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HEMODYNAMICS AND THE REGULATION OF SPLANCHNIC OXYGEN AND GLUCOSE METABOLISM

- The role of shear stress, nitric oxide, and prostaglandins.

By Chao Han

A thesis submitted to the Faculty of Graduate Studies in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

**Department of Pharmacology and Therapeutics, Faculty of Medicine,
University of Manitoba
Winnipeg, Manitoba**

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**Hemodynamics and the Regulation of Splanchnic Oxygen and Glucose Metabolism-
The Role of Shear Stress, Nitric Oxide, and Prostaglandins**

BY

Chao Han

**A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University
of Manitoba in partial fulfillment of the requirements of the degree
of
DOCTOR OF PHILOSOPHY**

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ABSTRACT

The regulation of local metabolism is intimately related to blood flow. Vasoactive substances, such as nitric oxide (NO), prostaglandins and adenosine, are involved in the regulation of hepatic and intestinal blood flow and metabolism. These substances and other vascular factors interact. The general hypothesis of the thesis is that the functional homeostasis of an organ is achieved through the interaction of different factors at the levels of both hemodynamics and metabolism, and the regulation of local hemodynamics meets the requirement of metabolism and maintains physiological integrity. Several aspects in hemodynamics-related regulation of glucose and oxygen metabolism in the liver and intestine were addressed in this thesis using *in vivo* animal models.

My results showed that the inhibition of NO potentiated the vasoconstrictor effect of norepinephrine, and norepinephrine slightly suppressed the vasodilator effects of adenosine and isoproterenol in the hepatic artery. However, blockade of NO substantially potentiated the suppression by norepinephrine of the vasodilation, suggesting that NO strongly inhibited the effects of norepinephrine in the interaction, although NO is not involved in control of basal hepatic arterial tone.

Having shown that NO inhibited the vascular effect of norepinephrine, the influence of NO on norepinephrine-induced hepatic glycogenolysis was further investigated. NO potentiated norepinephrine-induced glycogenolysis while it inhibited the vasoconstrictor effect.

NO antagonizes adenosine vasodilator effects in the intestine. The results in the thesis further demonstrated that NO, either released endogenously by shear stress or

introduced exogenously, inhibited the metabolic effect of adenosine in the intestine, suggesting that NO antagonized adenosine also at the metabolic level.

The mechanism of the regulation of intestinal glucose utilization is not clear. It was hypothesized that the release of autacoids by elevated shear stress, secondary to increased blood flow, mediates increased intestinal glucose uptake from the blood. My experiments discovered that prostaglandin F_{2α} mediated the increase in intestinal glucose uptake when blood flow was increased.

The results in this thesis provided more evidence that the vasoactive substances interact at hemodynamic and metabolic levels, supporting the concept that the system maintains a hemodynamic and metabolic homeostasis through the interaction of these substances and factors.

1. INTRODUCTION

Normal function of any organ depends on the homeostasis of its metabolic activities. A sufficient blood supply is critical to maintain the homeostasis of local metabolism. The regulation of blood flow in an organ must meet the requirements of local metabolism, and the function of an organ serves the integrity of the whole body. It is not surprising to find that many vasoactive substances possess dual properties: vascular effects and metabolic effects. It is essential to understand the metabolic responses under different hemodynamic environments and therefore to study the interaction of these vasoactive substances at both hemodynamic and metabolic levels.

1.1 OVERVIEW OF BASIC ASPECTS IN HEPATIC AND INTESTINAL CIRCULATION

An introductory overview of hepatic and intestinal circulation and metabolism in this section will highlight the very basic aspects under the topic. The intention of this overview is to provide a brief sketch of the multiple interacting regulatory systems that are then dealt with in more detail in areas where the topic of the research is focused.

1.1.1 Anatomy and microcirculation

1.1.1.1 Anatomy of splanchnic circulation

The blood supply to the liver normally accounts for about 25% of total cardiac output, although the liver constitutes only 2.5% of body weight (Greenway and Lautt, 1989). The liver is supplied by the portal vein and hepatic artery. Of the total hepatic blood flow, about a quarter, which is fully oxygenated, supplies the liver directly through the hepatic artery. The rest of the hepatic blood flow reaches the liver through the portal vein. The common hepatic artery derives from the celiac artery, from which the splenic

and gastric arteries also branch. The gastroduodenal artery derives from the common hepatic artery at the T-junction. The superior mesenteric artery supplies the small intestines and a part of the colon. The inferior mesenteric artery supplies the colon and rectum. The blood from the mesenteric vascular bed drains into the mesenteric vein that conducts the blood to the portal vein. Smaller amount of blood from the spleen (10%), pancreas (10%) and stomach (20%) also drains into the portal vein (Greenway and Lautt, 1989). The contributions from these organs to portal blood flow vary under different physiological conditions (Greenway and Lautt, 1989).

1.1.1.2 Hepatic microcirculation

The acinus, consisting of a cluster of parenchymal cells, forms the functional vascular unit of the liver. Of any cells in any organs the hepatic parenchymal cells are most richly perfused based on blood flow to tissue mass ratio (Lautt, 1996b). The acinus varies in size and shape at the end of vascular stalks comprising the terminal branch of the portal venules, hepatic arterioles, bile ductules, lymphatics and nerve endings (Greenway and Lautt, 1989; Rappaport, 1981). Blood enters the acinus in the central region, flows outward, and drains into the terminal hepatic venule at the periphery. The acinus is divided into three zones according to the direction of blood flow and degree of oxygenation. Zone one is the first zone to be perfused and most oxygenated. This unique microvascular arrangement gives no opportunity within the hepatic structure for diffusible materials to make a shortcut of the vascular pathway, and precludes the diffusion of metabolic products from zone 3 back to the central region affecting the vascular smooth muscle (Greenway and Lautt, 1989; Lautt, 1996b). The activity of the parenchymal cells develops a large substrate gradient across the zones, resulting in the

ability to almost completely extract selected substances.

There are about 20 sequential parenchymal cells in each sinusoid that makes up a special capillary connecting the inflow vessel to the hepatic venule. In the sinusoids, individual hepatocytes may be in contact with perfusate on two sides (Gumucio and Miller, 1981; Lautt, 1996b). The hepatic endothelial cells lining the sinusoids are special cells with flattened processes perforated by small fenestrations. The fenestrae allow connection between the sinusoidal lumen and the space of Disse and filter macromolecules of differing sizes (Wisse et al., 1985). The sieve plays an important role in lipoprotein metabolism (Fraser et al., 1995). The mechanism controlling the size of the fenestrae is not clear. Some substances, including alcohol, are known to influence the size of the fenestrae (Mak and Lieber, 1984). The size of fenestrae also differs in different zones (Nopanitaya et al., 1976). Other cell types, including the Kupffer cells, stellate cells and pit cells, are also found in the sinusoids (Bouwens et al., 1992). The Kupffer cells are the macrophages, and the pit cells are the lymphocytes in the liver. The stellate cells (Ito cells), also called fat-storing cells, are now known to be important in control of sinusoidal resistance by their contraction, in extracellular matrix metabolism and in production of mediators in the liver, as well as in fat- and retinoids-storage (Kawada, 1997).

1.1.1.3 Intestinal microcirculation

Blood supplies the gastrointestinal tract through the celiac, superior mesenteric and inferior mesenteric artery. The intestinal wall is composed of three layers: muscle, submucosa and mucosa. The branches of small mesenteric arteries penetrate the longitudinal and circular muscles and give rise to arterioles to the muscular, submucosal

and mucosal layer. The arterioles in the submucosa become the main arterioles to the tip of the villi (Gore and Bohlen, 1977). These arterioles perfusing the submucosal and mucosal layers are parallel to those perfusing the muscularis, and the blood flow perfuses the submucosal and mucosal layers in series (Greenway and Murthy, 1972). The mucosal and submucosal layers account for up to 80% of the tissue mass, and the ratio of blood flow to tissue mass in the muscularis is only about one tenth of that in mucosa and submucosa (Greenway and Murthy, 1972).

The physiological functions of absorption and metabolism in the intestines draw more attention to the intestinal microvilli. Each villus resembles a miniature vascular bed with a single arteriole that enters the villus in the center, and the venule drains the blood in opposite directions, which forms a "hairpin loop" (Casley-Smith and Gannon, 1984). The vessels in the hairpin loop are innervated, and neural control of vascular constriction of the arteriole may play an important role in control of intestinal blood flow in response to different requirements (Bohlen et al., 1975).

In the hairpin loop, diffusion distance is relatively short between afferent and efferent vessels conducting blood flow in opposite directions. An arteriole-venule shunting of oxygen may exist, resulting in a descending gradient of tissue oxygen from the base of the villus to its tip (Takala, 1996). The gradient is inversely related to the blood flow. This countercurrent shunting may make the villi more susceptible to hypoxia, or the tip of the villus may normally function in hypoxic conditions. A model for this countercurrent shunting was established (Shepherd and Kiel, 1992).

1.1.2 Blood flow and metabolism

1.1.2.1 In the liver

The hepatic circulation is unique because the liver receives blood supply from both the hepatic artery and portal vein. There are two principal determinants of hepatic blood flow. The resistance in the superior mesenteric vascular bed determines the portal blood flow to the liver. The portal vein is unable to control its flow to the liver (Greenway and Stark, 1971). The intra-hepatic portal venous resistance regulates only the portal pressure, not the flow; while the hepatic arterial resistance determines the arterial blood flow (Greenway and Lautt, 1989; Takala, 1996).

The liver is highly innervated. Electrical stimulation of the hepatic nerves decreases hepatic arterial conductance markedly, with blood flow and conductance approaching zero at maximum response (Greenway and Oshiro, 1972). The vasoconstriction is sympathetic nerve-mediated (Greenway and Lautt, 1989). The vascular escape, that is the recovery of vascular tone towards its control level during continued stimulation, occurs in the hepatic artery. The mechanism of autoregulatory escape is not fully understood. Glucagon was shown to block the vascular escape in the hepatic artery in cats (Lautt et al., 1985). Nitric oxide was recently suggested to be the mediator of vascular escape in the hepatic artery (Ming et al., 1999). The stimulation of sympathetic nerves to the liver increases portal pressure (Lautt, 1996c) due to increased resistance in the liver and unchanged portal flow which is mainly controlled by the superior mesenteric artery. Sympathetic nerve stimulation increases hepatic glucose output while decreasing the vascular conductance (Lautt, 1980b). The hepatic parasympathetic nerve is very important in control of the release of hepatic insulin sensitizing substance (HIS) which enhances insulin action in the skeletal muscles (Xie and Lautt, 1996). The effect of parasympathetic nerves on the hepatic vasculature appears to be minimal or absent

(Greenway and Lautt, 1989).

Two main intrinsic mechanisms are involved in the regulation of hepatic arterial blood flow. First, pressure-flow autoregulation in the hepatic artery results in a relatively constant blood flow over a wide range of perfusion pressure (Ezzat and Lautt, 1987). Secondly, the hepatic arterial buffer response (HABR), an interaction of the hepatic artery with portal venous blood flow, plays an important role in control of hepatic arterial blood flow (Lautt, 1996a). It is interesting that these two mechanisms are both mediated by adenosine, which will be discussed in more detail in section 1.5.2.

Evidence from different sources supports that hepatic arterial conductance is not controlled by parenchymal cell metabolism (Lautt, 1977a; Lautt, 1980a). This phenomenon should be attributed to the unique arrangement of hepatic microvasculature, which precludes diffusion of vasoactive substances upstream to the arterial resistance vessels. On the other hand, blood flow to the liver may influence hepatic metabolism and functions. A brief occlusion of the hepatic artery reduced hepatic oxygen consumption (Mathie, 1997), suggesting that the hepatic arterial blood is important for hepatic oxygenation. The clearance of a number of hormones, including aldosterone, cortisol, 11-deoxycorticosterone, corticosterone, and progesterone, depends on hepatic blood flow (Messerli et al., 1977). Change in portal blood flow may influence hepatic glucose metabolism. The decrease in hepatic glycogen content after a low dose of glucose gavage (Casado et al., 1992) may be related to increased portal blood flow. A change in blood flow may change shear stress state of the hepatic endothelial cells and Kupffer cells, and increase the release of substances, such as prostaglandins, which may stimulate glycogenolysis through intercellular communication (Casteleijn et al., 1988).

1.1.2.2 In the intestine

Blood flow in the mesenteric vascular bed is controlled by intrinsic factors such as locally released vasoactive substances and extrinsic factors including sympathetic innervation and circulating hormones. In contrast to the liver, intestinal blood flow is intimately controlled by local metabolism. Pressure-flow autoregulation, reactive hyperemia, hypoxic vasodilation and functional hyperemia in denervated preparations provided evidence for the existence of local control of intestinal blood flow (Granger et al., 1980). Adenosine is involved in many mechanisms in local control of intestinal blood flow. It has been suggested to mediate pressure-flow autoregulation in the intestine (Lautt, 1986a), which was demonstrated to be attenuated by nitric oxide (Macedo and Lautt, 1996a). These will be discussed in sections 1.5.3.1 and 1.2.6.2. Hyperemia occurs in the intestine after a brief period of arterial occlusion (Granger et al., 1980). After ingestion of food, the intestinal blood flow can increase 2-3 times and remain high for the next few hours (Burns and Schenk, 1969). Many mechanisms such as local reflexes and locally released substances including bile, vasoactive intestinal peptide, histamine and other hormones had been suggested to mediate postprandial hyperemia (Gallavan and Chou, 1985). Results from recent studies suggested that adenosine mediates, at least in part, both reactive (Lautt, 1986b; Pawlik et al., 1993) and postprandial hyperemia (Jacobson and Pawlik, 1994; Sawmiller and Chou, 1992). Nitric oxide was also shown to partially mediate functional hyperemia (Alemany et al., 1997). The roles of NO and adenosine in control of intestinal blood flow will be discussed in sections 1.2.6.1 and 1.5.3.1 respectively.

Other factors may also influence intestinal blood flow, but the mechanisms are not

clear. A myogenic mechanism was earlier suggested to be one of the mechanisms controlling the resistance vessels in the intestine (Granger et al., 1980). However, experiments showed that changes in portal pressure in the physiological range did not affect superior mesenteric arterial resistance in the cat (Lautt, 1986b). Decreased oxygen tension causes dilation in the superior mesenteric artery; however, adenosine was excluded as a mediator of the dilation (Lockhart and Lautt, 1990).

Blood flow is crucial for intestinal oxygenation. The relationship between oxygen uptake and blood flow has been described (Kviety and Granger, 1982). A minimum of about 25-30ml/min/100g tissue blood flow is necessary for sufficient oxygenation. A sufficient blood supply to the intestinal mucosa is essential for mucosal protection. Local release of nitric oxide and prostaglandins play very important roles in maintaining mucosal blood flow (Wallace and Granger, 1996) (see section 1.2.6.1 and 1.4.2.1).

The intestines are highly innervated by sympathetic nerves. Stimulation of the sympathetic nerves reduces intestinal blood flow (Granger et al., 1980), but vascular escape occurs during continued stimulation (Folkow et al., 1964; Greenway, 1984). Intestinal oxygen uptake was initially decreased by nerve stimulation and returned to pre-stimulation levels during the escape plateau (Lautt and Graham, 1977) due to increased oxygen extraction ratio. Pre-capillary sphincters were suggested to regulate the oxygen uptake during the escape phase. Some mechanisms were suggested for the escape (Greenway, 1984), but adenosine is not likely to be involved in the escape in the cat (Lautt et al., 1987). Also, circulating catecholamines are important vasoactive substances that influence intestinal blood flow. The superior mesenteric arterial vascular escape from the constriction induced by norepinephrine infusion was demonstrated as well (Lautt et

al., 1987).

Thus, different mechanisms and many important vasoactive substances such as norepinephrine, adenosine, nitric oxide and prostaglandins are involved in the regulation of intestinal and hepatic vascular resistance and metabolism. In the following sections, I will discuss the role of nitric oxide, prostaglandins and adenosine separately in the regulation in intestinal and hepatic hemodynamics and metabolism. I will also discuss the influences of different hemodynamic factors on vessel wall shear stress that is closely related to the release of many autacoids.

1.2 VASCULAR AND METABOLIC RESPONSE TO NITRIC OXIDE

1.2.1 The discovery of nitric oxide in vascular tissue

Nitric oxide (NO) is a small inorganic molecule. In 1980, Furchtgott and Zawadski first demonstrated that the intact endothelial cells lining the blood vessels are essential for acetylcholine-induced vascular smooth muscle relaxation. The substance released from the endothelial cells became known as endothelium derived relaxing factor (EDRF) (Furchtgott and Zawadski, 1980). The pharmacological and chemical properties of EDRF were studied and characterized during the next several years. A large amount of chemical and biological evidence provided by two groups working independently suggested that the EDRF is NO (Ignarro et al., 1987; Palmer et al., 1987).

NO is synthesized from an amino acid, L-arginine, in a variety of mammalian cells by several isoforms of NO synthase (NOS). In the presence of NADPH and other cofactors, the reaction utilizes oxygen and yields citrulline and NO (Knowles and Moncada, 1994). Chemically, NO is very reactive. It has a half-life in diluted solution of

less than 10 seconds and the oxidized products are nitrite and nitrate. In a biological system, NO binds to oxyhemoglobin and other heme-containing proteins (Ignarro et al., 1987; Martin et al., 1986), which terminates NO's biological action rapidly; the half-life estimated is less than 3 seconds (Palmer et al., 1987).

At least three NOS isoforms have been found: the constitutive isoforms including endothelial NOS (eNOS), neuronal NOS (nNOS), and the inducible NOS (iNOS). Activation of the constitutive form of NOS is calcium/calmodulin dependent and results in release of picomoles of NO for seconds to minutes, which mainly serves in the regulation of physiological functions. On the other hand, the inducible form is stimulated in many cell types at the transcription level by cytokines and lipopolysaccharide (endotoxin), which results in release of nanomoles of NO for hours to days and serves mainly for immunological defence. The L-arginine/NO pathway and NO are important in the regulation of vascular tone (Rees et al., 1989b), neurotransmission (Gillespie et al., 1989) and host defense (Nathan and Hibbs, 1991).

1.2.2 Biological stimuli of endothelial NOS

One of the principal roles of the endothelium is the regulation of vascular tone through release of NO. Biophysical stimuli such as fluid shear stress and pulsatile stretch were suggested to be two of the main determinants for generating NO (Pohl et al., 1986; Rubanyi et al., 1986). Shear stress is a biomechanical force that acts on the blood vessel wall to modulate its functions. Shear rate is defined as force per unit area of the vessel wall. The strength of shear stress on the vessel wall can be calculated according to the property of blood flow and hemodynamic indices (Kamiya and Togawa, 1980) (for discussion in more detail, see section 1.3). Change in flow and/or physical distortion of

endothelial cells could transmit a signal through one or a combination of the following mechanisms: conducting the signal through cytoskeletal network to the nucleus, opening mechanosensitive ion channels, and altering the concentration of endothelial agonists (for review (Busse and Fleming, 1998)).

Increased intracellular calcium concentration activates NOS in the endothelial cells (Moncada et al., 1991). This calcium dependent activation of eNOS is mediated by calmodulin (Busse and Mulsch, 1990). Recently, a calcium-independent mechanism of eNOS activation by shear stress was also suggested to be through a tyrosine kinase pathway (Fleming et al., 1998).

1.2.3 The mechanism of NO action

The activated NOS uses L-arginine as a substrate and converts it to nitric oxide and citrulline. NO is a small molecule, liposoluble gas, which simply diffuses through cell membranes and stimulates the soluble form of guanylate cyclase in the adjacent smooth muscle cells. The activation of guanylate cyclase increases cellular cGMP level (Lowenstein et al., 1994), which decreases the intracellular calcium concentration and causes smooth muscle relaxation.

A recent study (Weisbrod et al., 1998) showed that the level of cGMP under different pharmacological interference did not correlate well with the intracellular calcium concentration, and suggested that NO may affect calcium regulatory mechanisms by affecting one or more ion channels or transporters which have not yet been fully defined. NO also binds to many other enzymes, especially heme-containing proteins. Thus, NO may possess the ability to modulate other enzymatic pathways. For example, NO may inhibit the activity of aconitase in Krebs cycle (Stadler et al., 1991) resulting in

suppression of mitochondrial respiration. NO may also inhibit phosphoenolpyruvate carboxykinase (Ceppi and Titheradge, 1998) resulting in an inhibition of gluconeogenesis in sepsis. However, the mechanisms of these interactions are not clear.

1.2.4 Pharmacological manipulation of NO

The synthesis of NO by NOS is through L-arginine. A number of L-arginine analogues have been synthesized as competitive inhibitors of NOS (Moncada et al., 1991). In these synthetic NOS inhibitors, the guanidino amino group has been masked or modified so that the compound binds to NOS but does not produce NO. N^G -monomethyl-L-arginine (L-NMMA) was first used as a potent NOS inhibitor (Rees et al., 1989a). N^G -nitro-L-arginine methyl ester (L-NAME) (Rees et al., 1990) is the most potent NOS inhibitor available so far and has shown to have good *in vitro* and *in vivo* correlation in its pharmacological activities (Rees et al., 1990; Wang et al., 1993). Those compounds were used successfully in a variety of studies to block NO production. An *in vitro* study has indicated that L-NAME may have anti-cholinergic activity (Buxton et al., 1993). However, this may not occur *in vivo*. A recent study (Sadri and Lautt, 1998) demonstrated that intra-portal administration of a submaximal dose of L-NAME caused insulin resistance. Intra-portal administration of L-NAME or an equimolar dose of L-NMMA caused the same degree of insulin resistance. This experiment ruled out the anti-cholinergic effect of L-NAME.

