mm Hg respectively for control, L-NAME and L-arginine. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ by Analysis of Variance (ANOVA) for multiple comparisons followed by Tukey's HSD (honestly significant difference)($n = 5$).

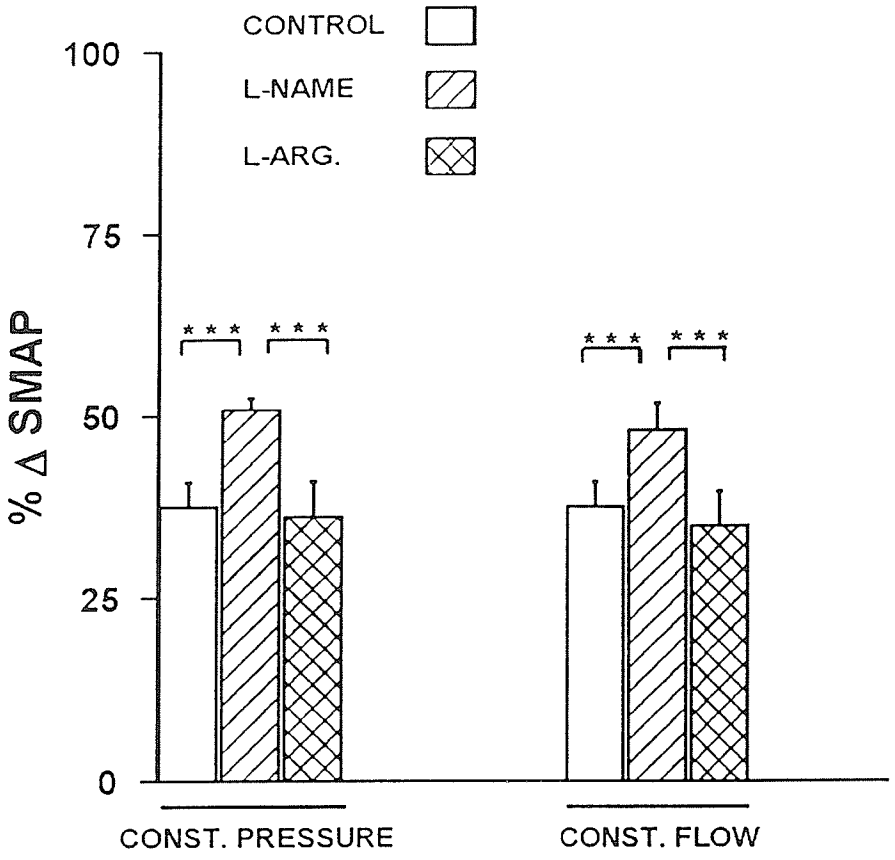

FIGURE 26: The effect of isoproterenol expressed as % change in superior mesenteric arterial perfusion pressure. The decrease in pressure in response to isoproterenol measured from the same basal pressure were of 37.4 $\pm$ 3.4%, 50.7 $\pm$ 1.7% and 30.0 $\pm$ 4.9% respectively for control, L-NAME and L-arginine. From the same basal flow the vasodilatory effects of adenosine decreased pressure by 37.4 $\pm$ 3.4%, 47.9 $\pm$ 3.7% and 34.8 $\pm$ 4.7% respectively for control, L-NAME and L-arginine. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ by Analysis of Variance (ANOVA) for multiple comparisons followed by Tukey's HSD (honestly significant difference) ($n = 5$).

The maximal vasodilation in response to intra-arterial ADO (0.4 mg· kg body wt^{-1}· min^{-1}) and ISO (0.2 μg· kg body wt^{-1}· min^{-1}) of the HA, with the responses expressed either as absolute changes or % change, were not significantly different comparing the control (6.6 $\pm$ 1.7 mm Hg, 6.2 $\pm$ 1.5% for ADO and 6.4 $\pm$ 2.0 mm Hg, 6.1 $\pm$ 1.8% for ISO) to the responses after L-NAME (7.2 $\pm$ 1.9 mm Hg, 7.3 $\pm$ 2.1% for ADO and 12.8 $\pm$ 2.8 mm Hg, 11.4 $\pm$ 2.6% for ISO).