By a competitive mechanism, L-arginine can be used to reverse NOS blockade by L-arginine analogues in many situations (Rees et al., 1989a; Wang et al., 1993). However, caution should be used, especially for *in vivo* study, because L-arginine, as an amino acid, is involved in quite a few biochemical pathways besides the production of

NO (Wu and Morris, 1998). The amino acid may stimulate the secretion of a number of different circulating hormones including insulin and glucagon (Giugliano et al., 1997).

Many NO donors such as sodium nitroprusside, nitroglycerin, and 3-morpholinosydnonimine (SIN-1) can be used to increase NO level or reverse NOS blockade. Other compounds such as methylene blue, a guanylate cyclase inhibitor (Zhang et al., 1997), can also be used to provide more information about cGMP, which is the second messenger of NO function.

1.2.5 The function of NO in the liver

1.2.5.1 NO in control of hepatic vascular tone

In addition to the NO released from endothelial cells, NO is generated in the liver from many other cells, including the hepatocytes (Zhang et al., 1997), and other nonparenchymal cells such as the Kupffer cells and stellate cells in physiological and pathological conditions. However, the contribution of NO to the regulation of hepatic vascular tone is not well understood.

The vasodilator effect of NO in the hepatic circulation has been demonstrated in different studies. The NO donor SIN-1 and L-arginine increased flow in a perfused rat liver model (Zhang et al., 1997). In another double perfused (through both hepatic artery and portal vein) rabbit liver model, acetylcholine but not sodium nitroprusside and adenosine-induced vasodilation in the hepatic artery was blocked by NOS inhibitors (Mathie et al., 1991), providing further evidence for the vasodilator effects of NO either released endogenously or provided exogenously.

However, NO seems not likely to be involved in control of hepatic vascular tone in the basal condition. Macedo and Lautt (1998 and 1997) showed in *in vivo* studies that the

blockade of NOS by L-NAME did not affect the basal vascular tone in the hepatic artery and portal vein in anesthetized cats. The results suggested that NO did not play a significant role in control of basal hepatic vascular tension in intact animals. Another *in vivo* study in pigs (Ayuse et al., 1995) confirmed that the blockade of NOS by L-NAME did not alter portal resistance, suggesting that NO was not involved in control of basal tone in the portal system. The hepatic arterial resistance, in the same study, was increased by L-NAME. However, the hepatic innervation was not disturbed in Ayuse's study (1995), which is different from Macedo's studies (1997 and 1998) where the anterior hepatic plexus was denervated. Since the administration of a NOS inhibitor could suppress the inhibition of central sympathetic outflow by NO (Owlya et al., 1997) and potentiate local responses to sympathetic stimulation, the increase in hepatic arterial resistance after NOS inhibition could result from potentiated sympathetic effects.

Some *in vitro* studies showed controversial results on the influence of NO on the basal portal vascular resistance. Intra-portal infusion of N^ω-nitro-L-arginine increased portal perfusion pressure in a perfused rat liver model (Mittal et al., 1994). In another double perfused (through both hepatic artery and portal vein) rat liver model, L-NAME had no effect on basal portal vascular tone (Mathie et al., 1996). The concern was raised about the normality of the portal perfused *in vitro* model. Besides, the perfusion rate used in Mittal's model was higher than that *in vivo* and in Mathie's study; the high perfusion rate might cause elevated shear stress during the experiment.

1.2.5.2 NO suppresses the hepatic vasoconstriction response

The endothelium-dependent regulation of vascular response to sympathetic stimulation has been reported in many different organs and tissues (Cohen and Weisbrod,

1988; Greenberg et al., 1990; Tesfamariam and Cohen, 1988). In a study in isolated rat-tail artery (Thorin and Atkinson, 1994), L-NAME and methylene blue blocked the endothelium-dependent inhibition of norepinephrine release suggesting a prejunctional inhibition by NO. Recently, Macedo and Lautt (1998) showed that NO inhibited the vasoconstriction effect of sympathetic stimulation, and the inhibition occurred only when shear stress was allowed to increase in response to the vasoconstriction. The inhibition was through a post-synaptic mechanism since shear stress-induced release of NO inhibited both norepinephrine infusion- and sympathetic nerve stimulation-induced vasoconstriction. All these data suggest that in addition to the vasodilator effect, NO is also involved in the regulation of vascular response to other vasoactive substances, such as norepinephrine, in the liver.

1.2.5.3 NO in control of hepatic metabolism

1.2.5.3.1 Energy metabolism. NO has been reported to inhibit the activity of aconitase (Stadler et al., 1991), nicotinamide adenine dinucleotide (NADH) ubiquinone oxidoreductase, and succinate ubiquinone oxidoreductase (Kurose et al., 1993) in cultured hepatocytes and perfused liver. The outcome of the inhibition by NO is inhibition of mitochondrial respiration resulting in decreased oxygen consumption in the hepatocytes as shown in Stadler's study. However, little *in vivo* data are available to elucidate the function of NO in hepatic oxygen consumption and basal hepatic energy metabolism.

1.2.5.3.2 Glucose metabolism. One of the important functions of the liver is its ability to store glucose, which is often utilized when glucose is needed to maintain systemic homeostasis. However, the role of NO in regulation of basal glucose output in

normal and pathological conditions is not clear. Change in basal glucose level by NO may be used to assess basal hepatic glucose output to some extent. The results, however, were controversial. An increase in arterial glucose level in normal and hypertensive rats after chronic administration of L-NAME was observed (Tong et al., 1997). In another study, basal glucose level was not found to be altered after L-NAME (Bursztyn et al., 1997). NO was also found to decrease the activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an enzyme crucial for both glycogenolysis and gluconeogenesis (Dimmeler and Lottspeich, 1992). NO activated glycogen phosphorylase to enhance glycogenolysis (Borgs et al., 1996), and inhibited glycogen synthesis by inhibition of the conversion of glycogen synthase *b* to glycogen synthase *a* (Sprangers et al., 1998). The NO effects may be concentration related (Sprangers et al., 1998) since the activation of glycogen phosphorylase happens at higher concentration.

Xie and Lautt (1996) showed the important role of the liver to control skeletal muscle response to insulin through the release of hepatic insulin sensitizing substance (HISS). Disruption of parasympathetic nerves in the liver caused insulin resistance. Sadri and Lautt (1998) demonstrated that the blockade of NO in the liver caused insulin resistance. The relation between NO pathway and cholinergic pathway is currently not clear.

One of the important functions of norepinephrine or sympathetic stimulation in the liver is to stimulate glycogenolysis and gluconeogenesis, which increases hepatic glucose output in response to stress. The inhibition of vasoconstriction, in response to sympathetic nerve stimulation or norepinephrine infusion, by shear stress-induced release of NO (Macedo and Lautt, 1998) was discussed in the previous section. Whether NO

inhibits norepinephrine-induced increase in hepatic glucose output is not clear. The formation of NO had no major influence on the glycogenolytic effect of norepinephrine but inhibited the vasoconstrictor effect of norepinephrine in a study using perfused rat liver (Weidenbach et al., 1997). Other *in vitro* studies using isolated perfused liver also showed that NO had no significant influence on phenylephrine-, an α_1 -agonist, induced increase in glucose output (Moy et al., 1991). However, there are little *in vivo* data available so far. As previously mentioned (section 1.2.5.1), the perfused liver model might not provide all the factors required for normal physiological functions, for example, normal vascular and metabolic response. A degradation of hepatic glycogen content, for example, was found following the preparation of the isolated liver model (Glinsmann et al., 1969). This may explain why NO did increase glucose output in another study, in which glycogen stores were preserved by glucose preloading (Borgs et al., 1996). More experiments, especially *in vivo* preparations that can mimic normal physiological situations more closely, are certainly needed for a better understanding of the function of NO in hepatic glucose metabolism.

1.2.6 The function of NO in the intestine

1.2.6.1 NO in control of intestinal blood flow

NO was suggested to be a major determinant of mesenteric vascular tone and perfusion of the gastrointestinal mucosa (Salzman, 1995). Blockade of NO release by N-nitro-L-arginine in a resting condition decreased intestinal blood flow in rat (Pawlik et al., 1995). In several *in vivo* studies in cats, the administration of L-NAME decreased basal conductance in the superior mesenteric artery (Macedo and Lautt, 1996a; Macedo and Lautt, 1997), but not in the hepatic artery (Macedo and Lautt, 1997). In a fast-feed

pig model, N^G -monomethyl-L-arginine decreased superior mesenteric arterial blood flow in sham-fed animals, after which the postprandial hyperemic response was diminished (Alemany et al., 1997). In a study in rat ileum, local application of L-NAME decreased resting blood flow by half (Bohlen and Lash, 1996), suggesting a dilator effect of NO on basal vascular tone. These results support that NO released in basal condition is involved in control of basal intestinal blood flow in different models and different species. Since the resistance increased more in distribution arterioles than in the terminal vessels and shear rate increased in the large arterioles after L-NAME in that study, the authors suggested that dilation of the large resistance vessels was caused by flow-mediated release of NO. Another study also demonstrated that NOS blockade by N^G -monomethyl-L-arginine decreased resting diameter of first and second-order arterioles in the intestine (Nase and Boegehold, 1996), which further supported that NO released in basal condition controls intestinal blood flow through dilation of intestinal large arterioles.

NO does not only have influence on the resting vascular tone in the intestine. Intraluminal administration of oleic acid doubled jejunal blood flow, while the blockade of NO release by N^G -nitro-L-arginine attenuated this increase and L-arginine reversed the attenuation (Pawlik et al., 1995). In another study in pig, the increase in superior mesenteric arterial blood flow induced by milk or water-feeding was diminished by the inhibition on NOS with N^G -monomethyl-L-arginine (Alemany et al., 1997). These data suggested that NO participates, at least partially, in mediating functional hyperemia in the intestines. NO is also known to increase mucosal blood flow, inhibit acid secretion and promote mucus secretion, thereby, indicating that NO is important in gastrointestinal mucosal defence (Wallace and Granger, 1996).

1.2.6.2 Interaction of NO with other vasoactive substances

In the intestine, the importance of NO is also evidenced by its ability to regulate the vascular response to other vasoactive substances in different hemodynamic conditions. NO inhibited sympathetic nerve stimulation-induced vasoconstriction in the superior mesenteric vascular bed (Macedo and Lautt, 1996b). In this study, the NOS inhibitor potentiated nerve stimulation-induced vasoconstriction only when shear stress was allowed to increase, suggesting that the inhibition of vasoconstriction by NO is shear stress dependent. Furthermore, blockade of NOS potentiated only nerve stimulation-induced, but not norepinephrine infusion-induced vasoconstriction, suggesting that the modulation of sympathetic activity by NO was through a pre-synaptic mechanism in the intestine. Nase et al. showed that local administration of NO inhibitor N^G-monomethyl-L-arginine increased the magnitude of sympathetic nerve stimulation-induced vasoconstriction in the intestine in rats (Nase and Boegehold, 1996). Potentiation of the magnitude of sympathetic constriction by the NOS inhibitor was demonstrated in small feed arteries and first order arterioles, and in second order arterioles only at strong stimulation, suggesting that the sites of modulation are small arteries and large arterioles.

More interestingly, NO did not only inhibit the vasoconstrictor effect of sympathetic nerve stimulation, it also antagonized vasodilator effects in the superior mesenteric artery (Macedo and Lautt, 1997). Macedo and Lautt showed that blockade of NO synthesis using L-NAME potentiated the vasodilator effect of adenosine and isoproterenol in the intestine; the potentiation seems not to be shear stress-dependent since the shear rate was not significantly changed in the experiment. In another study, a potentiation of pressure-flow autoregulation in the superior mesenteric artery by NOS

blockade was observed (Macedo and Lautt, 1996a). The autoregulation index was greater in the presence of L-NAME compared to control, and the potentiation by L-NAME was reversed by L-arginine. This observation is supported by the results from a previous study that NO antagonizes the vasodilator effect of adenosine. An earlier study (Lautt, 1986a) showed that pressure-flow autoregulation in the superior mesenteric artery could be blocked by adenosine antagonism, suggesting that adenosine mediated the autoregulation in the superior mesenteric artery.

1.2.6.3 Role of NO in intestinal motility

Normal intestinal peristalsis is essential for the provision of enteral nutrition, gastroenteric decomposition and parenteral hyperalimentation. It is now clear that the release of NO from nonadrenergic noncholinergic neurons plays a critical role in the coordinated propagation of gut contents and relaxation (Miller et al., 1993; Salzman, 1995). The spontaneous nonadrenergic noncholinergic jejunal motility was increased markedly by NOS inhibition with N^ω-nitro-L-arginine as demonstrated in a cat study (Gustafsson and Delbro, 1993). The inhibitory effect of NO on intestinal motility was shown in dogs (Alemayehu et al., 1994). In this study, L-NAME increased jejunal motility while decreasing blood flow and increasing oxygen uptake. A similar effect of L-NAME was also demonstrated in isolated dog ileal segments (Daniel et al., 1994). In this study, the removal of calcium from perfusate or addition of N-type calcium channel blocker reduced the response, and therefore the authors suggested that a calcium-dependent tonic release of NO inhibits intestinal motility. The authors also showed that NO inhibited the release of excitatory mediators such as vasoactive intestinal polypeptide (VIP).

1.2.6.4 Role of NO in intestinal metabolism

The critical roles of NO in control of intestinal blood flow and smooth muscle activity have been discussed. I also mentioned (in section 1.2.3 and 1.2.5.3) that NO might interact with mitochondrial enzymes to inhibit the energy metabolism. Based on these facts, NO may be expected to influence intestinal metabolism. However, relatively few data are available and there are many controversies in existing data. Alemayehu et al. (1994) demonstrated that the administration of L-NAME increased jejunal oxygen consumption while increasing intestinal motility in dogs. Another study in rat ileum (Bohlen and Lash, 1996) showed that L-NAME did not change oxygen consumption while the inhibitor increased vascular resistance in large arterioles. In the same study, isosmotic replacement of sodium chloride with mannitol decreased blood flow and oxygen consumption. These results suggested that under basal conditions NO is released mainly in the large arterioles and had no influence on intestinal metabolism. More data are necessary to elucidate if there is any direct effect of NO on intestinal metabolism. I have discussed the interaction of NO with other vasoactive substances, such as adenosine, in control of intestinal vascular tone. Whether the interaction happens at the metabolic level also needs to be addressed.

1.2.7 Organ specificity of NO function

NO is important in control of vascular resistance and may play a role in control of local metabolism. In addition to its direct effect on vascular smooth muscle, NO also interacts with other vasoactive substances such as norepinephrine and adenosine (see the discussions in section 1.2.6.2). The subtle differences of NO action in different organs have attracted attention from NO researchers. In an *in vitro* study, acetylcholine caused

relaxation in porcine aortic tissues but not coronary arteries (Shepherd and Vanhoutte, 1991). In the splanchnic vascular system, NO is involved in control of basal vascular resistance in the intestine but not in the liver (Macedo and Lautt, 1997). NO antagonizes vasodilation induced by adenosine and isoproterenol in the intestine but again, not in the liver (Macedo and Lautt, 1997). NO antagonizes the vasoconstriction through a post-synaptic mechanism in the liver while through a pre-synaptic mechanism in the intestine (Macedo and Lautt, 1998; Macedo and Lautt, 1996b). At this point, the biological and physiological implication of organ specificity of NO action is not fully clear. I believe that the biological and physiological importance of organ specificity of NO action will be clarified as more data are accumulated in future studies.

1.3 SHEAR STRESS, THE BIOMECHANICAL STIMULUS OF NO AND OTHER AUTACOID RELEASE

I have discussed that shear stress is one of the main stimuli of endothelium derived NO. It seems necessary to define shear stress and how to estimate the magnitude of shear stress before further discussion of the effect of shear stress on the release of NO and other autacoid release from the endothelium. To understand the factors that may influence shear stress, it is essential to assess the production of NO and other autacoids under different hemodynamic conditions.

1.3.1 Definition of blood vessel wall shear stress

When fluid flows inside the blood vessels, the flow (both laminar and turbulent flow) generates a physical force or a mechanical distortion, which tends to shear the inner layer of the vessel off its base in the direction of flow. This biomechanical stress acting on the endothelial cells that line the blood vessels to modulate their function and activity,

is called shear stress. The hemodynamic forces generating the stress also include hydrostatic pressure, cyclic strain and stretch of the vessel. Shear stress is defined as force per unit area of vessel wall.

1.3.2 Estimation of blood vessel wall shear stress

The shear stress on the blood vessel wall can be estimated by wall shear rate (γ) (Kamiya and Togawa, 1980) at the inner surface of the vessel:

$$\gamma = (m+2) \cdot Q / (\pi \cdot r^3) \quad (1)$$

and the wall shear stress (τ) is given by:

$$\tau = \eta \cdot \gamma \quad \text{or} \quad \tau = \eta \cdot (m+2) \cdot Q / (\pi \cdot r^3) \quad (2)$$

Where, η is the viscosity of blood, Q is total blood flow and r is the internal radius of the vessel; m is a constant indicating the property of the flow; with laminar flow $m=2$, and with turbulent flow the value would be greater than 2. It should be noted that this calculation is based on ideal fluid dynamics; in other words, the fluid should be a true solution, the vessel should be a length of rigid tubing.

Using equation (2), one can analyze the change of shear stress in a condition, such as vasoconstriction under constant flow rate, where vasoconstrictor-induced decrease in the radius of the blood vessel will increase shear stress. However, the answer will not be so obvious when the flow and the radius of the vessel change together or the change of radius is not directly assessable. In fact, in most *in vivo* hemodynamic studies, the indices most easily obtained are changes in flows and pressures. For a general purpose, the calculation of shear stress can be rearranged according to Poiseuille's law (Berne and Levy, 1994):

$$Q = \pi \cdot \Delta P \cdot r^4 / (8 \cdot \eta \cdot l) \quad (3)$$

Where, ΔP is the pressure gradient when blood flows through an ideal tubing with length of l . Rearrange Poiseuille's law as follows:

$$r = [(8 \cdot \eta \cdot l \cdot Q) / (\pi \cdot \Delta P)]^{1/4} \quad (4)$$

Replace r in equation (2) to obtain:

$$\tau = \eta \cdot (m+2) \cdot Q / \{\pi \cdot [(8 \cdot \eta \cdot l \cdot Q) / (\pi \cdot \Delta P)]^{3/4}\} \quad (5)$$

$$\text{or simplify to } \tau = C \cdot Q^{1/4} \cdot \Delta P^{3/4} \quad (6)$$

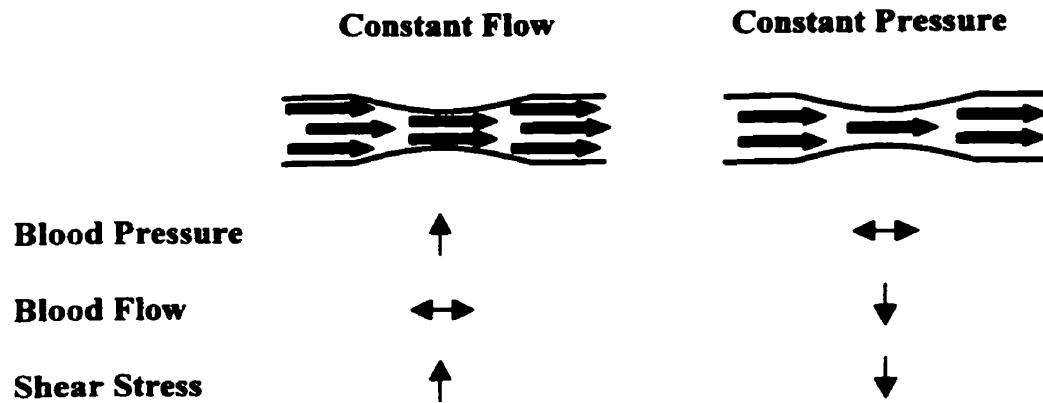
Where, C is a constant of the property of flow, viscosity of blood and length of the vessel are assumed not to change significantly under physiological conditions. Here, it is clear that if pressure is held constant, shear stress will be proportionally related to a quarter power of blood flow. On the other side of the coin, if flow is held constant, shear stress will be proportionally related to three quarter power of pressure gradient. Experimental results proved this conceptual analysis of wall shear stress. Either vasoconstriction or increased flow increased the production of NO and PGI₂ from the endothelium of rabbit femoral arteries (Hecker et al., 1993). It should be pointed out that the estimation of change in shear stress obtained from equation (6) is calculated under the assumption of a parabolic flow profile, a situation never fully true *in vivo*. In addition, a) the blood is not a true solution; b) the blood vessel is compliant under physiological conditions; c) there are many branches and bifurcations along the vascular tree; and d) the vessel diameter gradually decreases and vasoconstriction or vasodilation-induced change in diameter is not identical along the vessel. Buess and Fleming (1998) pointed out that the shear stress value calculated according to Poiseuille's law is under-estimating the actual values *in situ* because of the reasons discussed above.

1.3.3 Change in shear stress in the vascular bed *in vivo*

Based on the analysis in the previous section, blood vessel wall shear stress changes when blood flow or pressure changes. When sympathetic nerve stimulation or infusion of vasoconstrictors induces vasoconstriction, the vascular resistance increases, which results in an increase in pressure and/or a decrease in blood flow. If the perfusion pressure is maintained, blood flow decreases. Since increased resistance is balanced by the decrease in flow at the site of constriction, shear stress is not changed at this site. Shear stress at other sites, however, is decreased due to decreased flow. On the other hand, if the flow is maintained, perfusion pressure will increase due to increased vascular resistance, and shear stress is increased. These approaches were used by Macedo and Lautt (1996b and 1998) to manipulate shear stress and the production of NO successfully. Under a constant flow condition, vasoconstriction increased perfusion pressure and shear stress, as well as the production of NO. Under constant pressure condition, the results showed that shear stress and the production of NO were not changed. The changes in shear stress under different conditions are illustrated in Figure 1-1.

Use of vasodilators can increase shear stress as well, but the circumstance would be different during vasodilation. In this case, the flow increases in response to the vasodilation when perfusion pressure is held constant, and wall shear stress increases. If blood flow does not change and perfusion pressure drops due to vasodilation, shear stress decreases. Changes in shear stress during vasodilation are also illustrated in Figure 1-1. If perfusion pressure does not change, blood flow increases in response to vasodilation, and shear stress increases due to the increase in blood flow. The site at which shear stress increases the most is not likely to be the site with most dilation; instead, it is more likely to be the site without or with less dilation, for example, the capillaries that have no

Vasoconstriction



Vasodilation

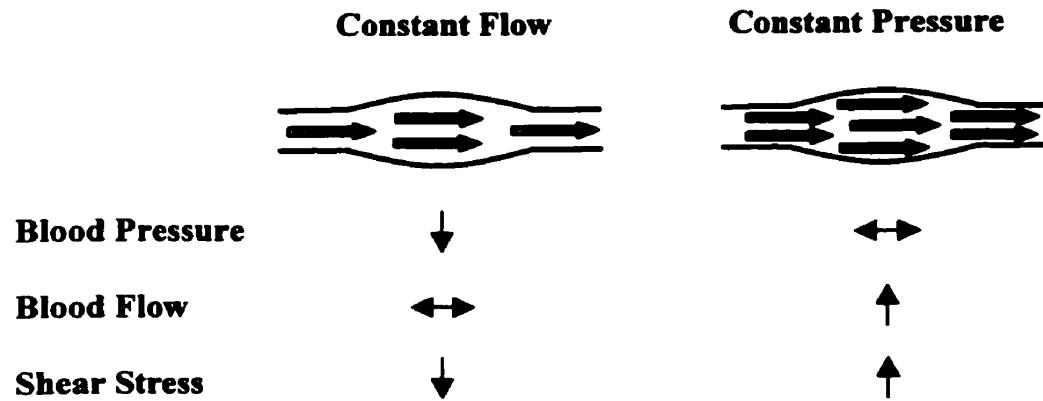


Figure 1-1. Shear stress in different hemodynamic conditions.

→ Blood flow.

smooth muscle to respond. The NO released in the capillary may have less effect on smooth muscle; nevertheless, NO released there has the nearest access to the metabolic site. It is important to study the change in shear stress and the site of shear stress under different hemodynamic circumstances for interpreting related experimental data.

1.4 PHYSIOLOGY OF PROSTAGLANDINS IN HEPATIC AND INTESTINAL CIRCULATION AND METABOLISM

A special family of substances related to the metabolism of lipid acids, named prostaglandins, contribute to the modulation of almost every biological function in the body. Discovery of prostaglandins and the research on its biological functions have much longer history than that of NO. As early as 1930, the first observation was made by Kurzok and Lieb (1930), who found that human uterus strips relax or contract when exposed to human semen. The finding of shear stress-induced release of prostaglandins from endothelial cells expanded the area (Busse and Fleming, 1998). In this thesis, I will focus on the function of prostaglandins in hepatic and intestinal metabolism and the possible interaction of prostaglandin and NO in the liver and intestine.

1.4.1 Biosynthesis and metabolism of prostaglandins

Three naturally occurring eicosapolyenoic acids, namely trienoic acid, arachidonic acid and pentaenoic acid, can be the precursors of prostaglandins, thromboxane and leukotrienes (Dyerberg et al., 1978). Arachidonic acid, which exists ubiquitously in the human body as a component of phospholipids of cell membrane and other complex lipids, is the most common precursor of prostaglandins. The hydrolysis of arachidonic phosphate ester provides the first rate-limiting step in prostaglandin formation. A variety of physical, chemical and neurohormonal stimuli may activate the enzyme, phospholipase

A_2 , which hydrolyzes the ester and free arachidonic acid. Once released, arachidonic acid can follow two pathways: the cyclooxygenase pathway leading to the formation of prostaglandins and thromboxanes (Vane et al., 1998), or the lipoxygenase pathway produces leukotrienes and other hydroxyl acids.

The first product of the cyclooxygenase pathway is an unstable cyclic endoperoxide, PGG_2 , which can be further converted (spontaneously or by peroxidase) to PGH_2 . PGH_2 is the intermediate for PGD_2 , PGE_2 , $PGF_{2\alpha}$, PGI_2 and thromboxanes (Konturek and Pawlik, 1986).

The cascade leading to the release and metabolism of arachidonic acid from cell membranes can be initiated by many factors such as nerve stimulation, neurotransmitters, neuropeptides, bradykinin, and mechanical strain of the cell (Gimbrone and Alexander, 1975; McGiff et al., 1972; McGiff et al., 1976; Ramwell et al., 1966). Shear stress or pulsating flow is also a stimulus to cells to release prostaglandins (Klein-Unlend et al., 1996). Fluid shear stress can increase the production of prostaglandins in vascular endothelial cells (Davies and Hagen, 1993), smooth muscle cells (Alshihabi et al., 1996) and many other cell types (Ajubi et al., 1996; Smalt et al., 1998). The mechanism of shear stress induced release of prostaglandins may relate to the increase in intracellular Ca^{2+} concentration (Carter and Pearson, 1992) and calcium-dependent activation of phospholipase A_2 (Chang et al., 1987). The prostaglandin released from endothelial cells is mainly PGI_2 (Schrör, 1985). Release of other prostaglandins such as PGE_2 and $PGF_{2\alpha}$ is also found in endothelial cells (Charo et al., 1984).

Prostaglandins are rapidly degraded to inactive oxidized products by 15-hydroxy-PG-dehydrogenase in biological systems. This enzyme is mainly distributed in the lung,

which results in about 95% of PGE and PGF series, but not PGI, being destroyed during a single passage through the lung. PGI_2 can pass unchanged from the venous to the arterial circulation. However, all prostaglandins, including PGI_2 , are inactivated in gastrointestinal mucosa and in the liver when they pass through the portal circulation. The biosynthesis and metabolism of prostaglandins were reviewed by Vane et al. (1998) and Konturek and Pawlik (1986).

1.4.2 Functions of prostaglandins in splanchnic circulation and metabolism

1.4.2.1 Vascular response to prostaglandins

Individual prostaglandins have various effects on the vasculature, which may change from tissue to tissue and species to species. PGE_2 and PGI_2 are generally believed to be vasodilators in most vascular beds, including coronary, renal and mesenteric vascular systems (Konturek and Pawlik, 1986). However, the results from different studies are quite inconsistent and dependent on different species and preparations. Bolus injection of PGE_2 in both renal and mesenteric vascular beds of the dog caused vasodilation (Chapnick et al., 1978; Feigen et al., 1977), while direct administration of this prostaglandin into rat kidney produced vasoconstriction (Gerber and Nies, 1979). PGE_2 was also found to be a vasodilator (Boroyan, 1976) or a vasoconstrictor (Karmazyn and Dhalla, 1982) in the coronary vascular beds. Prostacyclin, PGI_2 , causes relaxation of coronary artery strips from human (Davis and Grinsburg, 1980), dog (Palik et al., 1982) cat (Ogletree et al., 1978), but not that from pig (Dusting et al., 1977).

1.4.2.1.1 In the intestine. In the mesenteric arterial vascular bed, the evidence for the vasodilator effects of PGE_2 and PGI_2 is solid and consistent in most species, including the dog (Chapnick et al., 1978), pig (Houvenaghel et al., 1979), cat (Smith III et al.,

1978) and human (Hassan and Pickles, 1983). Chapnick et al. (1978) demonstrated that the intra-arterial infusion of PGE₂ or PGI₂ decreased mesenteric vascular resistance in the dog. In the study, the vasodilation induced by PGI₂ was maintained for the duration of the infusion, while that of PGE₂ lasted for only 4-6 minutes in the mesenteric vascular bed. These data suggested that these two vasodilator prostaglandins might play different roles in the regulation of blood flow.

PGI₂ increases mucosal blood flow, and PGE₂ inhibits acid secretion in the stomach. They both are protective against gastric mucosal injury (Wallace and Granger, 1996).

By contrast to the vasodilator effects of PGE₂ and PGI₂, the data for the vasoconstrictor effect of PGF_{2α} are more consistent. This prostaglandin contracted isolated coronary arteries (Ogletree et al., 1978; Palik et al., 1982), increased blood pressure in the dog (Ducharme et al., 1968), and acted as a potent vasoconstrictor in the mesenteric vascular bed in the dog (Chapnick et al., 1978) and cat (Lippton et al., 1987). However, the vascular effect of PGF_{2α} may be concentration dependent. Infusion of lower doses decreased mesenteric vascular resistance, whereas higher doses produced an increase in the mesenteric vascular resistance (Houvenaghel et al., 1979).

1.4.2.1.2 In the liver. It is known that the Kupffer cells (Birmelin and Decker, 1984) and endothelial cells (Eyhorn et al., 1988; Tomasi et al., 1978) in the liver are capable of producing prostanoids. Zymosan, the lipids from yeast membrane, and arachidonic acid caused vasoconstriction in perfused rat liver (Dieter et al., 1987). The vasoconstrictor effects of these two lipids were blocked by indomethacin suggesting the involvement of prostaglandin(s) in control of hepatic circulation, and suggested that the prostaglandin(s)

produced in the presence of the lipids in the liver might be vasoconstrictor(s). In a perfused rat liver model (Iwai et al., 1988), infusion of PGF_{2α}, PGE₂, and PGD₂ all reduced portal flow. The potency of PGF_{2α} is comparable with 20 Hz prevascular nerve stimulation. PGF_{2α} showed a similar effect in another study using perfused rat liver (Weidenbach et al., 1995), where the addition of this prostaglandin decreased portal flow and bile secretion.

1.4.2.1.3 Modulation of adrenergic activity. In addition to the direct action of prostaglandins on vascular smooth muscle, these autacoids may modulate adrenergic nervous activities and vascular response to the nerve stimulation or norepinephrine infusion. Malik et al. demonstrated that E series prostaglandins reduced the vasoconstrictor response to sympathetic nerve stimulation in rabbit kidney at concentrations not high enough to cause direct vascular actions (Malik and McGiff, 1975). The response to injected norepinephrine was unaffected in the same study, suggesting a presynaptically inhibitory mechanism. However, in rat kidney, PGE₂ augmented the vasoconstrictor responses to sympathetic nerve stimulation to a greater degree than did injected norepinephrine, suggesting that the prostaglandin may also have a facilitator effect. PGF_{1α} and PGF_{2α} were also reported to potentiate the response to sympathetic nerve stimulation in various tissues in the dog (Brody and Kadowitz, 1974). The modulation of sympathetic nervous system by prostaglandins seems to be tissue and species dependent. In a perfused rat liver, sympathetic nerve stimulation, norepinephrine and ATP-induced decrease in perfusion flow were reduced by a phospholipase A₂ inhibitor and a cyclooxygenase inhibitor (Iwai and Jungermann, 1987), indicating that prostaglandin(s) may mediate or modulate constrictor effects in the liver.

1.4.2.2 Prostaglandins and glucose metabolism

A full appreciation of the roles of prostaglandins in maintaining glucose homeostasis requires understanding of the influences of these compounds on glucose production, insulin and glucagon secretion, and glucose absorption and utilization. Prostaglandins were reported to have influence on insulin (Johnson et al., 1973; Pek et al., 1975) and glucagon (Pek et al., 1975) secretions. A PG_{I₂} analogue, iloprost, increased whole body and skeletal muscle glucose uptake in response to insulin (Paolisso et al., 1995), suggesting the prostaglandin may regulate the response to insulin. The influences of prostaglandins on glucose homeostasis have been reviewed in many different aspects by Robertson (1983). However, in this thesis, I would like to concentrate only on the influence of prostaglandins on hepatic and intestinal glucose metabolism.

1.4.2.2.1 Glucose metabolism in the liver. Glinsmann demonstrated the intrinsic capability of the liver to regulate glucose output (Glinsmann et al., 1969), and cAMP was suggested to mediate the hormonal control of hepatic glucose mobilization. In a model using perfused rat liver, Iwai et al. (1988) showed that PGD₂, PGE₂, PGF_{2α} and a thromboxane A₂ analogue were able to enhance glucose and lactate release from the liver. The PGF_{2α}-induced increase in glucose output mimicked that by sympathetic nerve stimulation in dynamics and kinetics. With this evidence, the authors suggested that the prostaglandin was the mediator of sympathetic hepatic nerve-induced change in hepatic glucose production. Another study confirmed the effect of PGF_{2α} on hepatic glucose production in a similar model (Weidenbach et al., 1995). The results that both phospholipase A₂ inhibitor and cyclooxygenase inhibitor blocked nerve stimulation-induced increase, and cyclooxygenase inhibitor blocked arachidonic acid-induced

increase in hepatic glucose output (Iwai and Jungermann, 1987) supported the theory that prostaglandins partially mediate or modulate the sympathetic effects on hepatic glucose metabolism. That the metabolic effects of norepinephrine were not affected while the hemodynamic actions were clearly reduced by indomethacin in Iwai's study may suggest a pre-synaptic modulation of the metabolic effects and post-synaptic modulation of the vascular effects. However, the norepinephrine outflow was not altered by indomethacin in the study.

Since prostanoids are synthesized only in non-parenchymal cells, the control of metabolism appears to depend on complex intra-organ and intercellular communications. Endotoxin increased PGD₂ production from the Kupffer cells and increased glycogenolysis in perfused liver, but endotoxin did not influence glycogenolysis in isolated parenchymal cells (Casteleijn et al., 1988). These results provided evidence for the cell-cell interaction in prostanoids-mediated communication in control of hepatic glucose metabolism.

1.4.2.2.2 In the intestine. The role of prostaglandins in control of mesenteric blood flow was discussed in a previous section (section 1.4.2.1). However, there are few data available on prostaglandins in control of intestinal glucose metabolism. Gallavan and Chou (1985) noted that prostaglandins attenuate intestinal vascular and metabolic response to food ingestion. Early studies showed that PGE₂ and F₂ may inhibit glucose absorption by the intestine (Balint et al., 1979; Coupar and McColl, 1972). Overall, the mechanisms for control and regulation of intestinal glucose uptake and utilization is not clear, and this area has not yet been well studied.

1.4.3 NO and prostanoids

NO and prostaglandins are both found to play important roles in control of hepatic and intestinal blood flow and metabolism. It is interesting to note a few facts that highlight the relationship of NO and prostaglandins. NO and prostaglandins are synthesized in many common cell types, for example, the Kupffer cells and endothelial cells. Both NO and prostaglandins are not stored but are synthesized as required. Shear stress is a stimulus for the release of NO and prostaglandins (Davies and Hagen, 1993; Small et al., 1998), and the activations of both phospholipase A₂ (Chang et al., 1987) and eNOS (Moncada et al., 1991) are calcium dependent. Henrion et al. (1997) observed, in the mesenteric artery isolated from rats with chronic inhibition of NOS, an overproduction of vasodilator prostaglandins, which compensated the decrease in flow-induced vasodilation. Another study (Koller et al., 1993) demonstrated that L-arginine analogues blocked the production of NO and vasodilator prostaglandin(s) from the endothelial cells but had less influence on the direct effect of PGE₂ on the smooth muscle cells. NO and prostaglandin(s) function synergistically to prevent hepatic damage in a rat endotoxemia model (Harbrecht et al., 1994). In the model, the blockade of cyclooxygenase alone had no effect on the LPS-induced hepatic injury; whereas, the additional blockade of NO production caused more severe hepatic damage than NO blockade alone. The biosynthesis of prostaglandins appears to be modulated by the L-arginine/NO pathway; and on the other hand, prostaglandins may regulate NO production in a variety of cell types too. The possible interactions between NO and cyclooxygenase were reviewed by several researchers (Kaley and Koller, 1995; Rosa et al., 1996).

1.5 ADENOSINE, ANOTHER IMPORTANT ENDOGENOUS SUBSTANCE

1.5.1 Introduction

Adenosine is an endogenous adenine nucleoside, which was first discovered about seventy years ago by Drury and Szent-Gyorgyi (1929). Interest in the vasodilator effect of adenosine was really expanded by the suggestion that adenosine was the mediator linking cardiac metabolism and coronary vascular tone (Berne, 1963). Adenosine is involved in the metabolic regulation of blood flow in the heart, brain (Collis, 1989) and skeletal muscle (Lash, 1996). As a metabolic product, adenosine has influence on the metabolic state of the tissues. It has been called a "retaliatory metabolite". The protective role of this nucleoside is supported by that adenosine can form from extracellular ATP and other sources (Newby et al., 1990), and that adenosine can decrease oxygen consumption and increase oxygen supply (through vasodilation) (Belardinelli and Shryock, 1992) to help balance the ratio of oxygen demand to supply.

1.5.1.1 Biological formation of adenosine

Since all cells can use free energy derived from the catabolism of adenosine triphosphate (ATP) during normal function and under some pathological conditions, all cells are possible sources of adenosine. There are three major pathways of adenosine formation. Adenosine can form from breakdown of ATP, where the utilization of ATP increases 5'-AMP level, and 5'-nucleotidase located on cell membrane converts it to adenosine (Feigl, 1983) intracellularly. Adenosine also forms by the same pathway extracellularly by ecto-5'-nucleotidase (Newby et al., 1990; Shryock and Belardinelli, 1997). The second pathway to produce adenosine is from the hydrolysis of S-adenosylhomocysteine to adenosine and homocysteine (Deussen et al., 1989). The demethylation of S-adenosylmethionine forms S-adenosylhomocysteine. The reverse

process might serve as a reservoir for excessive adenosine (Bontemps et al., 1983). Recently, another pathway of adenosine formation from cAMP extracellularly was suggested and found in many different tissues (Brundage et al., 1997; Mi and Jackson, 1995).

Many biological and pathological situations enhance the production of adenosine and may increase local and blood adenosine concentration. Hypoxia and ischemia increased production of adenosine in the heart (Lasley and Mentzer Jr, 1993), brain (Latini et al., 1996) and skeletal muscle vasculature (Marshall, 1995). Hemorrhage induced a marked increase in adenosine production and adenosine plasma concentration in the venous blood (Zhang and Lautt, 1994).

1.5.1.2 Adenosine metabolism

Free adenosine levels in the blood and interstitial spaces are tightly controlled and maintained at low concentrations. The half-life of adenosine in blood is only about a few seconds (Möser et al., 1989). Adenosine is removed from plasma mainly by two processes. The uptake by the nucleoside transporters located on cell membranes in many cell types, including erythrocytes and endothelial cells, takes up adenosine very efficiently (Pearson et al., 1978; Plagemann et al., 1985). Adenosine transported into the cells is then either converted to adenine nucleotides (to ATP) by adenosine kinase or to inosine by adenosine deaminase (Meyskens and Williams, 1971; Plagemann et al., 1985). Intracellular adenosine is more likely to be phosphorylated since the kinase has much greater affinity than the deaminase does (Sparks and Bardenheuer, 1986). Extracellular deamination of adenosine by ecto-adenosine deaminase is another metabolic pathway to diminish adenosine (Meghji et al., 1988). The extracellular deamination of adenosine

yields inosine, and then it is sequentially metabolized to hypoxanthine, xanthine and uric acid by different enzymes (Shryock and Belardinelli, 1997).

1.5.1.3 Adenosine receptors and second messengers

It is believed that adenosine acts through receptors that are present on the surface of the cell membranes in most cells in the body. Adenosine receptors belong to the P₁ purinoceptor family. So far, four subtypes of adenosine receptors have been recognized: A₁, A_{2A}, A_{2B} and A₃ adenosine receptors (Fredholm et al., 1994). Adenosine receptors are G-protein coupled. The A₁ receptors link to inhibitory G-protein and inhibit adenylate cyclase activity, and therefore decrease intracellular cAMP levels. A₂ receptors link to stimulatory G-protein, and agonism of the receptor stimulates the activity of the cyclase and increases cAMP levels (Fredholm et al., 1994; Shryock and Belardinelli, 1997) for reviews). Evidence suggested that agonism of A₃ receptors inhibits the cyclase activity and decreases cAMP levels (Salvatore et al., 1993); other evidence suggested that it stimulates the production of inositol 1,4,5-triphosphate and increases the activity of phospholipase C in mast cells (Ramkumar et al., 1993).

The A₁ adenosine receptors are mainly distributed in the brain, testis, adipose tissue, heart and kidney. A_{2A} adenosine receptors are distributed in the brain, while A_{2B} receptors are abundant in the gastrointestinal tract ((Fredholm et al., 1994; Shryock and Belardinelli, 1997) for reviews).

The activation of A₂ adenosine receptors on vascular smooth muscle cell increases the intracellular cAMP level. This mechanism has been generally accepted for the vasodilator effect of adenosine (Komas et al., 1991). On the other hand, A₁ receptors are more closely linked to the metabolic effects of adenosine (Van Schaick et al., 1998).