5.4. DISCUSSION

Our studies provide evidence that there is a significant organ-specific interaction between NO and the vasodilator responses to ADO and ISO in pentobarbital-anesthetized cats. Inhibition of NO production did not alter basal vascular tone or the vasodilation to ADO or ISO in the HA. The SMA, in contrast, showed a basal vasoconstriction and potentiation of the maximal vasodilatory effect of both ADO and ISO during the inhibition of NOS; the effects were reversed by L-arginine. These observations indicate that the SMA, but not the HA, expresses a compensatory mechanism in which the absence of NO promotes a potentiation of other vasodilators possibly acting through a cyclic nucleotide "cross-talk".

Methodological considerations: Studies were attempted at standardized perfusion pressures. However a degree of variability in the pressures is reported because of the necessity to correct the circuit pressure for the resistance in the cannula determined from a calibration curve at the end of each experiment.

L-NAME in the SMA promoted a change in baseline vascular tone. To

compensate for the baseline shift, the responses were tested at pre-L-NAME flow and separately at pre-L-NAME pressure with adjustments made using the pump-controlled long circuit. The use of a constrictor agent to change baseline tone to the same extent as produced by L-NAME was not possible because basal tone could not be predicted accurately and the compound would have had to be given before L-NAME, since L-NAME was long acting.

Comparisons of the dilator response to ADO and ISO could be made at the same dose when blood flow was held at basal control level after L-NAME and L-arginine. However, in a constant pressure protocol, when flow was adjusted with the pump to hold pressure at the control level, flow to the SMA was dramatically different in the control state, after L-NAME, and after L-arginine. The same dose infused into different blood flows would yield different concentrations at the receptors. To compensate for this potential artifact, the dose of drug used was ascertained to produce a maximal dilation and then a dose of double and quadruple was tested. All three doses were tested after L-NAME and L-arginine. Because the drug concentration varied over a four fold range at each flow level, and all doses produced a maximal response, changes in response could not be attributed to changed concentrations of drug at the receptors site.

Another aspect to consider in our study is that the HA seems to be in a more dilated state due to the use of the pump-controlled long circuit. Norepinephrine was initially considered to raise the basal tone, however, this procedure was not possible to pursue since norepinephrine-induced vasoconstriction was potentiated

after L-NAME, thus complicating interpretation of the results (data not shown). Although the basal tone of the HA was reduced by the circuit, all arteries reported showed additional dilation in response to both ADO and ISO. The reduced tone in the HA but normal tone in the SMA using identical testing conditions is another example of vascular bed selectivity.

Influence of L-NAME on the vasodilatory effects of adenosine and isoproterenol in the SMA: The potentiated effect of ADO observed in the SMA is in agreement with the results shown by Gardiner et al. (1991) who observed an increase in the hyperemic response to NECA, a selective ADO A2 agonist, in the presence of L-NAME in the mesenteric bed. Inhibition of NO results in an increase in ADO release in the coronary artery (Woolfson et al., 1995). However, this has not been shown for the SMA and would not account for the potentiated vasodilation observed with ISO. Relavic et al. (1992) showed an increase in the release of ATP in response to shear stress in the rat mesenteric arterial bed. This increase could lead to a subsequent degradation of the ATP to ADO and potentiation of the vasodilators. Shear stress is not altered by changes in the vascular tone if perfusion pressure is constant (chapter 1 and 2). In this study we tested the dilators at the same pressure so that basal shear stress would be similar before and after L-NAME. Any change in shear stress that would occur would be a reduction, during dilator infusion, which would tend to counter not to potentiate dilation. Further, the demonstrated potentiation was to a maximal response that was obtained to a four fold range of doses so that increased concentrations of ADO

at the receptors would not be capable of causing a greater dilation. Thus, it is unlikely that altered ADO levels, even if they occurred, could account for the observed potentiation.