Some adenosine effects on cellular metabolism may also link to adenosine metabolites such as inosine (Haun et al., 1996). However, adenosine receptors and their subtypes involved in vascular and nonvascular functions have not been fully explored in the liver and intestine. One of the reasons might be lack of selective tools for adenosine receptors for the purpose of *in vivo* studies.

1.5.2 Adenosine in hepatic circulation and metabolism

1.5.2.1 The hepatic arterial buffer response

The liver is perfused by the portal vein and hepatic artery. The hepatic artery can regulate its blood flow, the portal vein cannot. It was interesting to find that the control of hepatic arterial blood is independent from metabolic activities of the liver (Lautt, 1980a). This is quite different from what happens in other organs. Besides the necessity of oxygen supply, a constant blood flow to the liver seems necessary for controlling metabolic rates of circulating hormones (Lautt, 1977a), which are mainly metabolized in the liver. The mechanism of intrinsic regulation of hepatic arterial blood flow: the hepatic arterial buffer response (HABR) was proposed (Lautt, 1983b), and adenosine was suggested to be the mediator of the hepatic arterial buffer response (Lautt et al., 1985). According to the adenosine washout hypothesis, adenosine is constantly released into the space of Mall, where the hepatic arteriole, portal venule and bile ductule meet before entering the sinusoids. Adenosine dilates the arterioles. The local concentration of adenosine is regulated by washout into the portal blood vessels. When portal blood flow decreases, the concentration of adenosine increases due to less washout and decreases the resistance of hepatic arterioles to increase blood flow ((Lautt, 1996a) for review). It is interesting that the production of adenosine is not metabolically controlled at this site.

1.5.2.2 Autoregulation of hepatic arterial blood flow

Blood pressure-flow autoregulation is the intrinsic ability of an organ to adjust its blood flow in the face of the change of perfusion pressures. When perfusion pressure drops, the resistant vessels in an organ dilate, resulting in a relatively constant blood flow perfusing the organ over a wide range of perfusion pressure. Under physiological conditions, autoregulation exists in cerebral, coronary, hepatic, intestinal and other circulations.

In the liver, some studies have demonstrated the nonlinearity of arterial pressure-flow relationship (Takeuchi et al., 1966). Ezzat and Lautt (1987) showed, using an *in vivo* model, that the hepatic artery of the cat is an autoregulating blood vessel. This phenomenon occurred in the range of perfusion pressure from 70 up to 120 mmHg that was the control pressure. A nonselective adenosine receptor blocker, 8-phenyltheophylline, abolished the pressure-flow autoregulation in the liver, suggesting that adenosine mediated this physiological phenomenon (Ezzat and Lautt, 1987).

It is interesting that the hepatic arterial pressure-flow autoregulation is also adenosine-mediated and through a washout mechanism. Thus, both forms of intrinsic control of hepatic arterial blood flow, the hepatic arterial buffer response and autoregulation, are through the same mechanism. The washout of adenosine by arterial blood affects hepatic arterial resistance in the autoregulation; whereas, the washout of adenosine in the space of Mall by portal venous blood affects hepatic arterial resistance in the buffer response. Therefore, both portal venous and hepatic arterial blood flow affects hepatic arterial resistance through the same washout mechanism and the same mediator.

1.5.2.3 Modulation of vasoconstriction

In addition to its direct vasodilator effect, adenosine plays a role in the modulation of sympathetic nerve activities through activation of the purinergic receptors. The suppression effect of adenosine on sympathetic activities could be acting at different sites including sympathetic ganglia, nerve terminal and the end organ (Evoniuk et al., 1986). Adenosine inhibited the sympathetic vasoconstrictor responses in a gracilis nerve-muscle preparation in the dog (Klabunde, 1987), although the concentration of adenosine used was high. During hemorrhagic hypotension, the release of adenosine suppressed the noradrenergic response but did not affect the responses to norepinephrine infusion suggesting that adenosine inhibited neurotransmission (Jackson et al., 1994). Other studies confirmed that the inhibitory effects of adenosine on sympathetic nerve stimulation are by a presynaptic mechanism in the pulmonary artery (Tamaoki et al., 1997) and femoral vascular bed (Hom and Lokhandwala, 1981), and also in frog muscle vascular diameter (Fuglsang et al., 1989).

Lautt and Legare (1986) found that intra-arterial infusions of adenosine caused dilation of the hepatic artery and inhibition of arterial vasoconstriction induced by both nerve stimulation and norepinephrine infusion in the liver. The infusion of adenosine also inhibited the vasoconstrictor effect of angiotensin and vasopressin in the cat (Lautt and Legare, 1986). These results suggest that adenosine plays a more general role in suppression of vasoconstriction and control of blood flow in the liver. It is interesting to note that the modulation of vasoconstriction by adenosine occurred only in the hepatic artery but not in the portal vein, even within the same organ.

Neither intra-arterial nor intra-portal venous infusion of adenosine affected portal pressure (Lautt and Legare, 1986) in the cat, suggesting that no adenosine receptors are

present in the portal venous vascular smooth muscle. However, adenosine-induced increase in portal pressure was seen in isolated perfused liver (Nukina et al., 1994). The cause of these differences is not clear. Abnormality of *in vitro* preparations and species difference may all be encountered. In addition, the adenosine concentration reaching tissues could be much higher in *in vitro* studies where a biological solution was used as perfusate rather than the blood. Stimulation of A₃ adenosine receptors in Mast cells with high adenosine concentration increased occurrence of vasoconstriction in hamster cheek pouch arterioles (Shepherd et al., 1996). However, the applicability of this mechanism in the liver is not clear.

1.5.2.4 Adenosine and hepatic metabolism

Adenosine receptors have been found in the liver, and it is believed that the activation of these receptors stimulates gluconeogenesis in the liver (Stiles, 1986). However, most data available on this issue are from *in vitro* experiments. Adenosine was demonstrated to stimulate glucose release from rat hepatocytes (Hoffer and Lowenstein, 1986). Glycogen loss and activation of glycogen phosphorylase were also found in this study. Other studies also showed similar effects of adenosine in hepatic glucose metabolism (Nukina et al., 1994; Vanstapel et al., 1991) in isolated perfused rat livers. In the two latter studies, the increase in glucose production, however, was found to be transient and dropped quickly. It is not yet clear if this biphasic pattern of glucose production in response to adenosine is due to direct effects of adenosine or is secondary to the deprivation of glycogen storage in the model. Controversial results were also reported by Lavoinne et al., in which adenosine inhibited hepatocyte gluconeogenesis (Lavoinne et al., 1987). The differences of the results seem to be adenosine concentration

dependent since a similar study using micromolar concentration of adenosine stimulated gluconeogenesis (Hoffer and Lowenstein, 1986), whereas the concentration used in Lavoinne's study was a thousand times higher.

Adenosine was found to decrease oxygen consumption and increase lactate production in many other organs, for instance, in the heart (Wannenbarg et al., 1994). There are not enough data to make a clear conclusion of adenosine effects on oxygen consumption in the liver. An increased adenosine level was found to be important for protection of the liver from ischemia-reperfusion injury (Nakayama et al., 1997). The results may suggest that adenosine has an effect on oxygen demands. Another study suggested that adenosine released during preconditioning could act through the stimulation of NO to protect the liver against the injury caused by ischemia-reperfusion (Peralta et al., 1997). Administration of adenosine to perfused rat liver caused a decrease in oxygen consumption (Vanstapel et al., 1991). In other studies, however, a transient increase followed by a biphasic pattern in oxygen consumption was observed (Nukina et al., 1994). Since adenosine-mediated regulation of hepatic blood flow is not metabolic dependent (Lautt, 1980a), it would be very interesting to find out the role of adenosine in hepatic oxygen metabolism in an *in vivo* experiment.

1.5.3 Adenosine in intestinal circulation and metabolism

1.5.3.1 Vasodilator effect of adenosine

Adenosine-induced vasodilation has been shown in many different species including the dog (Granger and Norris, 1980), cat (Lautt et al., 1987), rat (Bohlen, 1987; Pawlik et al., 1993), rabbit (Bohlen, 1987) and pig (Norlen, 1988) in *in vivo* experiments. Adenosine increases blood flow in the intestinal wall as well as in the mucosal layer

(Sawmiller and Chou, 1991; Walus et al., 1981). Endogenous adenosine and its vasodilator effect in the mesenteric vascular bed have been suggested to be involved in many regulatory mechanisms in the intestines ((Jacobson and Pawlik, 1994) for review).

1.5.3.1.1 Role of adenosine in autoregulation. The mechanism of pressure-flow autoregulation in the gut involves the vasodilation of resistant vessels in response to reduced perfusion pressure, which results in a relatively constant blood flow within a wide range of perfusion pressures. The autoregulation was suggested to be adenosine mediated (Lautt, 1986b). It was shown that an adenosine receptor blocker, 8-phenyltheophylline, blocked pressure-flow autoregulation in fasted cat (Lautt, 1986a). The ability of 8-phenyltheophylline to block the autoregulation was dose-dependent, and the doses used in the study also showed a dose-dependent blockade of exogenous adenosine-induced vasodilation, confirming the mediator role of adenosine in the mesenteric vascular autoregulation.

1.5.3.1.2 Role of adenosine in intestinal hyperemia. Postprandial hyperemia in the intestine is a local hemodynamic response to the presence of food in the intestinal lumen. Many mechanisms mediating this phenomenon were proposed during the past years, and the possible involvement of metabolic demands, the effect of bile acids, intrinsic nerve regulation, vasoactive intestinal peptide (VIP) and other hormones like neuropeptides, histamine and prostaglandins were studied (Gallavan and Chou, 1985). Adenosine was suggested to be the major mediator in regulation of postprandial hyperemia (Sawmiller and Chou, 1992). The administration of an adenosine uptake blocker, dipyridamole, enhanced the hyperemia and decreased oxygen consumption, while the treatment with adenosine deaminase abolished the hyperemia, which supported the hypothesis.

However, I would not agree with the authors in attributing the attenuated increase in oxygen consumption by adenosine deaminase during food placement to decreased metabolic activity. The oxygen consumption during the period seems more likely to be limited by the blood flow, which did not increase as much in the presence of adenosine deaminase (also see the discussion in section 1.5.3.3). Adenosine is also suggested to mediate, at least in part, the reactive hyperemia in the intestine (Lautt, 1986b; Pawlik et al., 1993; Sawmiller and Chou, 1992).

1.5.3.2 Interaction of adenosine with other vasoactive substances

I have discussed that adenosine suppresses the vasoconstriction induced by sympathetic nerve stimulation and other vasoconstrictors in the liver (Lautt and Legare, 1986) in a previous section (section 1.5.2.3). The inhibitory effect of adenosine on the vasoconstriction induced by sympathetic nerve stimulation and norepinephrine infusion was also demonstrated in cat mesenteric arteries (Lautt et al., 1988). In this study, isoproterenol induced the same vasodilation as adenosine did but did not affect the vasoconstriction. Since adenosine inhibited both nerve stimulation and norepinephrine-induced constriction, adenosine seems to act through a postsynaptic mechanism. In another study, an adenosine receptor agonist was shown to inhibit the noradrenergic neurotransmission in rat mesentery (Kuan and Jackson, 1988), but endogenous adenosine was not involved in the modulation in the study.

I have mentioned before (section 1.2.6.2) that the vasodilator effect of adenosine was antagonized by nitric oxide in the superior mesenteric artery in the cat (Macedo and Lautt, 1997). I also discussed the mediator role of adenosine in pressure-flow autoregulation in the intestine. A recent study showed that nitric oxide attenuated

autoregulation capacity in the superior mesenteric artery in cat (Macedo and Lautt, 1996a), providing evidence for the interaction of adenosine and NO produced endogenously.

1.5.3.3 Adenosine effects on oxygen consumption

Adenosine possesses dual properties: the effect on hemodynamics and the effect on metabolism. The direct effects of adenosine on intestinal oxygen consumption were shown in cats (Granger et al., 1978). Local infusion of adenosine decreased arterial-venous oxygen difference and oxygen consumption across the ileum, and these effects could be blocked by aminophylline in the study. Reduced oxygen consumption by adenosine infusion was also shown in the small intestine in dog (Granger and Norris, 1980). Shepherd et al. studied the effects of isoproterenol and adenosine on intestinal oxygen uptake and hemodynamics. The results showed that both isoproterenol and adenosine infusion reduced intestinal oxygen consumption (Shepherd et al., 1984). Based on the observation that adenosine increased the muscularis blood flow while isoproterenol increased mucosal blood flow, the authors proposed a blood stealing theory to explain adenosine-induced reduction of oxygen consumption in addition to its direct effect.

There are also controversial results as to adenosine effect on oxygen consumption. Adenosine and one of its receptor agonists were reported to increase oxygen uptake and blood flow to the gut (Walus et al., 1981). In a study in dog jejunum (Sawmiller and Chou, 1992), the administration of adenosine deaminase attenuated the increase in oxygen consumption and blood flow during food placement. The authors, however, attributed the effect of adenosine deaminase on oxygen consumption to decreased

metabolic activity. I would not agree with the explanation since oxygen consumption after the food placement in the presence of adenosine deaminase is still much higher than in the resting state. The oxygen consumption after the food placement seems more likely to be limited by blood flow. The food placement-induced increase in intestinal blood flow was abolished in the presence of adenosine deaminase. The changes in blood flow could underline the difficulties in interpretation of the results in Chou's study, and may have influenced the interpretation of other published data.

1.6 REMARKS ON THE INTRODUCTION

1.6.1 The interrelations of these vasoactive substances

In this chapter, I have discussed a few aspects of NO, prostaglandins and adenosine in the control of hepatic and intestinal blood flow and metabolism. Besides the direct actions of each substance, almost every candidate discussed has the ability to modulate the action of others somehow. The interrelationships are also linked by shear stress, which is one of the stimuli for the release of NO and other autacoids such as prostaglandins from the endothelium. All vasoactive substances have the potential to cause changes in hemodynamics and affect shear stress under certain conditions. Changing the shear-stress state may release NO and other vasoactive substances.

NO inhibits vasoconstriction caused by sympathetic nerve stimulation or norepinephrine infusion in a shear stress dependent manner. Adenosine suppresses the vasoconstriction induced by nerve stimulation, norepinephrine and other constrictors in the hepatic artery and mesenteric artery. Prostaglandins can modulate sympathetic activity as well, although different individual substances in the family may have different

effect, and their actions may also be dependent on tissue and species difference. Here, it seems that each one has influence on sympathetic activities. However, nerve stimulation or infusion of norepinephrine may increase shear stress resulting in an increase in NO and/or prostaglandin production from the endothelium.

Furthermore, it was found that NO antagonizes the vasodilator effect of adenosine in the intestine. They are both vasodilators in the intestine. Even before we know where this piece of the puzzle fits, it can easily be figured out that adenosine can increase shear stress through increasing blood flow by its vasodilator effect.

NO and prostaglandins may regulate each other in their production, and/or may use each other to mediate their respective function. In the normal vascular system, they are both linked to shear stress at least in some aspects.

These tangled relationships are figuratively summarized in Figure 1-2.

1.6.2 Tissue and organ specificity of the actions of these substances

Organ or tissue specificity makes the puzzle even more complicated for us to resolve. The inhibitory mechanism of NO on norepinephrine is presynaptic in the intestine and postsynaptic in the liver. NO antagonizes adenosine and isoproterenol-induced vasodilation in the intestine but not in the liver. Adenosine suppresses vasoconstriction in the liver but only in the hepatic artery, not in the portal vein. Prostaglandins act differently in different tissues and species. For example, PGF_{2α} causes vasoconstriction in the liver but vasodilation at lower concentration in the mesenteric artery. These "inconsistencies" in different tissues have given scientists more fun and headaches as well, and perhaps, the inconsistencies are the mechanisms making true physiological sense by incorporating the functions of different tissues or organs in a

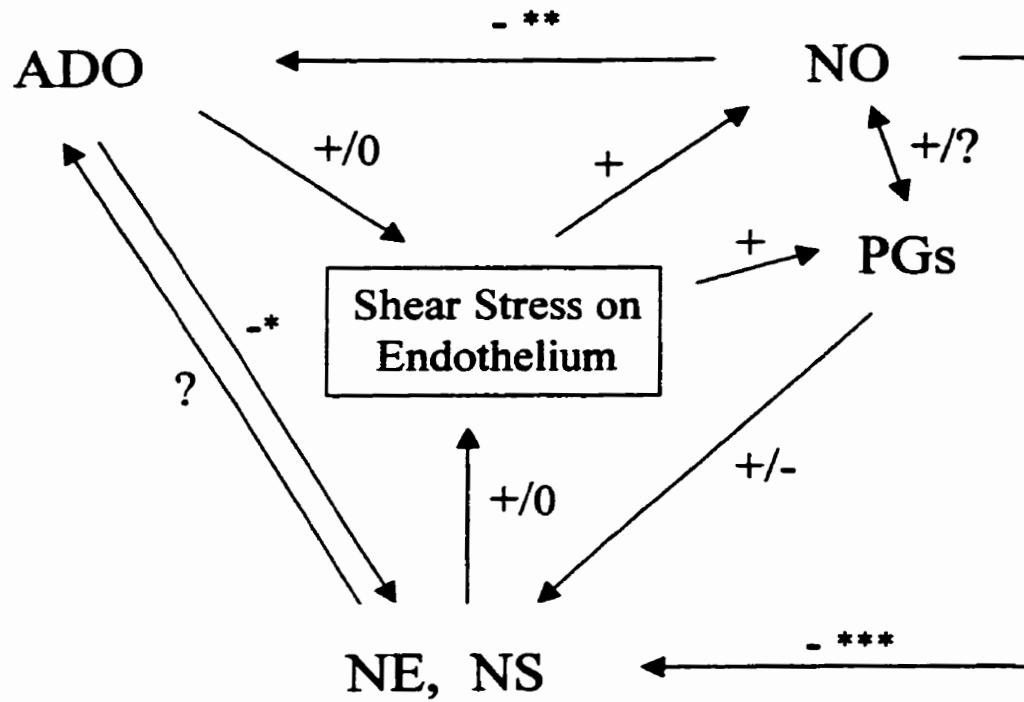


Figure 1-2. The relationship of the vascular responses to adenosine (ADO), nitric oxide (NO), prostaglandins (PGs), and norepinephrine (NE) or sympathetic nerve stimulation (NS). ADO, NO and some PGs cause vasodilation while NE or nerve stimulation causes vasoconstriction. Changes in hemodynamics in response to constrictors or dilators result in changes in endothelium shear stress, and NO and/or PGs production.

+, increase the effect or response;

0, no influence;

-, decrease the effect of response;

?, not clear yet;

*, tissue specific, antagonizes only in the hepatic artery not in portal vein;

**, organ specific, only in the superior mesenteric artery not in the liver;

***, presynaptic in the intestine, postsynaptic in the liver.

body.

1.6.3 The missing pieces of the puzzle

We know that adenosine suppresses sympathetic activity; we do not know if there is any modulation of adenosine vasodilator effect by sympathetic action. We know that NO antagonizes adenosine-induced vasodilation directly in the intestine but not in the liver; we do not know if there is any indirect effect in the liver, and we do not know if this antagonism occurs at the metabolic level. We know that NO antagonizes the vascular response to sympathetic stimulation in the liver; we do not know if NO antagonizes the metabolic response. We know that some of these vasoactive substances act differently in different tissues and organs, but we do not know why; and the list goes on.

In the following chapters of this thesis, I will address some of these questions.

2. HYPOTHESIS AND OBJECTIVES OF THE THESIS

2.1 GENERAL HYPOTHESIS OF THE THESIS

In the previous chapter, I have discussed several endogenous substances and some biological factors that are important in control of hepatic and intestinal blood flow and metabolism. It is obvious that those substances and factors interact, either by their direct effects or through the influence on other factor(s). A body of published evidence for the interaction of these vasoactive substances in control of hemodynamics in the liver and intestine was discussed in the introduction. However, few data are available to demonstrate the interaction at the level of local metabolism.

In the regulation of a single physiological activity, a number of redundant mechanisms and interaction of several factors are normally involved. It would have been very difficult to understand many physiological phenomena if we had studied only one factor or one response. Further, without the knowledge of integrative physiology and pharmacology, we would have had difficulty interpreting the data from a study on a single factor. To have a better understanding of the physiology and pharmacology of a substance, it is essential to study the property of this substance as well as the interaction with others at different levels, for instance, in control of blood flow and metabolism.

Based on current knowledge, previous publications from our laboratory, and my understanding of the importance of integrative physiology and pharmacology, a general hypothesis of the thesis is formulated:

Control and regulation of normal functions in an organ requires more than one mechanism, and the homeostasis is achieved through the interaction of different factors.
The interaction of these factors occurs at the levels of both hemodynamics and

metabolism. The regulation of local hemodynamics meets metabolic requirement and maintains physiological integrity.

The working hypotheses for different studies will be stated in the corresponding chapters.

2.2 OBJECTIVES OF THE THESIS

In this thesis, several working hypotheses were tested in the liver and intestine in whole animal preparations. To gain more knowledge of physiology and pharmacology about these two organs and to provide more materials for testing the hypothesis, I studied hemodynamics and related metabolic activities in the two organs in several different aspects.

1. The influence of norepinephrine on vasodilator responses was investigated in the hepatic artery. Since NO antagonizes the vasoconstrictor effect of norepinephrine, and NO does not affect the vasodilator effect of adenosine in the liver, the possibly indirect influence of NO on the vasodilators was studied.
2. Since NO antagonizes the vascular response to norepinephrine in the liver, whether NO also antagonizes the hepatic glycogenolysis response to norepinephrine was investigated.
3. It is known that NO antagonizes the vasodilator effect of adenosine in the intestine. An *in vivo* model in the small intestine was designed to investigate whether NO antagonizes adenosine at the metabolic level.
4. I observed that the intestinal glucose uptake from the blood increased when blood flow increased. A possible mechanism mediating this increase in intestinal glucose uptake was investigated.

3. METHODOLOGY

3.1 GENERAL APPROACH

Vascular and metabolic responses to different vasoactive substances, in almost all situations, involve more than two different tissues. The intercommunication between different types of cells or different tissues requires certain anatomic arrangement. Furthermore, to study the influence of hemodynamics on local metabolism, the anatomic integrity of the organ is definitely necessary. For those reasons, the goals of the thesis would be difficult to reach using an *in vitro* model in the experiments. I decided to use *in vivo* models in whole animal preparations for the purposes of the studies.

The experiments required measurements of all hemodynamic indices including blood flow and pressure in the organ while monitoring systemic conditions and providing life support. Catheters were connected to pressure transducers and flow probes were placed in experimental animals. In order to control blood flow or pressure in an organ, the original blood flows were manipulated with a pump-controlled circuit. All these required extensive surgical preparation before the experiment. In the studies dealing with metabolic responses, a certain amount of blood needed to be sampled for the assessment of blood contents or concentrations of different substances. I chose the cat as the animal model in the studies because, first, this species is very close to human in terms of circulation and anatomy; second, the species gives a reasonable size for the study of hemodynamics and metabolism.

There was no special requirement of using conscious animals in the study. All surgical preparations and experiments were done under anesthesia in this thesis. The

anesthetic level was checked frequently during the experiment.

3.2 METHODS

3.2.1 Preparation of the experiment

3.2.1.1 Animal conditions

All animals (either gender) used in the experiments in this thesis were housed in the Central Animal Care at the University of Manitoba. Vets in the Animal Care preconditioned the animals before they were available for the experiments. All the animals were treated according to the guidelines of the Canadian Council of Animal Care and all the protocols in the studies were approved by the Animal Use Ethics Committee at the University of Manitoba. The animals were fed with standard cat chow. The animals were fasted for 18 hours (over night) but allowed free access to water before the experiment. Fasting of the animal reduces respiratory accidents due to vomiting during the induction of anesthesia, makes more room in the abdominal cavity and increases the success of surgery, and the fasting is important to reduce the variability of intestinal glucose uptake in the intestinal glucose metabolism study. Each individual animal was weighed before the experiment.

3.2.1.2 Surgical preparation

The animal was anesthetized using sodium pentobarbital (32.5 mg/kg, in saline solution, 32.5 mg/ml) injected intraperitoneally. After the induction, the deepness of anesthesia was checked by corneal reflex and jaw tension. As soon as surgical anesthesia was reached, the animal was put on a surgical table. Hairs in surgical areas were shaved. A rectal temperature probe was inserted to monitor body temperature. The temperature

was maintained at 37.0 ± 0.5 °C using a thermal control unit (Model 72, Yellow Springs Instruments, Yellow Springs, Ohio) operating a heater under the surgical table. The right brachial vein was first cannulated for intravenous infusions. Anesthesia was maintained using a continuous infusion (~1.0 ml/min, 0.78 mg/ml of sodium pentobarbital in saline) via the venous cannula. Supplemental anesthetics (6.5 mg in 0.2 ml once) and fluid (10% Dextran 40 intravenous solution mixed with equal volume of saline) were also given through that route during the experiment when required. A respirator (Harvard Apparatus, Millis, MA) was used to assist ventilation via a tracheal cannulation. The left carotid artery and right femoral vein were cannulated for monitoring systemic arterial pressure and central venous pressure respectively. Laparotomy was performed through a midline incision. The spleen was removed after a gentle massage of the organ followed by ligation of all connecting vessels. The inferior mesenteric artery was ligated. Two intravenous catheters (24G Optiva™, Medical Inc., Arlington, TX) were inserted into the portal vein. One for monitoring portal venous pressure and the other was used for either blood sampling or drug infusion in different experiments. The animal was heparinized (200 IU/kg) before a vascular circuit was established.

All other surgical procedures for special purposes of different experiments will be described in detail in each chapter according to the studies. When all the surgical procedures were done, the animals were allowed to stabilize for at least 45 min in hemodynamic studies and at least 1 hour in metabolic studies.

3.2.1.3 Preparation of catheter and arterial circuit

Polyethylene tubing of different sizes was used for vascular catheters. One side of the tubing was sharpened at a 45-degree angle for cannulation while the other side was

connected to a stopcock. All catheters were washed with distilled water and filled with heparin solution (200 IU/ml) before surgery.

An arterial circuit was used to control superior mesenteric arterial blood flow or pressure. The circuit consists of a pump (Masterflex, Cole Parmer Instrument Co., Barrington, IL) controlling flow rate, an air bubble trap also serving as a pulsation buffer, a filter to prevent clots from entering the circulation, and an electromagnetic flow-probe (EP608, Carolina Medical Electronics, Inc., King, NC) measuring the flow rate. The circuit is shown in Figure 3-1. During the experiment, the circuit acquired blood from double cannulation of the abdominal aorta (except in hepatic hemodynamic studies in which the circuit acquired blood from femoral arterial cannulations). The circuit was washed, sterilized and filled with 5 % Gentran 40 saline solution before each experiment. The sterilization includes the following steps: 1. Circulate the circuit with 10 % formaldehyde for 40 minutes; 2. Flush the circuit with 2 litre of distilled water; 3. Flush it with 1 liter of 0.5 % ammonia solution; 4. Flush it with a large amount of distilled water; 6. Flush it with saline.

3.2.1.4 Calibration of pressure transducers

All pressures were monitored by pressure transducers (Gould and Statham, Gould Inc., Oxnard, CA) in all experiments. The transducers had been set to zero at the level of the central vena cava. The transducers were calibrated using a mercury manometer to generate a standard pressure in millimeters of mercury. The sensitivity of the amplifiers in a dynograph recorder (R611, Sensor Medics, Anaheim, CA) were then adjusted to desired responses. For those transducers used for venous pressure, a water manometer was used for the standard, and the height of water column was converted to

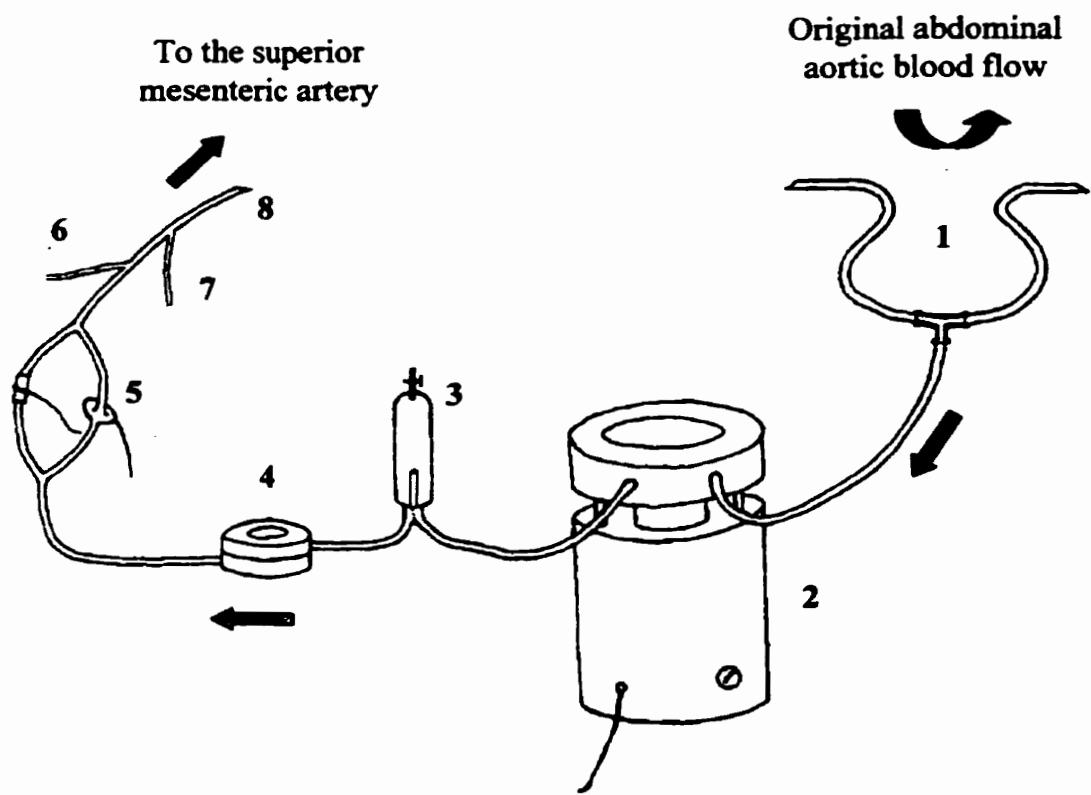


Figure 3-1. The arterial circuit. The circuit was used to control superior mesenteric arterial blood flow or pressure. The circuit consists of

- 1) Double cannulation at abdominal aorta (cannulations of femoral arteries in Chapter 4); 2) Pump controlling blood flow; 3) Bubble trap and pulsation buffer; 4) Silk filter; 5) Electromagnetic flow-through flow probe and its ground; 6) Drug infusion line; 7) Pressure monitoring and sampling line; 8) Superior mesenteric arterial cannulation;

← Direction of blood flow.

corresponding value in mercury by the specific weight of mercury. The calibrations of all transducers used in the study were checked once each half a year.

3.2.1.5 Calibration of flow-probes

The flow rates were measured using electromagnetic flow probes. Two types of flow probes were used in the study, which are the flow through type (EP608, Carolina Medical Electronics, Inc., King, NC) and the clamp type (size 6 or 8, EP408, Carolina Medical Electronics, Inc., King, NC). Calibration of the flow probes was carried out at the end of each experiment. At the end of each experiment, the blood from the animal was collected in a reservoir and used for the calibration.

3.2.1.5.1 Calibration of flow through probe and correction of circuit resistance. The arterial circuit with the flow-probe was taken out of the animal. The superior mesenteric arterial cannula was taken out with a small piece of the artery still attached to the tip. The circuit was then operated to recirculate the blood in the reservoir at different flow rates that cover the range used in the experiment. The responses to different flow rates were used to calculate a response factor of the probe. The factor was used to calculate flow rates according to the responses during the experiment. The time elapsed during a certain volume of blood being pumped out of the circuit was measured using a stopwatch. The time and volumes calculated the flow rates. The circuit pressures recorded were regressed against different flow rates. The slope and interception were used to correct the circuit resistance at different flow rates. A true perfusion pressure is calculated by subtraction of the pressure generated by circuit resistance at the flow rate from measured circuit pressure.

3.2.1.5.2 Calibration of clamp type probe. An extra surgical procedure was

performed to reconnect the blood vessels with the flow-probe into a loop after each experiment. A couple of cannulations and ligations of branching vessels were made to allow blood to be pumped through the loop and drained into a reservoir. A pump was used to control the flow rate. The time elapsed during which a certain volume of blood was pumped out was measured using a stopwatch. The time and volumes calculated the flow rates. The response factor of the flow probe was calculated using the responses to different flow rates. The factor obtained was used for the calculation of blood flows during that experiment.

3.2.1.6 Other instruments used in the study

3.2.1.6.1 Glucose analyzer. Blood glucose levels were analyzed using a glucose analyzer (YSI Sports Industrial analyzer, Yellow Springs, Oh). The analyzer uses a glucose oxidase membrane that oxidizes glucose to hydrogen peroxide to which the electrode responds. A 200 mg/dL glucose solution was used as a standard for the calibration of the analyzer. The calibration was performed before each experiment and checked several times during the experiment. Re-calibration was also performed if necessary during the experiment. A 25 μ l sample is needed for the assay.

3.2.1.6.2 Lactate analyzer. Blood lactate levels were analyzed using a lactate analyzer (YSI Sports Industrial analyzer, Yellow Springs, OH). The analyzer uses a membrane with the enzyme that oxidizes lactate to hydrogen peroxide to which the electrode responds. A commercially available 5 mmol/L lactate standard was used for calibration. The calibration procedure is the same as that used for glucose. A 25 μ l sample is needed for the assay.

3.2.1.6.3 Oxygen content. Blood oxygen content was analyzed using a LexO2 Con-

K oxygen content analyzer (Lexington Instruments, Waltham, MA). The atmospheric air saturated with water was used as a standard for calibration of the machine. The oxygen content in the air was corrected according to the atmosphere pressure and temperature at the time. A volume of 20 µl blood is needed for the assay.

3.2.1.6.4 Blood gas. Blood gases and pH were measured using a blood gas analyzer (system 1302, Instrumentation Laboratory, Lexington, MA). The instrument calibrates automatically using commercial available pre-mixed gas standards and pH standard solutions. The reliability of the machine was also checked using Sigma Clinical Blood Gas standards (Sigma Chemical, St. Louis, MO).

3.2.1.7 Data analysis

All the data in this study were expressed as mean ± SEM. Student *t* test was used to compare two groups of means. If the data from the same animal in an experiment can be paired, paired *t* test was used. When more than one group was compared to control or the comparison was among three or more groups, analysis of variance was used for detection of any difference. Tukey's test or linear contrast was used following the analysis to show where the difference was. Paired test was also used if applicable. P<0.05 was used as the criterion for significance to reject the null hypothesis. Computer programs, GraphPad Prism and Microsoft Excel were used to analyze the data and compute the statistics.

3.2.1.8 Chemicals and drugs

Adenosine, L-NAME, L-arginine, methylene blue, indomethacin, PGF_{2α}, PGI₂, norepinephrine and isoproterenol were purchased from Sigma (Sigma Chemical, St. Louis, MO). 3-morpholinosydnonimine (SIN-1) was purchased from Alexis Corporation (San Diego, CA). Adenosine, L-NAME, L-arginine, and methylene blue solutions were

made fresh in saline before each experiment. Indomethacin was dissolved in 2 ml of 5 % NaHCO₃ solution and then diluted to 10 ml with saline. Norepinephrine was dissolved in distilled water at a concentration of 1 mg/ml as a stock solution. Isoproterenol was dissolved in 0.2 mg/ml ascorbic acid solution at a concentration of 1 mg/ml as a stock solution. Stock solutions were stored at -20 °C, and were diluted to desired concentration before the experiment. The concentrations of adenosine and other drugs were calculated according to body weight of each individual animal. PGE₂ was purchased from the Upjohn Co. (Kalamazoo, MI). Prostaglandins were dissolved in 95% ethanol at 1 mg/ml concentration as stock solutions separately, which were stored as small aliquots at -20°C. Fresh solutions were made by dilution of the stock solution to desired concentrations using saline in each experiment. One tube of stock solution was used at a time, and all prostaglandin solutions were protected from light using aluminum foil.

Other methods used specifically for each experiment will be described and discussed elsewhere in the following chapters.

4. BLOCKADE OF NITRIC OXIDE SYNTHASE POTENTIATES THE SUPPRESSION OF VASODILATORS BY NOREPINEPHRINE IN THE HEPATIC ARTERY

4.1 INTRODUCTION

I have discussed in Chapter 1 that NO was involved in control of basal vascular tone in the superior mesenteric artery but not in the hepatic artery (Macedo and Lautt, 1997) although the release of NO from the endothelium is controlled by shear stress in both the hepatic and superior mesenteric arteries. Shear stress-dependent release of NO suppressed the sympathetic nerve-induced vasoconstriction in the liver by a post-synaptic mechanism (Macedo and Lautt, 1998); on the other hand, NO suppressed the vasoconstriction by a pre-synaptic mechanism in the superior mesenteric artery (Macedo and Lautt, 1996b). The blockade of NO synthase by L-NAME potentiated the vasoconstriction induced by sympathetic nerve stimulation or norepinephrine infusion in the liver, but only the vasoconstriction induced by nerve stimulation, not by norepinephrine infusion, in the superior mesenteric artery when shear stress was allowed to increase.

Adenosine is another potent endogenous vasodilator in control of splanchnic blood flow. This substance is involved in the hepatic arterial buffer response (Lautt, 1985), blood pressure-flow autoregulation in the liver (Ezzat and Lautt, 1987) and intestine (Lautt, 1986a). Adenosine is a potent vasodilator in the intestinal vasculature (Granger et al., 1978; Granger and Norris, 1980); it may also regulate intestinal postprandial hyperemia (Sawmiller and Chou, 1992). That adenosine suppressed the vasoconstriction by norepinephrine or sympathetic nervous stimulation in the superior mesenteric artery

(Lautt et al., 1988) and hepatic artery but not in the portal vein (Lautt and Legare, 1986) was discussed in the previous chapter.

The two endogenous vasodilators, adenosine and NO, suppress the constriction induced by norepinephrine or sympathetic nerve stimulation in the liver and intestine. It is interesting that the vasodilation induced by adenosine was inhibited by NO in the superior mesenteric artery but not in the hepatic artery (Macedo and Lautt, 1997). This tangled relationship of these vascular regulators and the high organ specificity with distinct mechanisms of NO in the modulation of other factors suggests that these major vascular regulators interact and modulate each other. The working hypothesis in this study is that NO suppresses the vasoconstrictor effect of norepinephrine, therefore modulates the effect of norepinephrine in the interaction with adenosine and other vasodilators in the control of hepatic arterial conductance. The objective of this study was to determine if norepinephrine suppresses the vasodilation induced by vasodilators such as adenosine and isoproterenol, and if NO plays a role in the interaction in control of hepatic arterial vascular tone. The results showed that only a small portion of the maximum vasodilator effects of adenosine and isoproterenol were suppressed by norepinephrine at a dose that produced maximum constrictor effects. After blockade of NO synthase, the suppression of the vasodilators by norepinephrine was substantially potentiated. The results are consistent with the hypothesis that NO plays a very important role in the modulation of interaction between norepinephrine and vasodilators.

4.2 METHODS AND PROTOCOLS

4.2.1 Surgical procedure

Cats of either gender, weighing 4.5 ± 2.6 kg, were fasted and anesthetized as described in Chapter 3. After the general surgical preparation, the gastroduodenal artery was ligated to ensure that the celiac arterial blood was exclusively into the liver. The anterior hepatic nerve plexus was cut to prevent any influence of systemic reflex on the liver. The gastric artery was cannulated for the infusion of norepinephrine into the hepatic artery, and the splenic artery was cannulated for monitoring hepatic arterial blood pressure. Two intravenous catheters (24G OptivaTM, Medical Inc.) were placed into the portal vein for adenosine infusion and monitoring portal venous pressure. The celiac artery was cleared of surrounding tissues, and an electromagnetic flow probe (EP408, Carolina Medical Electronics, Inc., King, NC) was placed on the artery for monitoring hepatic arterial blood flow. The superior mesenteric artery was cannulated, and the blood flow was controlled using a pump-controlled arterial circuit (see Chapter 3) to obtain a pressure comparable with the systemic arterial pressure. The circuit acquired blood from femoral arterial cannulations (Macedo and Lautt, 1997). A flow-through electromagnetic flow probe (EP608, Carolina Medical Electronics, Inc., King, NC) was incorporated into the circuit to monitor the blood flow. Calibration of the flow probes was carried out *in situ* as described before. All pressures were monitored by pressure transducers (Gould and Statham, Gould Inc., Oxnard, CA) that had been set to zero at the level of the central vena cava.

4.2.2 Protocols

4.2.2.1 The influence of NO on norepinephrine-induced vasoconstriction

The maximum response of the hepatic artery to norepinephrine infusion was tested and compared in the presence of L-NAME and L-arginine in six animals. Norepinephrine

was infused into the hepatic artery at a starting dose of 0.25 µg/kg/min; then the dose was doubled until there was no further decrease in hepatic arterial blood flow. The second-to-last dose was taken as the maximum dose. This maximum response to NE infusion was then compared after the administration of L-NAME (2.5 mg/kg, iv infusion over 10 min). The effect of L-NAME was reversed by the administration of L-arginine (75 mg/kg, iv infusion over 10 min), and the NE infusion was tested again. The portal blood flow was maintained using the pump in the circuit. Hepatic arterial conductance and a percent change from basal conductance induced by norepinephrine infusion were calculated.

4.2.2.2 The influence of norepinephrine on adenosine-induced vasodilation

The maximum vasodilator effect of adenosine was tested and compared in the presence and absence of norepinephrine before and after the blockade of NO synthase in seven animals. An adenosine dose response curve was obtained by infusion of adenosine into the portal vein. The doses of adenosine were increased cumulatively in a stepwise manner until the maximum dilation was reached before recirculation occurred (judged by decrease in systemic arterial pressure); the dose producing maximal effect before the recirculation was taken as the maximum dose. The dose response curve was repeated under a background infusion of the maximal dose of norepinephrine when the vasoconstriction reached a plateau. The dose of norepinephrine was pre-tested as described in protocol 4.2.2.1. The animals were then treated with L-NAME (2.5 mg/kg, iv infusion over 10 min), and the maximum vasodilator effects in the presence and absence of NE were tested again. The portal blood flow was maintained constant through the experiment.