NOS inhibitors or removal of the endothelium potentiate the vasodilatory responses to the NO donors, sodium nitroprusside in the thoracic aorta, SMA and HA bed, sodium nitrate in the thoracic aorta, and SIN-1 in the femoral artery and cerebellar cortex (Busse et al., 1989; Ralevic et al., 1991; Shirasaki & Su, 1985; Li & Iadecola, 1994). Possible mechanisms include the following. Inhibition of endogenous NO formation could increase the available pool of guanylate cyclase - cGMP for activation; a cGMP phosphodiesterase might be inhibited; decreased extrusion of cGMP from the cells might occur and NO continuously released from the endothelium could act as an inhibitor of the relaxant effects of exogenous nitrovasodilators (Ralevic et al., 1991; Luscher et al., 1989; Busse et al., 1989). Even though the removal of the endothelium seems to reduce the levels of cGMP, nitrovasodilators promote a higher increase of the cyclic nucleotide in the absence of NO production by the endothelial synthase (Busse et al., 1989). Pearl et al. (1984) made use of aminophylline as a phosphodiesterase inhibitor and observed a potentiation in the hypotensive properties of sodium nitroprusside concomitant with elevations in plasma cGMP levels. They concluded that sodium nitroprusside, through a potentiated activation of the guanylate cyclase, increased intracellular levels of cGMP (Pearl et al., 1984). Maurice et al. (1990,1991) reported that elevation of cGMP by nitrovasodilators potentiated cAMP levels by stimulating

adenylate cyclase. Another group (Heuzé-Jouber et al., 1992) observed that NO directly or indirectly activates the production of cAMP in the renal circulation. The novel observation that cGMP inhibits phosphodiesterase III which enhances the effect of phosphodiesterase IV and leads to elevated cyclic AMP levels suggests a regulatory relationship between the two cyclic nucleotides (Eckly & Lugnier, 1994; Komas et al., 1991). Furthermore, as NO has recently been observed to induce cAMP, this nucleotide has also been reported to prolong the half-life of cGMP (Eckly & Lugnier, 1994; Komas et al., 1991; Hernandez et al., 1994; Vigne et al., 1994; Murthy & Makhlouf, 1995). Additionally, a few studies have investigated the cross-talk between the two cyclic nucleotides. Activation of ß-adrenergic receptors by isoproterenol enhanced cGMP accumulation in intact pinealocytes and cG-kinase activity in extracts of smooth muscle cells (Murthy & Makhlouf, 1995; White & Klein, 1995). In this latter study the increased activity of cG-kinase was observed only after cAMP basal levels were raised (White & Klein, 1995). Cross-activation of G-kinase by cAMP seems to be a mechanism present in tissues and extracts of vascular smooth muscle (Lincoln et al., 1977; Lincoln et al., 1990; Ito & Karachot, 1990).

Jacobson and Pawlik (1994) suggested that ADO, through its actions on the A2 receptor of the vascular smooth muscle of mesenteric arterial resistance vessels, activates both adenylate cyclase and guanylate cyclase. The results obtained with ISO, which is known to activate adenylate cyclase, lead us to the conclusion that the mechanism by which vasodilation is enhanced is not exclusive for ADO and

that a common pathway might exist. Based on the previous discussion, the potentiation of ADO and ISO, in the presence of L-NAME in the SMA, might be explained either by a potentiated activation of the guanylate cyclase directly or indirectly or by an increment of the cyclic GMP levels due to the inhibition of its hydrolysis through cyclic AMP effects on the phosphodiesterases.

Influence of L-NAME on the vasodilatory effects of adenosine and isoproterenol in the HA: In the present experiments the ADO and the ISO-induced dilation, known to be mediated by a P1-purinoceptor of the A2 sub-type for the former and through beta-2 receptors for the latter, were neither antagonized nor potentiated by L-NAME. These *in vivo* results are in agreement with those observed by Mathie et al. (1991a) in which ADO vasodilatory properties were not affected by either L-MMA (N-monomethyl-L-arginine, a NOS inhibitor) or L-NAME in livers of New Zealand White rabbits perfused in vitro. The HA is reported to have low levels of guanylate cyclase (Ignarro, 1989). Thus, a possible interaction between the two cyclic nucleotides would be compromised and the hypotensive effect of both vasodilators in this specific arterial bed would not be expected to be potentiated. Accordingly, the HA appears to be unique in the lack of these responses to L-NAME and L-arginine.