4.2.2.3 The influence of norepinephrine on isoproterenol-induced vasodilation

The maximum vasodilator effect of isoproterenol was tested and compared in the presence and absence of norepinephrine in the same group of animals and using the similar procedures as in protocol 4.2.1.2. These tests were repeated after the blockade of NO synthase by L-NAME (2.5 mg/kg). An isoproterenol dose response curve was obtained by infusion of the drug into the portal vein.

4.2.3 Calculation of conductance

To assess vascular tone and the interaction of vascular response to different drugs, vascular conductance was used (Lautt, 1989). The hepatic arterial conductance (HAC) was calculated as $HAC = [\text{Flow rate (normalized to body weight)}] / [\text{Hepatic arterial pressure} - \text{Portal venous pressure}]$.

4.3 RESULTS

4.3.1 The response of hepatic artery to norepinephrine infusion

The hepatic arterial responses to norepinephrine infusion varied slightly in different animals, and the intra-arterial dose to induce maximum vasoconstriction was 0.45 ± 0.13 $\mu\text{g}/\text{kg}/\text{min}$ ($n=6$). The infusion of norepinephrine into the hepatic artery decreased blood flow from 15.8 ± 2.6 to 10.5 ± 1.7 $\text{ml}/\text{kg}/\text{min}$ ($p < 0.01$). The hepatic arterial pressure increased from 102 ± 8 to 145 ± 16 mmHg ($p < 0.01$). The hepatic arterial conductance was decreased by $50.0 \pm 5.2\%$ (by 0.088 ± 0.022 from 0.164 ± 0.033 $\text{ml}/\text{kg}/\text{min}/\text{mmHg}$, $p < 0.05$) during norepinephrine infusion as shown in Figure 4-1. The administration of L-NAME (2.5 mg/kg) increased the systemic arterial pressure and therefore hepatic arterial pressure (93 ± 4 before and 121 ± 3 mmHg after L-NAME, $p < 0.001$), but did not change basal hepatic arterial conductance (0.164 ± 0.033 before versus 0.166 ± 0.037 $\text{ml}/\text{min}/\text{kg}/\text{mmHg}$

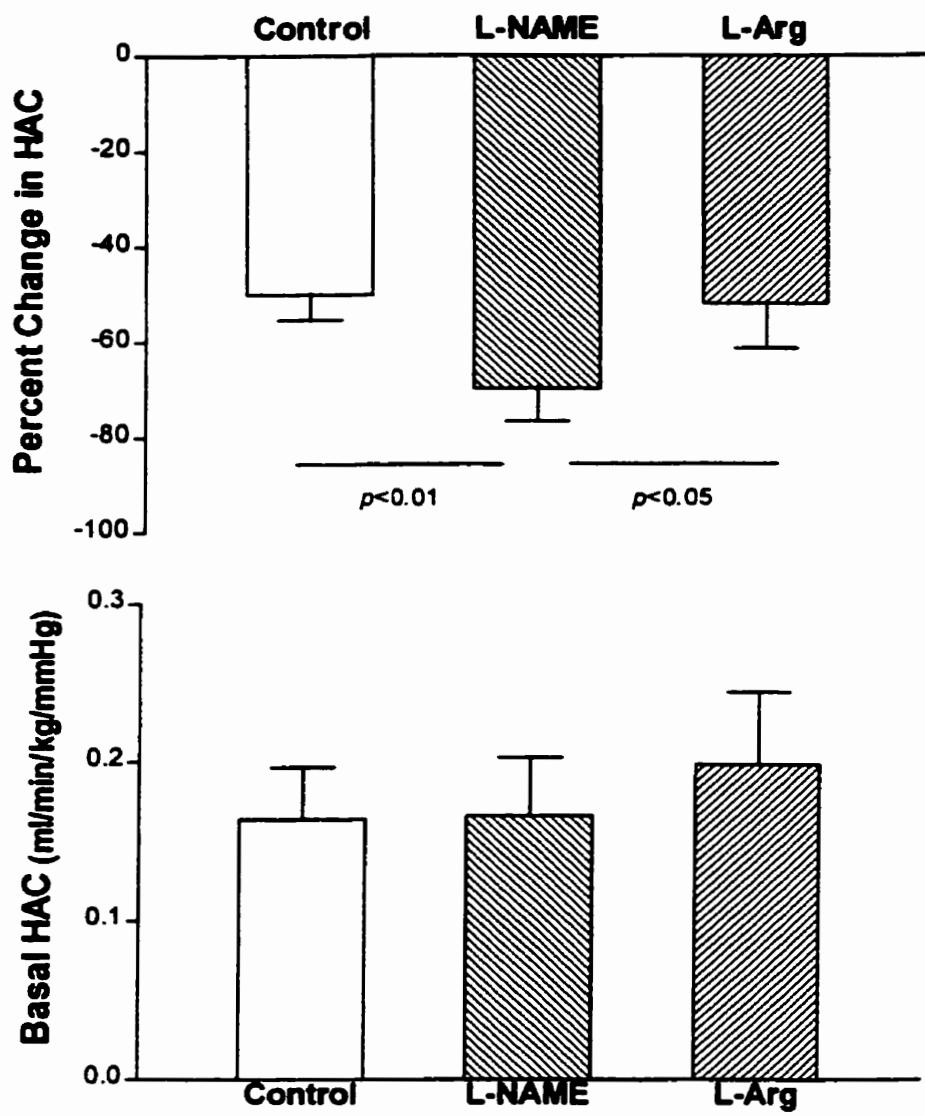


Figure 4-1. The percent change in hepatic arterial conductance (HAC) induced by norepinephrine infusion ($0.45 \pm 0.13 \text{ } \mu\text{g/kg/min}$) was potentiated by the administration of L-NAME (2.5 mg.kg); the potentiation was reversed by L-arginine (L-Arg). The administration of L-NAME and L-arginine did not change the basal hepatic arterial conductance (n=6).

after, NS). However, in the presence of L-NAME, the infusion of norepinephrine decreased hepatic arterial conductance by $69.7\pm6.8\%$ (reduced to 0.045 ± 0.015 ml/min/kg/mmHg, $p<0.01$). The maximum vasoconstrictor effect of norepinephrine on the hepatic artery was potentiated after L-NAME ($p<0.01$, Figure 4-1).

The administration of L-arginine (75 mg/kg) reversed the effect of L-NAME on systemic arterial pressure and hepatic arterial pressure (96 \pm 5 mmHg after L-arginine, $p<0.001$ when compared to L-NAME). The administration of L-arginine did not change the basal hepatic arterial conductance but reversed the potentiation of the vasoconstrictor effect of norepinephrine by L-NAME. In the presence of L-arginine, norepinephrine infusion decreased hepatic arterial conductance by $51.9\pm9.4\%$, which is significantly different ($p<0.05$, Figure 4-1) from that in the presence of L-NAME alone and not different from the control constriction by norepinephrine infusion.

Therefore, norepinephrine infusion caused a decrease in the hepatic arterial conductance, which was potentiated by L-NAME, and the potentiation could be reversed by L-arginine.

4.3.2 The effect of norepinephrine infusion on the vasodilators

The maximum vasodilator effect of adenosine in the hepatic artery was determined from an adenosine dose-response curve for each experiment. The maximum hepatic arterial conductance reached by adenosine infusion (at a dose of 1.6 ± 0.2 mg/kg/min) was 0.222 ± 0.027 ml/min/kg/mmHg ($n=7$). This maximum vasodilator effect of adenosine was slightly suppressed (by $14.0\pm5.8\%$, to 0.194 ± 0.031 ml/min/kg/mmHg, $p<0.05$) by a background infusion of the maximum dose of norepinephrine (0.49 ± 0.12 μ g/kg/min, Figure 4-2). Similar to adenosine, the infusion of isoproterenol at a dose of 0.91 ± 0.09

$\mu\text{g}/\text{kg}/\text{min}$ caused maximum hepatic arterial dilation with a conductance of 0.249 ± 0.035 $\text{ml}/\text{min}/\text{kg}/\text{mmHg}$. When the dose response curve of isoproterenol was repeated against a background infusion of norepinephrine, the maximum hepatic arterial conductance reached was 0.206 ± 0.034 $\text{ml}/\text{min}/\text{kg}/\text{mmHg}$, which was slightly but significantly depressed ($17.4 \pm 3.8\%$, $p < 0.01$) by the infusion of norepinephrine as shown in Figure 4-2.

4.3.3 The influence of L-NAME on the suppression of vasodilation by norepinephrine

The administration of L-NAME (2.5 mg/kg) increased hepatic arterial pressure (92 ± 4 before versus 105 ± 16 mmHg after L-NAME, $p < 0.005$) but did not affect the maximum hepatic arterial conductance induced by adenosine infusion (0.217 ± 0.033 $\text{ml}/\text{min}/\text{kg}/\text{mmHg}$ after L-NAME, NS) or by isoproterenol infusion (0.232 ± 0.029 $\text{ml}/\text{min}/\text{kg}/\text{mmHg}$ after L-NAME, NS). However, the maximum hepatic arterial conductance induced by adenosine infusion was reduced by norepinephrine infusion by $45.2 \pm 9.1\%$ (by 0.098 ± 0.026 $\text{ml}/\text{min}/\text{kg}/\text{mmHg}$ $p < 0.01$) after L-NAME; the suppression was substantially potentiated when compared to that before L-NAME ($p < 0.05$). Similarly to adenosine, isoproterenol infusion-induced maximum hepatic arterial conductance was suppressed by $45.6 \pm 7.2\%$ (by 0.101 ± 0.015 $\text{ml}/\text{min}/\text{kg}/\text{mmHg}$, $p < 0.0005$) by norepinephrine. The suppression was potentiated ($p < 0.05$). The effects of norepinephrine on vasodilations induced by adenosine and isoproterenol were significantly potentiated by the administration of L-NAME as shown in Figure 4-3. The dose response curves of adenosine and isoproterenol infusion in control and during the infusion of a maximum dose of NE before and after L-NAME from one representative experiment were plotted in

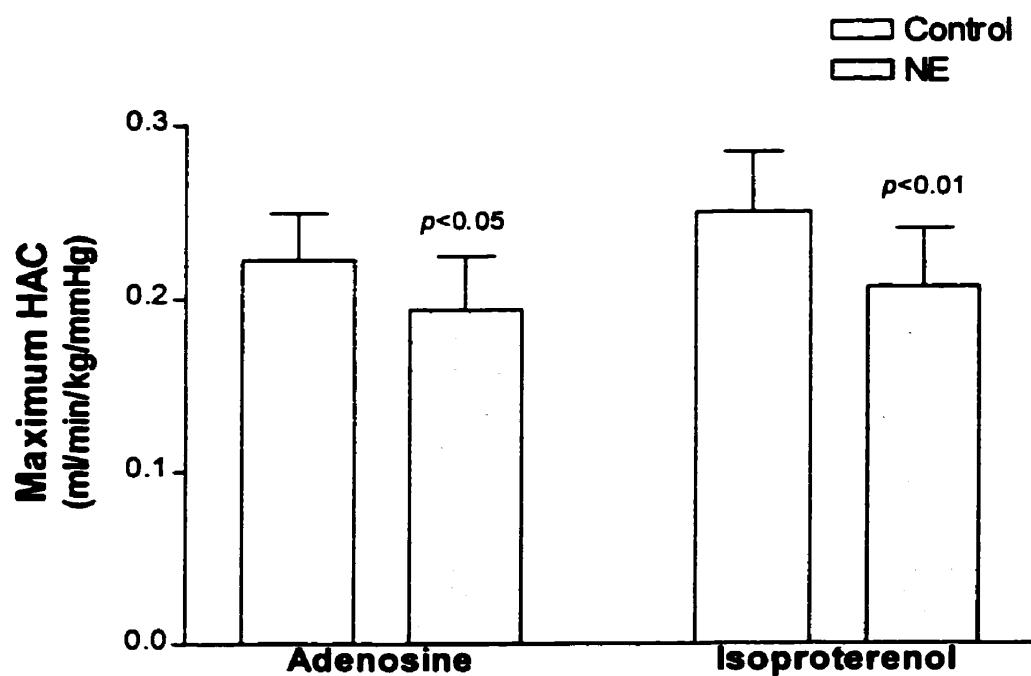


Figure 4-2. The maximum hepatic arterial conductance (HAC) induced by adenosine (1.57 ± 0.20 mg/kg/min) and isoproterenol (0.91 ± 0.09 μ g/kg/min) infusion. The maximum conductance was slightly suppressed during a background infusion of maximum dose of norepinephrine (0.49 ± 0.12 μ g/kg/min, $n=7$).

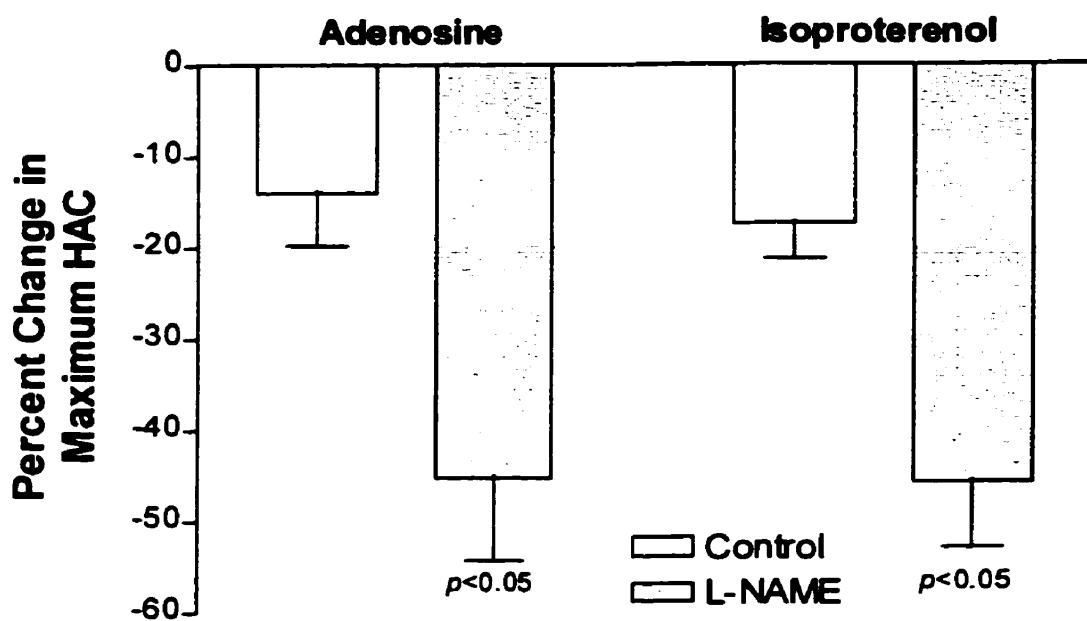


Figure 4-3. The percent changes in adenosine- and isoproterenol-induced maximum hepatic arterial conductance (HAC) in response to norepinephrine infusion. The suppression of adenosine- and isoproterenol-induced maximum hepatic arterial vasodilation by norepinephrine was potentiated in the presence of L-NAME ($n=7$).

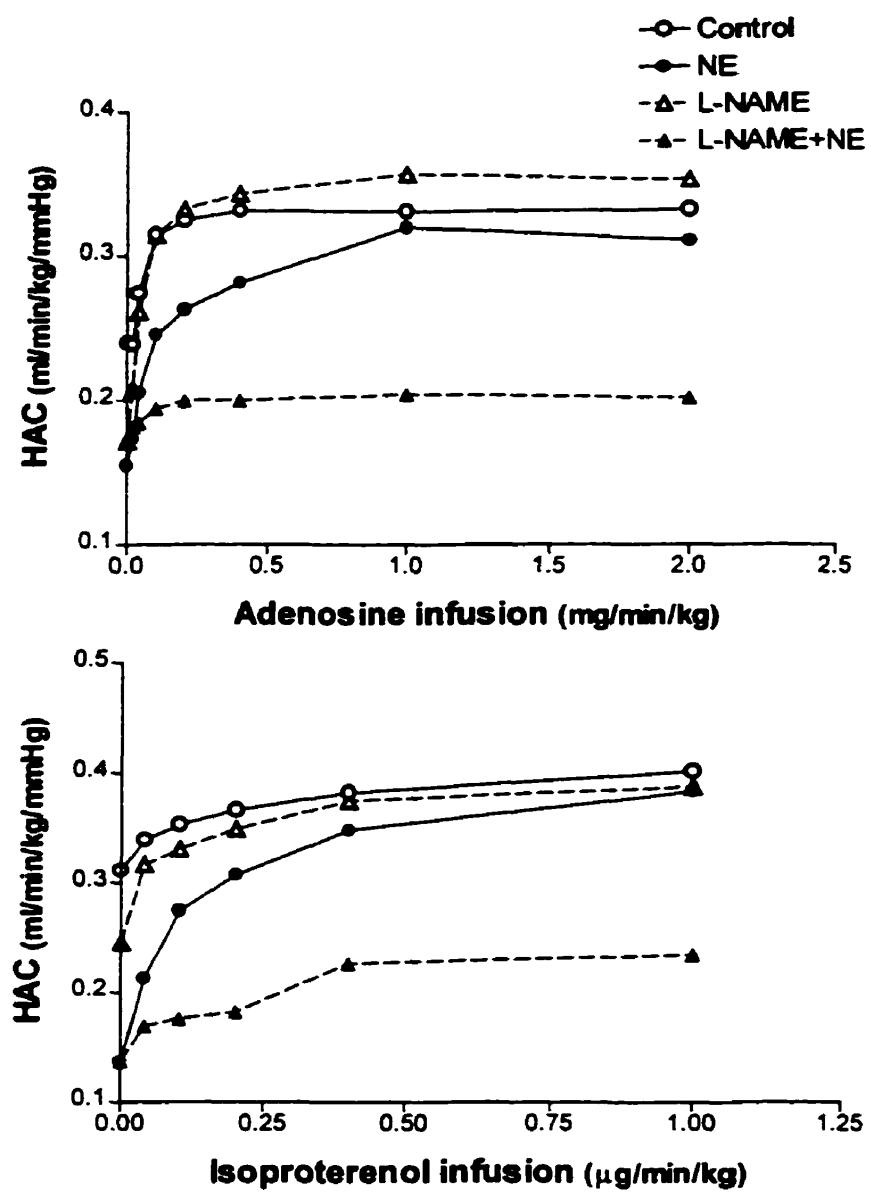


Figure 4-4. The dose response curves of adenosine and isoproterenol infusion in the absence and presence of norepinephrine infusion before and after L-NAME from one animal. The maximum responses in hepatic arterial conductance (HAC) were slightly suppressed by norepinephrine but not changed by L-NAME; however, after L-NAME, norepinephrine substantially suppressed the maximum responses. The effects of L-NAME and norepinephrine were similar for adenosine and isoproterenol.

Figure 4-4.

Thus, the vasodilator effects of adenosine and isoproterenol were slightly suppressed by norepinephrine. The blockade of NO synthase by L-NAME did not affect the vasodilator effect of adenosine or isoproterenol but potentiated both the constriction induced by NE and the suppression of the vasodilators by norepinephrine.

4. 4 DISCUSSION

In this chapter, I demonstrated the modulation of the norepinephrine vasoconstrictor effect by NO. The blockade of NO synthase by L-NAME potentiated the vasoconstrictor effect of norepinephrine, which confirmed the previous finding from our laboratory that shear stress-induced release of NO antagonized the vasoconstrictor effect of sympathetic nerve stimulation and norepinephrine infusion in the liver (Macedo and Lautt, 1998). In agreement with the previous results, the administration of L-NAME did not change the basal hepatic arterial tone, suggesting that NO was not involved in the control of basal hepatic arterial tension. The infusion of norepinephrine at a dose to produce maximum constriction, on the other hand, suppressed the vasodilator effect of adenosine, only to a small extent, implying the possibility that sympathetic activation modulates the vasodilation induced by vasodilators. Furthermore, the most important finding in the current study was the crucial role of NO in modulation of this interaction between noradrenergic vasoconstriction and vasodilators. My results showed that the blockade of NO synthase substantially potentiated the suppression of adenosine vasodilator effect by norepinephrine. The suppression was not adenosine specific since the vasodilation induced by isoproterenol was suppressed as well. In addition, I confirmed that L-NAME

per se did not affect the vasodilator response (Macedo and Lautt, 1997). The data support the hypothesis that NO suppresses not only the noradrenergic responses, but also the ability of norepinephrine to suppress the response to vasodilators.

4.4.1 Considerations of methodology

4.4.1.1 Surgery

The surgical procedures including removal of spleen and ligation of the gastroduodenal artery and gastric artery were to ensure that the blood flow through the celiac artery was exclusively into the liver. A flow probe was placed on the celiac artery to measure hepatic arterial blood flow. The anterior hepatic nerve plexus was surgically denervated to avoid any influence of systemic reflexes on the liver. Changes in portal blood flow could have influence on hepatic arterial conductance due to the hepatic arterial buffer response (Lautt, 1985). The inferior mesenteric artery was ligated and the superior mesenteric arterial blood flow was maintained using a pump to control total portal blood flow throughout the experiment.

4.4.1.2 Dose and route of administration

To study the interaction between vasodilators and norepinephrine, I infused adenosine or isoproterenol into the portal vein, so that the blood concentration of the vasodilator was not affected by change in blood flow. Norepinephrine was infused directly into the hepatic artery, but the effect of norepinephrine would not change when hepatic arterial blood flow was decreased in response to the vasoconstrictor since a maximum dose was used. To prevent any influence of baseline effect by norepinephrine infusion on the response of the vasodilators, a full dose response curve of the vasodilator was obtained and the maximum responses were used as the index.