In conclusion, this study suggests that there is a compensatory mechanism with vasodilators that is highly organ-specific. In the absence of the vasodilator, NO, the magnitude of the ADO and ISO vasodilator responses increased for the SMA but not for the HA. The splanchnic circulation accounts for about 25% of the

cardiac output. Of this, roughly 3/4 supplies the arterial beds of the organs that drain into the PV. The liver does not control PV blood flow. The HA blood flow is modulated locally through the PV blood flow by the HABR. In this case, if the HA basal tone and the ADO vasodilator effects were dependent on NO levels, the HABR would become not only dependent on the PV blood flow but also on the basal levels of NO. The lack of NO effect on the HA would appear to uniquely allow the HA to be under local regulation that has evolved to maintain a total hepatic flow as constant as possible. This compensatory mechanism becomes relevant for the SMA in the prevention of ischemic states and provides the capacity to increase autoregulatory potency by ADO when an increased basal tone is due to reduced NO (chapter 4). Lack of production of NO might be related to some types of hypertension and might account for the higher resistance observed in the SMA that is seen as one of the diabetic vascular dysfunctions (Taylor & Poston, 1994; Taylor et al., 1994). During high mesenteric resistance states, this dilator compensatory mechanism would allow ADO to partially offset the elevated resistance in the gut. The liver would be expected to show a normal buffer response to any alteration in PV blood flow, uncomplicated by altered NO-induced basal tone or dilator compensation. Further studies are required to determined functional consequences of the unique difference in the vascular regulation of the HA and SMA in normal and pathological states.

6. OVERVIEW AND FUTURE DIRECTIONS

In light of the present thesis, insights about the HA and SMA vascular resistance need to be revised. Both NO and ADO play relevant roles as local modulators of resistance in both beds. Because of the differences present between the two vascular beds, several questions remain unanswered. The first enigma relates to the mechanism by which the SMA, but not the HA, shows basal tone to be dependent on NO, however both show a modulation of vasoconstriction that is NO and shear stress dependent. Therefore, these experiments suggest that there are two different mechanisms involved in the release and physiological function of NO, one that is shear stress independent but is responsible for the basal tone, and a second one that is shear stress dependent and modulates vasoconstriction. To clarify these two mechanisms, further experiments using a 0.02% solution of glutaraldehyde dimer (DGA), that will decrease endothelium sensitivity to shear stress by increasing cell stiffness, can be performed (Melkumyants, 1995). If NO-mediation of basal tone is independent of shear stress, an increase in basal tone should not be observed after infusion of the solution of DGA either in the SMA or HA. However, in the presence of L-NAME, an increase in basal tone should be observed for the SMA but not for the HA, as shown in this thesis. Because of the expected impairment in shear stress, vasoconstriction would be potentiated after treatment with DGA in the intestine and the liver. Since the site of release of NO due to shear stress is not yet determined, further work is needed to clarify this point. In the intestine, our studies suggest a prejunctional effect of NO. However,

it remains unclear if this NO modulation of nerve endings is through suppression of norepinephrine release or any of the other cotransmitters that are released with norepinephrine, such as neuropeptide Y or ATP.

A "yin-yang" relationship between ADO and NO accounts for the autoregulation in the intestine. However, the mechanism by which NO antagonizes autoregulation in the intestine is not known. I suggest that it is by a shear stress dependent mechanism. However, the liver, which also shows shear stress dependent release of NO during vasoconstriction, does not show modulation of autoregulation by NO. The DGA technique for eliminating shear stress-dependent responses should clarify this issue.

Another "yin-yang" relationship between ADO and NO, as discussed in chapter 5, is that in the absence of NO, there is a potentiation of ADO vasodilatory properties in the intestine but not the liver. Further experiments clarifying the complex relationship between the two vasodilators can be followed with a focus on nucleotide "cross talk".

Although my studies provided further knowledge about ADO and NO regulation of vascular resistance in the intestine and liver, further work is required to clarify not only the mechanisms involved in this regulation but also its relevance in disease states. Considering the known relationships between hypoxia and inflammation and ADO and NO, it will be important to understand these roles in specific parts of the intestine including the colon and the involvement in Crohn's disease and ulcerative colitis. Because the "health" of the endothelial cells can be

related to hypertensive states, impairment in the shear stress dependent release of NO might explain some types of hypertension. Since the splanchnic bed receives about 25% of the cardiac output, the increase in resistance in this bed due to an impairment of the endothelial cells might account in a significant way for pathological conditions. Conditions of both increases and decreases in NO or ADO production in this vascular bed are anticipated to result in a wide range of vascular and metabolic perturbations in different disease states.

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