4.4.2 The interaction of adenosine, norepinephrine and NO

The results in this study showed that these vasoregulators do not act independently. Adenosine attenuated the vasoconstrictor response to the stimulation of sympathetic nerves and norepinephrine infusion in the superior mesenteric artery (Lautt et al., 1988) and in the hepatic artery but not portal vein (Lautt and Legare, 1986). In the latter study, adenosine also suppressed the vasoconstrictor effects of angiotensin and vasopressin, suggesting that the interaction generally happens between adenosine and vasoconstrictors. Adenosine inhibited sympathetic vasoconstriction also in gracilis muscle (Klabunde, 1987), and femoral vasculature by a presynaptic mechanism (Hom and Lokhandwala, 1981) in dogs. Evoniuk et al. (1986) suggested that adenosine could affect sympathetic activity at sympathetic ganglia, nerve terminals and innervated end organs. The results in this chapter showed that a maximum dose of norepinephrine suppressed the vasodilation response to adenosine and isoproterenol, suggesting that norepinephrine suppresses the vasodilation and that there is a balance between the response to vasodilators and to vasoconstrictors in the hepatic artery.

NO attenuated adrenergic vasoconstriction in the hepatic artery (Macedo and Lautt, 1998) and sympathetic nerve-induced constriction in the superior mesenteric artery (Macedo and Lautt, 1996b) in a shear stress-dependent manner. A study (Owlya et al., 1997) in men showed that NO was also involved in the regulation of central sympathetic outflow, and the authors suggested that both neuronal and endothelial NO synthesis contribute to the regulation of vascular tone. Cellek and Moncada (1997) demonstrated that both neuronal and inducible NO modulated adrenergic activity in rabbit anococcygeus muscle. The results from the current study confirmed the suppression of

norepinephrine-induced vasoconstriction by NO in the hepatic artery. Based on the results, I am able to take this one step further to show that NO strongly modulated the suppression by norepinephrine of the vasodilation induced by adenosine and isoproterenol. The weight of evidence supports that NO is a very powerful regulator in control of vascular tone, where NO suppresses not only the vasoconstrictor response to adrenoceptor agonists but also the inhibitory role of adrenoceptor agonists on responses to vasodilators.

4.4.3 Organ specificity

NO not only attenuates vasoconstriction, it also inhibits the vasodilation induced by adenosine and isoproterenol, however, in the superior mesenteric artery and not the hepatic artery (Macedo and Lautt, 1997). In this chapter, I demonstrated that NO potentiated the vasodilator effect in the hepatic artery in an indirect way through the suppression of norepinephrine interaction with vasodilators. In the first chapter of the thesis, I discussed organ specificity in the interaction of these vasoregulators. NO was involved in the control of basal tone in the superior mesenteric artery but not in the hepatic artery (Macedo and Lautt, 1997). Data from the current study also demonstrated that blockade of NO release did not affect basal hepatic arterial conductance. Adenosine suppressed vasoconstriction induced by norepinephrine, angiotensin and vasopressin in the hepatic artery but not in the portal vein, thus demonstrating selectivity of modulation even within the same organ (Lautt and Legare, 1986). NO antagonized pressure-flow autoregulation in the superior mesenteric artery (Macedo and Lautt, 1996a) but not in the liver (unpublished data, Macedo, Han and Lautt). Autoregulation is adenosine mediated in both organs (Ezzat and Lautt, 1987; Lautt, 1986a). The effect and the role that NO

plays seem quite different according to different organs and different tissues.

Another important effect of norepinephrine in the liver is to stimulate glycogenolysis. I can not, however, answer the question if NO also inhibits the glycogenolysis effect of norepinephrine with the data from the current experiment. This question will be addressed in the next chapter. Having shown that NO modulated the vascular response to norepinephrine and the suppression was through a post-synaptic mechanism, it would be very interesting to know the metabolic effect of NO in the liver.

Many vascular diseases, such as heart failure (Kubo et al., 1991), atherosclerosis (Zeiher et al., 1991) and hypertension (Cardillo et al., 1998) are related to an impairment in NO synthesis or release. The data from the current study suggest that NO is very important for the modulation of sympathetic activities and other factors in addition to its essential vasodilator effect. Impaired NO function could result in an exaggerated cardiovascular as well as central and peripheral neuronal response to sympathetic stimulation, vasoconstrictors and vasopressors. The high organ specificity of NO function further implies the importance of NO in the control of both metabolism and vascular tone. In conclusion, NO suppressed norepinephrine induced vasoconstriction but did not affect the basal tone and vasodilation induced by adenosine and isoproterenol in the hepatic artery. The vasodilator effects of adenosine and isoproterenol were slightly suppressed by norepinephrine, and the suppression was substantially potentiated by NO blockade.

5. NITRIC OXIDE POTENTIATES NOREPINEPHRINE-INDUCED HEPATIC GLUCOSE OUTPUT

5.1 INTRODUCTION

Norepinephrine released from the terminal of sympathetic synapses and the adrenal glands plays a crucial role in the regulation of hepatic circulation and glucose metabolism. Norepinephrine elicits vasoconstriction of the hepatic artery and portal vein as well as an increase in glucose output due to the promotion of hepatic glycogenolysis mainly through the activation of α_1 -adrenergic receptors (Lautt, 1983a). The vascular effects of norepinephrine on hepatic circulation can be modulated by NO. Blockade of NO formation with L-arginine analogues, the competitive NO synthase inhibitors, potentiated norepinephrine-induced vasoconstriction in perfused rat liver (Mittal et al., 1994; Weidenbach et al., 1997). The potentiation could be reversed by L-arginine. NO inhibited the vasoconstrictor effect of norepinephrine by shear stress-dependent and post-synaptic mechanism in the liver *in vivo* (Macedo and Lautt, 1998). In that study, sympathetic nerve stimulation or norepinephrine infusion-induced vasoconstriction was potentiated by L-NAME when shear stress was allowed to increase. In the previous chapter, the potentiation of norepinephrine-induced maximal vasoconstriction in the hepatic artery by NO synthase blockade was confirmed. In addition, I also showed that the blockade of NO formation markedly potentiated the suppressor effect of norepinephrine on other vasodilators.

In the intestine, shear stress-induced NO release inhibited sympathetic nerve stimulation but not norepinephrine infusion-induced vasoconstriction (Macedo and Lautt, 1997), suggesting a pre-synaptic modulation. In contrast, in the liver, NO suppressed the

vascular action of norepinephrine by a post-synaptic mechanism on the vascular smooth muscle with no alternation in norepinephrine release (Macedo and Lautt, 1998). Therefore the metabolic effects of norepinephrine were anticipated to be unimpaired by NO release in the liver. However, increasing evidence suggests that NO affect hepatic carbohydrate metabolism through multiple pathways under physiological or pathological conditions although the published data were not consistent. Infusion of NO donor into the portal vein stimulated hepatic glucose production in perfused rat liver, the mechanism of which was ascribed to a partial activation of glycogen phosphorylase (Borgs et al., 1996). NO also inhibited glycogen synthase activity resulting in an increase in intracellular glucose 6-phosphate in cultured rat hepatocytes (Sprangers et al., 1998). Gluconeogenesis from lactate and pyruvate and glycogenolysis was inhibited by lipopolysaccharide or cytokines-induced NO formation (Ceppi and Titheradge, 1998; Stadler et al., 1995) and by exogenous NO in a time and dose-dependent manner (Horton et al., 1994) in rat hepatocyte cultures. The mechanism was related to the inhibition of phosphoenolpyruvate carboxykinase (Casado et al., 1996). NO can also affect hormone-stimulated glucose metabolism. While inhibiting glycogenolysis induced by platelet-activating factor in perfused rat liver (Moy et al., 1991) and by glucagon in rat hepatocyte culture (Ceppi and Titheradge, 1998), NO had no effect on phenylephrine (Miura et al., 1992), norepinephrine (Weidenbach et al., 1997) or PGF_{2α} (Weidenbach et al., 1995) induced changes in glucose metabolism in perfused rat liver. So far, no systematic study on the effects of NO on norepinephrine-induced glucose metabolism *in vivo* has been reported.

The purpose of this study was to investigate whether NO has regulatory effects on norepinephrine-induced glucose metabolism *in vivo*. The data in this chapter indicated

that whereas NO inhibits hepatic vascular responses to norepinephrine, it potentiates the glycogenolytic response *in vivo*.

5.2 METHODS AND PROTOCOLS

5.2.1 Surgical preparation

General surgery and the instrumentation of the animals were previously described. Briefly, seven cats of either sex, weighing 3.4 ± 0.2 kg, were anesthetized. Systemic arterial pressure was monitored and arterial blood samples were taken from a catheter in the right carotid artery. Central venous pressure was monitored from a cannula inserted via the right femoral vein into the central vena cava with the cannula tip located about 2 cm above the diaphragm. The same cannula was used for taking blood samples from the hepatic vein. After laparotomy, a snare was put around the inferior vena cava at the level above both the renal veins so that a brief occlusion by the snare will block blood flow coming from the inferior vena cava, leaving the only source of blood above the snare being from the hepatic vein.

The gastroduodenal artery was ligated. The superior mesenteric nerve plexus and anterior hepatic nerve plexus were gently separated and cut to prevent any reflex influences. The celiac artery was cleared of surrounding tissues and all its branches were ligated except the common hepatic artery. This methodology assured that celiac arterial blood flow exclusively perfused the liver through the hepatic artery. The inferior mesenteric artery was ligated to assure that all portal blood flow is from the superior mesenteric artery as previously described (Lautt et al., 1985). The superior mesenteric artery was separated and cleared of surrounding tissues. Two clamp-type electromagnetic

flow probes (EP408, size 6 or 8, Carolina Medical Electronics, Inc., King, NC) were placed around the superior mesenteric and celiac arteries, respectively, for the measurements of blood flows. Two i.v. catheters (24G OptivaTM, Medical Inc.) were inserted into the portal vein for drug delivery and portal venous pressure measurement. The arterial pressure, central venous and portal pressure were measured using pressure transducers (Gould and Statham, Gould Inc., Oxnard, CA) as described in the methodology chapter.

5.2.2 Sampling

The blood samples from carotid artery, portal vein and hepatic vein were taken at different times during the experiment for the analysis of blood glucose concentration using the glucose analyzer (Yellow Springs Instruments). The order of sampling is artery, portal vein and then hepatic vein. The hepatic venous blood samples were taken 8 seconds after constricting the snare. This time was estimated for the hepatic venous blood to wash out mixed central venous blood (Lautt, 1982), so that a pure hepatic venous sample can be collected through the venous cannula, with its tip located at 2 cm above the diaphragm.

5.2.3 Hepatic glucose output calculation

Total hepatic glucose output (THGO) was calculated as follows:

$$\text{THGO} = [(C_{HV} - C_A) \times F_{HA} + (C_{HV} - C_{PV}) \times F_{SMA}] / \text{body weight}$$

Where C is glucose concentration measured during the experiment; the subscripts indicate the site of measurement, HV for the hepatic vein, A for the carotid artery, PV for the portal vein. F is the blood flow in the celiac artery (subscripted by HA) and in the superior mesenteric artery (subscripted by SMA); the latter was equal to the portal blood

flow in the experimental setup.

5.2.4 Protocols

The animals were allowed to stabilize after the surgery for at least 1h until stable basal hemodynamics and arterial glucose concentration were achieved. Then the animals were pre-challenged twice with norepinephrine infusion (0.15 µg/kg/min, i.p.v.) to ensure the stabilization of the glycogenolytic responses to norepinephrine thereafter. Preliminary experiments in four cats showed that the glucose responses were stable in the following five infusions of norepinephrine. Then the following protocols were tested.

5.2.4.1 Effects of SIN-1 on basal and norepinephrine induced hepatic glucose output

In seven animals, the effects of the NO donor, SIN-1 (0.1 mg/kg, i.p.v.), on norepinephrine-induced hepatic glucose output were examined when stable hemodynamics and arterial glucose concentrations were achieved. Intra-portal infusions of norepinephrine (0.15 µg/kg/min) were administered before SIN-1 as a control response and 10 min after SIN-1 administration. Blood samples from the carotid artery, portal vein and hepatic vein for the glucose determination were taken in the control condition and 2 minutes after norepinephrine infusion.

5.2.4.2 Effect of cGMP blockade

To further investigate the involvement of NO and its second messenger cGMP in SIN-1 induced changes in basal and norepinephrine-induced glucose metabolism, the same protocol was repeated 10 min after administration of a guanylate cyclase inhibitor, methylene blue, (300 µg/kg/min, for 10 min, i.p.v.) in 5 of 7 cats in the same group.

5.3 RESULTS

5.3.1 Hemodynamic response to norepinephrine infusion

Norepinephrine infusion caused vasoconstriction in both hepatic and systemic vascular beds, as indicated by the increases in portal venous pressure (by 1.5 ± 0.2 from 6.8 ± 0.3 mmHg, $p < 0.01$) and arterial pressure (by 26 ± 4 from 112 ± 5 mmHg, $p < 0.005$). After the administration of SIN-1, basal arterial pressure fell to 74 ± 4 mmHg ($P < 0.01$) whereas portal venous pressure was not altered. The vasoconstriction induced by norepinephrine infusion in the portal circulation was attenuated after SIN-1. The increases in portal venous pressure only reached 1.2 ± 0.2 mmHg, significantly less than that before SIN-1 ($P < 0.05$, Figure 5-1).

The superior mesenteric arterial blood flow was increased from 12.9 ± 1.2 to 14.5 ± 1.4 ml/min/kg ($p < 0.05$), while hepatic arterial flow decreased from 13.1 ± 1.7 to 10.9 ± 1.9 ml/min/kg ($p < 0.01$) during norepinephrine infusion. SIN-1 decreased hepatic arterial flow (from 14.2 ± 2.0 to 9.3 ± 1.3 ml/min/kg, $p < 0.005$) but did not alter the hepatic arterial conductance. The change in hepatic arterial blood flow and conductance induced by norepinephrine infusion were similar to those before SIN-1 (Figure 5-1).

Therefore, the introduction of exogenous NO had no significant influence on basal vascular tone but attenuated norepinephrine-induced vasoconstriction in portal circulation.

Methylene blue used after SIN-1 restored the norepinephrine-induced response in portal venous pressure (2.0 ± 0.3 mmHg, $P < 0.01$ vs SIN-1) and potentiated the vasoconstrictor effect of norepinephrine in the hepatic artery (Figure 5-1).

5.3.2. Response in hepatic glucose metabolism to norepinephrine

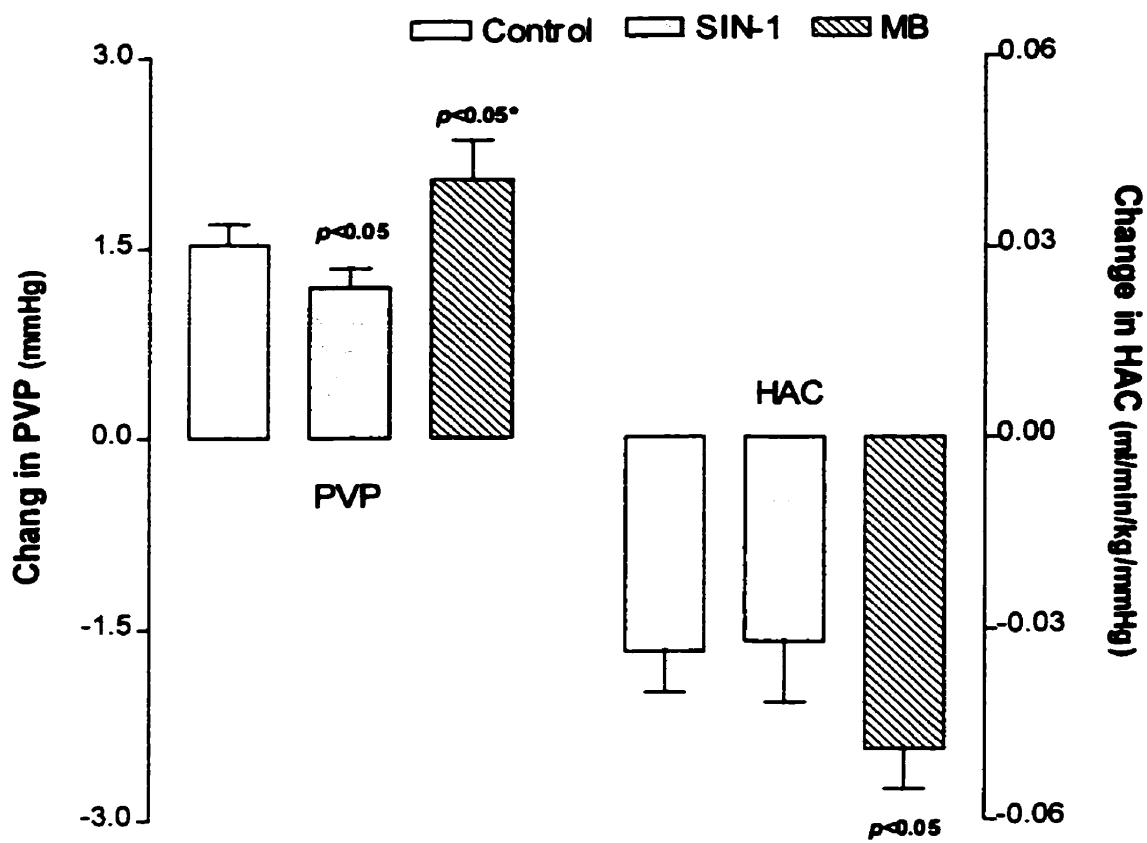


Figure 5-1. Change in hepatic hemodynamics induced by norepinephrine infusion (0.15 $\mu\text{g}/\text{kg}/\text{min}$, i.p.v.) in the presence of SIN-1 and methylene blue (MB). SIN-1 inhibited norepinephrine-induced increase in portal pressure (PVP) ($n=7$) and did not change norepinephrine-induced decrease in hepatic arterial conductance (HAC). Methylene blue reversed the inhibition by SIN-1 in the portal vein and potentiated the vasoconstrictor effect of norepinephrine in the hepatic artery ($n=5$, *vs SIN-1).

Intra-portal infusion of norepinephrine for 2 min increased arterial glucose concentration by 5 ± 1 from 113 ± 7 mg/dl ($P<0.05$), hepatic venous glucose concentration by 18 ± 1 from 130 ± 7 mg/dl ($P<0.05$). The total hepatic glucose output calculated increased by 5.3 ± 0.4 from 5.0 ± 0.5 mg/min/kg ($P<0.01$). Portal venous glucose concentration remained unchanged after norepinephrine infusion. SIN-1 stimulated glucose output from the liver. SIN-1 increased arterial glucose concentration by 3 ± 1 mg/dl ($P<0.05$), hepatic venous glucose concentration by 9 ± 3 mg/dl ($P<0.01$) and total hepatic glucose output by 1 ± 0.4 mg/min/kg ($P<0.05$) while no changes in portal venous glucose concentration occurred. Furthermore, SIN-1 significantly augmented NE-induced glucose release from the liver. After SIN-1, arterial glucose concentration increased by 11 ± 2 from 114 ± 8 mg/dl ($p<0.01$), portal glucose concentration by 7 ± 2 from 104 ± 6 mg/dl ($p<0.01$), hepatic venous glucose concentration by 40 ± 2 from 133 ± 9 mg/dl ($p<0.01$) and total hepatic glucose output increased by 7.2 ± 0.6 from 5.3 ± 0.7 mg/min/kg ($p<0.01$) during norepinephrine infusion. The values are significantly higher than those before SIN-1. The changes in glucose concentration at different sites are shown in Figure 5-2. Norepinephrine induced total hepatic glucose outputs before and after SIN-1 are shown in Figure 5-3.

Methylene blue used after SIN-1 significantly inhibited the effects of SIN-1 on potentiation of norepinephrine-induced hepatic glucose responses (Figure 5-2 and 3).

The NO donor, SIN-1, slightly increased hepatic glucose output while substantially potentiated norepinephrine-induced increase in hepatic glucose output.

5.4 DISCUSSION

The results in this chapter provided evidence that NO plays an important role in

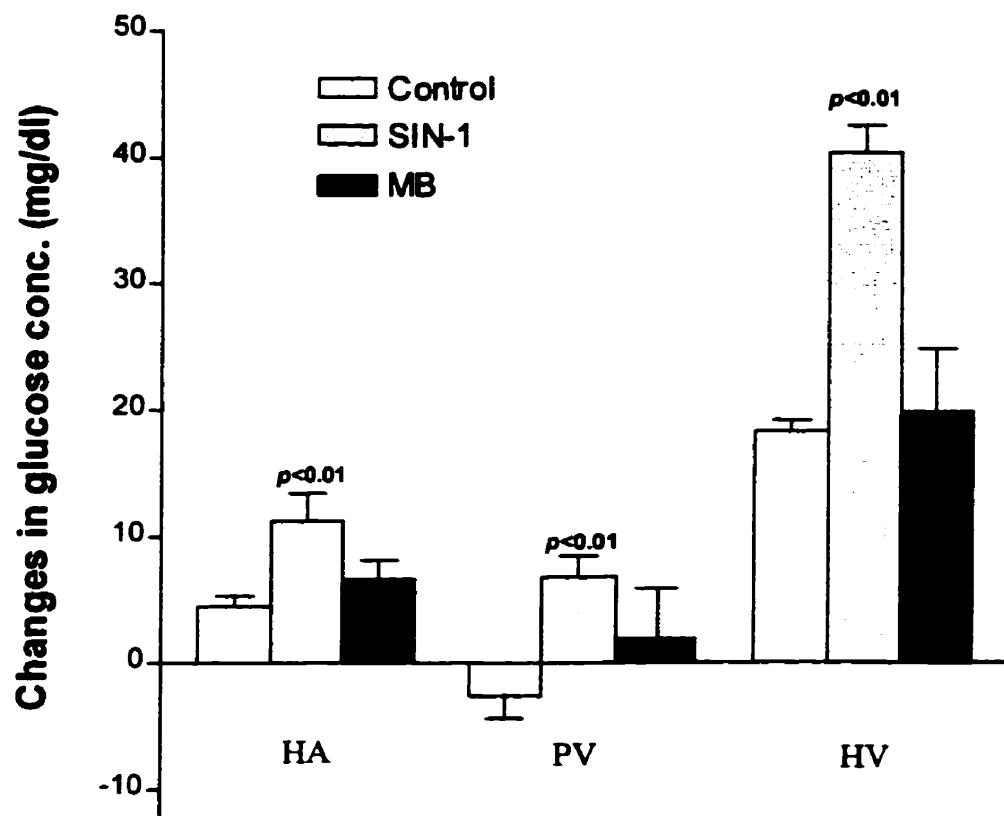


Figure 5-2. Changes in hepatic arterial (HA), portal venous (PV) and hepatic venous (HV) glucose concentrations induced by norepinephrine infusion (0.15 $\mu\text{g}/\text{kg}/\text{min}$, i.p.v.) in the presence of SIN-1 ($n=7$) and methylene blue (MB, $n=5$). SIN-1 potentiated norepinephrine-induced increases in glucose concentration in the hepatic artery, venous and portal venous. Methylene blue reversed the potentiation.

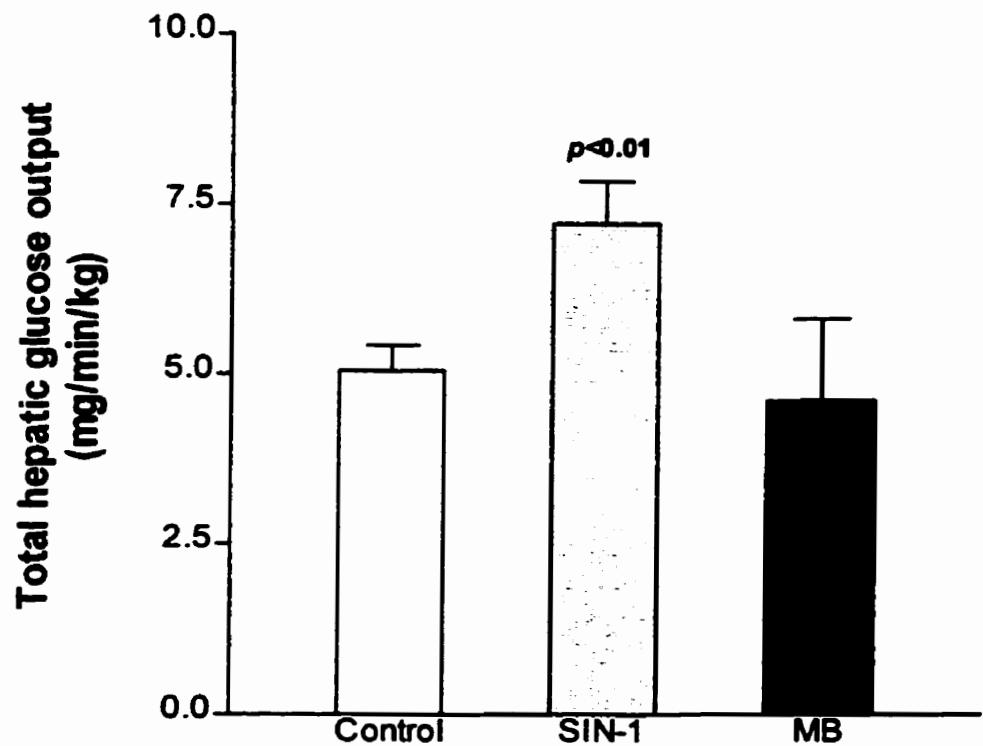


Figure 5-3. Norepinephrine-induced increase in hepatic glucose output in the presence of SIN-1 (n=7) and methylene blue (MB, n=5). The calculated total hepatic glucose output induced by norepinephrine infusion (0.15 μ g/min/kg) was potentiated by SIN-1 and the potentiation was reversed by methylene blue.

regulating both hepatic vascular and glucose metabolic responses to norepinephrine in the cat *in vivo*. The NO donor, SIN-1, potentiated norepinephrine-induced increase in arterial and hepatic venous blood glucose concentration and hepatic glucose output, while significantly decreasing portal vascular response to norepinephrine. Furthermore, the potentiation by NO of norepinephrine-induced increases in hepatic glucose output as well as arterial and hepatic venous blood glucose concentrations was inhibited by a guanylate cyclase inhibitor, methylene blue, confirming the regulatory role of NO and suggesting that the action was through cGMP pathway. That the administration of SIN-1 slightly increased hepatic glucose output suggested NO might also have direct effect on hepatic glucose output. To my knowledge, this is the first study performed *in vivo* showing NO as a regulator participating in norepinephrine-induced glycogenolysis in the liver.

5.4.1 Data from a study in rats

The data from a simultaneous ongoing study in rats (Ming et al., 1999) in our laboratory demonstrated that norepinephrine-induced elevation in arterial glucose content was inhibited by blockade of NO formation with L-NAME but potentiated by SIN-1. The potentiation of glucose metabolic response to norepinephrine by SIN-1 was abolished after cyclooxygenase blockade using indomethacin, suggesting that the cyclooxygenase product, prostaglandin(s), mediated these reactions. These results are consistent with the finding in the current study and further suggest the involvement of prostaglandin(s).

The vascular response to norepinephrine in the portal vein was also potentiated by L-NAME and inhibited by SIN-1. The blockade of prostaglandin formation with indomethacin prevented the inhibitory effect of NO on norepinephrine-induced vasoconstriction in the portal vein. The data from the rat study suggested that a

vasodilator prostaglandin released secondary to NO participated at least in part in this interaction.

5.4.2 Influence of NO on portal response to norepinephrine

The contribution of NO to the regulation of hepatic vascular tone was reviewed in Chapter 1. The results from the current study showed that the NO donor, SIN-1, had insignificant influence on basal portal venous pressure, confirming our previous observation that NO is not important in maintaining basal portal vascular tone (Macedo and Lautt, 1998; Macedo and Lautt, 1997). The NO donor, SIN-1, inhibited norepinephrine-induced portal vasoconstriction in the present study. In contrast, NOS inhibitor, L-NAME, significantly enhanced norepinephrine-induced portal vasoconstriction as demonstrated in the rat study (Ming et al., 1999). These results supported that NO inhibited the vasoconstrictor effects of norepinephrine. Similarly, it has been shown that, in perfused rat liver, either direct infusion of NO donor into the portal vein (Miura et al., 1992) or endogenous activation of NO synthase (Weidenbach et al., 1997) inhibited the vasoconstrictor effects of norepinephrine. Although norepinephrine may directly target its receptors located on the endothelial cells of portal vasculature to cause NO formation, the most likely mechanism responsible for NO formation during vasoconstriction is the increase in shear stress. Indeed, L-NAME only potentiated norepinephrine-induced hepatic vasoconstriction when shear stress was allowed to increase (Macedo and Lautt, 1998).

Furthermore, the results from our study in rat (Ming et al., 1999) suggested the involvement of prostaglandins in the inhibition of norepinephrine-induced portal vasoconstriction by NO. Blockade of prostaglandin formation with indomethacin

prevented the inhibitory effect of NO in the portal circulation. The data suggested that vasodilator prostaglandins released secondary to NO mediated the reaction. It has been shown in rat that NO increased cyclooxygenase activity thereby promoting the production of PGI₂ (Salvemini et al., 1996), and in rat mesenteric vasculature that L-NAME decreased the production of PGI₂ enhancing norepinephrine-induced vasoconstriction (Soma et al., 1996).

5.4.3 Influence of NO on basal glucose metabolism

The results from the present study and in our rat study (Ming et al., 1999) showed that the NO donor, SIN-1, slightly but significantly elevated basal hepatic glucose output. In the latter study, we also showed that acute administration of L-NAME slightly but significantly decreased baseline arterial glucose concentration in rats. These data strongly support the regulatory role of NO on basal glucose metabolism in the liver.

The published results on the influence of NO on basal hepatic glucose metabolism were not consistent. Tong et al. observed an increase in arterial glucose concentration in normal and hypertensive rats after chronic administration of L-NAME (Tong et al., 1997). An unchanged basal glucose level was found after L-NAME by other investigators (Bursztyn et al., 1997). Previous studies indicated that NO might affect glycogenolysis, glycogen synthesis and gluconeogenesis to regulate hepatic glucose metabolism (Ayuse et al., 1995; Borgs et al., 1996; Sprangers et al., 1998). Consistent with our data, Borgs et al observed a transient dose-dependent increase in glucose production during NO infusion in perfused liver from fed rats (Borgs et al., 1996). The enhanced glycogenolysis was due to a partial activation of glycogen phosphorylase as indicated by a simultaneous increase in the activity of glycogen phosphorylase and lack of NO effects on glucose

output in the phosphorylase-kinase-deficient rats.

5.4.4 Influence of NO on norepinephrine-induced changes in glucose metabolism

The results in this chapter demonstrated that NO potentiated norepinephrine-induced increase in hepatic glucose production. The data from our rat study (Ming et al., 1999) supported this observation. However, previous investigations suggested that NO had no significant influence on the α_1 -agonist-, phenylephrine (Moy et al., 1991) or norepinephrine (Weidenbach et al., 1997) induced glucose metabolism using isolated perfused liver. The reason for this discrepancy in results is currently unclear. The isolated livers may have a different reaction pattern compared with *in vivo* animals. For example, the degradation of hepatic glycogen following the preparation of the isolated liver perfusion model (Glinsmann et al., 1969) may result in a blunted glucose response to the stimuli. This might explain why in another study, where preserved glycogen stores were attained by glucose preloading, NO did increase glucose output (Borgs et al., 1996). Furthermore, the release of NO following norepinephrine stimulation is a shear stress dependent phenomenon in the hepatic vasculature (Macedo and Lautt, 1998). Our data also suggested the involvement of prostaglandin. The endothelium in response to elevated shear stress is the major source of NO and prostaglandin formation. Shear stress might not change in isolated constant-pressure-perfused liver (Weidenbach et al., 1997).

Increased hepatic glycogenolysis following norepinephrine stimulation is due to an inositol 1,4,5-trisphosphate (IP_3)-linked, Ca^{2+} -dependent activation of glycogen phosphorylase (Miura et al., 1992). NO has been recognized as an important regulator in controlling different cellular Ca^{2+} homeostatic processes (Clementi and Meldolesi, 1997). Guihard et al observed an unique phenomenon in the hepatocytes that NO/cGMP

potentiated norepinephrine and IP₃-evoked Ca²⁺ release from the intracellular stores (Guighard et al., 1996), which is distinct from the inhibitory effect of NO on Ca²⁺ release observed in other tissues including the smooth muscle, myocytes, and platelets. This is a mechanism relevant for increasing the apparent affinity of IP₃ receptors for IP₃, which induced sustained [Ca²⁺]_i oscillations (Guighard et al., 1996; Rooney et al., 1996). The unique action of NO in the hepatocytes could explain, at least partially, the blockade by the guanylate cyclase inhibitor, methylene blue, of the NO-mediated potentiation of NE-induced hepatic glucose production.

5.4.5 Involvement of prostaglandin in the modulator effects of NO on glucose metabolism

NO-stimulated production of glucose may be a prostaglandin-dependent phenomenon (Borgs et al., 1996). Indomethacin decreased basal glucose level and inhibited norepinephrine-induced glycogenolysis (Miller et al., 1985; Ming et al., 1999). Indomethacin also inhibited NO-induced glucose increases in basal condition, and furthermore, indomethacin abolished the potentiating effect of NO on norepinephrine-induced glycogenolysis (Ming et al., 1999). The data suggest that prostaglandin mediated the effect of NO on hepatic glucose metabolism both in basal and stimulated conditions.

In the liver, prostaglandins are mainly synthesized by Kupffer cells and cause glycogenolysis through the cAMP-linked Ca²⁺ mobilization (Decker, 1990). It has been demonstrated in most but not all previous studies that NO can promote the release of prostaglandins such as PGI₂ and E₂ from different tissues, and the mechanism of which has been attributed to the direct activation of cyclooxygenase by NO (Salvemini, 1997). Prostaglandin(s) involvement is secondary to the activation by NO since SIN-1 effects are not seen if prostaglandin(s) production is blocked (Ming et al., 1999). The precise

mechanism involved in NO/prostaglandin-mediated potentiation on norepinephrine-induced glucose production is currently unclear, however, it may relate to the augmented effects of cGMP/cAMP (the intracellular messengers for NO/prostaglandins) on IP₃ (the intracellular messenger for norepinephrine) evoked intracellular Ca²⁺ mobilization. In cultured hepatocytes, it has been found that both cGMP and cAMP potentiated IP₃-evoked intracellular Ca²⁺ mobilization (Guillard et al., 1996; Hajnóczky et al., 1993).

In response to a variety of stresses, norepinephrine released from sympathetic nerve endings or adrenal glands would cause severe hepatic vasoconstriction, which would increase shear stress at the endothelium, leading to the release of NO. The role of NO in modulation of the effect of norepinephrine fully fits the physiological requirement in both hemodynamics and metabolism in that post-synaptic action of NO in the liver (Macedo and Lautt, 1998) permits NO to protect the vasculature against elevated shear stress but maintains and potentiates the glycogenolysis that leads to increased glucose supply to extra-hepatic tissues. In conclusion, the present study showed that the influence of NO on hepatic hemodynamics is distinct from that on glucose metabolism in response to norepinephrine: while NO inhibited hepatic vascular response to norepinephrine, NO potentiated norepinephrine-evoked hepatic glucose output. Data from our other study suggest that prostaglandins intervene in these reactions.

6. SHEAR STRESS-INDUCED NITRIC OXIDE ANTAGONIZES ADENOSINE EFFECTS ON INTESTINAL METABOLISM

6.1 INTRODUCTION

Adenosine and NO are endogenous substances playing important roles in the control of splanchnic blood flow (Macedo and Lautt, 1996a; Macedo and Lautt, 1996b; Sawmiller and Chou, 1992). Adenosine dilates resistant vessels (Collis, 1989) to increase blood flow and oxygen supply to local tissues. NO released from endothelial cells by shear stress results in vascular smooth muscle relaxation through a cGMP dependent mechanism (Moncada et al., 1991). It has been shown in our laboratory that NO release in the intestine is shear stress dependent (Macedo and Lautt, 1996b). An interesting aspect of that study was that shear-dependent NO suppressed the constrictor response to nerve stimulation but did not act on norepinephrine-induced constriction, thus leading to the conclusion that the shear-dependent release of NO caused by these constrictors did not result in a direct effect on vascular smooth muscle but rather acted by decreasing transmitter release.

The metabolic effects of adenosine include the suppression of oxidative metabolism leading to a reduction of oxygen consumption (Granger et al., 1978), as well as the promotion of anaerobic glycolysis (Lasley and Mentzer Jr, 1993; Zhang and Lautt, 1993). These effects of adenosine further balance the ratio of oxygen demand and supply. The hemodynamic and metabolic effects of adenosine have been interpreted to be protective in different pathological situations (Ely and Berne, 1992). NO has been demonstrated *in vitro* to inhibit aerobic metabolism through inhibition of mitochondrial enzymes (Geng et al., 1992; Stadler et al., 1991), but the function of NO on metabolism is still unclear *in*

vivo.

Adenosine and NO may interact in many physiological and pathological conditions. Adenosine is released during hypoxic and ischemic conditions. NO synthase activation and increased NO production have been reported under similar conditions (Xu et al., 1995). NO as well as adenosine has been suggested to be involved in ischemia reperfusion (Peralta et al., 1997) and hypoxia-induced vasodilation (Pelligrino et al., 1995). The potential use of NO and adenosine in a therapeutic approach to reduce surgical ischemic-reperfusion injury has been discussed (Vinten-Johansen et al., 1995).

It was found previously in our laboratory that NO antagonizes the vasodilator effects of adenosine (Macedo and Lautt, 1997) and attenuates pressure-flow autoregulation (Macedo and Lautt, 1996a) that is known to be mediated by adenosine in the superior mesenteric vascular bed. However, the interaction of adenosine and NO in control of intestinal metabolism is unknown. Based on our early observations (Macedo and Lautt, 1997), I hypothesized that NO also antagonizes adenosine effects in the control of intestinal metabolism. In this chapter, the hypothesis was tested in an intestinal segment in cats. The effects of adenosine on local metabolism and the possible role of NO were investigated. The role of NO was explored by stimulating endogenous NO formation with elevated shear stress, by decreasing endogenous NO production by blocking NO synthase, or by administration of a NO donor. My results demonstrated that adenosine-induced decrease in oxygen consumption and increase in lactate production in the small intestine were antagonized by either shear stress-released or exogenously administered NO.

6.2 METHODS AND PROTOCOLS

6.2.1 Surgical preparation

Cats, either gender, weighing 3.9 ± 0.2 kg, were prepared as described previously. Briefly, the animal was anesthetized and cannulated for monitoring the pressures. The respiration was mechanically assisted. After removal of the spleen, the large intestine including ascending, transverse and part of the descending colon was removed after ligation of the inferior mesenteric artery and vein. The upper end of the intestinal segment was defined by double ligation and transection at the duodenum at a location that allowed pancreatic venous drainage. The superior mesenteric nerve plexus was separated and cut. A length of lower abdominal aorta was separated and double cannulated below the renal branches providing a shunt through a T-connector to the superior mesenteric artery through a pump-controlled (Masterflex, Cole Parmer Instrument Co., Barrington, IL) vascular circuit (see Figure 3-1). The superior mesenteric artery was cannulated, and the circuit flow rate was gradually increased to generate a circuit pressure that was slightly higher than systemic pressure to countervail circuit resistance. The pressure line in the circuit was also used for sampling. A venous catheter unit was placed into the portal vein for monitoring portal venous pressure and for blood sampling. The flow rate was measured using a flow-through electromagnetic flow probe (EP608, Carolina Medical Electronics, Inc., King, NC). All pressures were monitored by pressure transducers (Gould and Statham, Gould Inc., Oxnard, CA). The calibration of the instrument was described previously. At the end of each experiment, the defined intestinal segment was removed from the carcass, and a wet tissue weight was obtained after intestinal contents were cleaned out.

6.2.2 Experimental protocols

6.2.2.1 The effects of adenosine during constant flow perfusion

In order to study the effect on intestinal metabolism, adenosine at a dose providing maximum vascular response (0.4 mg/kg/min) (Macedo and Lautt, 1997) was infused into the superior mesenteric artery via an infusion line in the circuit during constant flow perfusion. The superior mesenteric arterial flow rate was maintained while circuit pressure dropped due to the vasodilator effect of adenosine during the constant flow perfusion. Blood samples from the arterial circuit sampling line and a portal venous catheter were taken simultaneously, 5 minutes before the infusion as control and after 5 minutes of adenosine infusion. The infusions of adenosine were then repeated after NO synthase blockade by L-NAME given as an intravenous infusion over 10 minutes (2.5 mg/kg in 10 ml). The first control sample after L-NAME was taken 10 minutes after completion of administration.

6.2.2.2 The effects of adenosine during constant pressure perfusion

In order to assess the role of NO, constant perfusion pressure was also maintained to elevate the shear stress during adenosine infusion. During a constant pressure perfusion, the blood flow rate was adjusted using the pump to maintain a circuit pressure as close to the control as possible. To compensate for the vasodilator effect of adenosine, the blood flow was increased. Blood samples were taken, and adenosine infusion, under constant pressure perfusion, was repeated after NO synthase blockade as described in protocol 6.2.2.1. Protocol 6.2.2.1 and 2 were performed in the same animal. A ten-minute interval was allowed between each test. The order of constant pressure and constant flow infusion was randomly assigned.

6.2.2.3 The influence of SIN-1 on adenosine metabolic effect during constant flow perfusion

The effects of exogenous NO were investigated using a NO donor, SIN-1, in another group of animals. The effects of adenosine infusion under constant flow perfusion were compared in the absence and presence of SIN-1. Two doses of SIN-1 were given as accumulative bolus injections (0.1 and 0.2 mg/kg, i.v., injected over 1 min). Adenosine infusion was tested 5 minutes after each dose of SIN-1.

6.2.2.4 The effect of isoproterenol on intestinal metabolism

In order to assess the effect of hemodynamic changes on intestinal metabolism, the effects of another vasodilator, isoproterenol, on intestinal hemodynamics and metabolism were studied in the same experimental design as adenosine during constant flow perfusion. The dose of isoproterenol (0.2 µg/kg/min) was selected to generate similar hemodynamic effects as adenosine infusion did. Blood samples from arterial and portal venous sites were taken at 5 minutes of the infusion.

6.2.3 Sampling and analysis

The hemodynamics in the superior mesenteric artery during adenosine infusion was normally stabilized within two minutes. In a previous study (Granger et al., 1978), adenosine effects on oxygen consumption in cat ileum were found to stabilize in about two minutes. The blood samples were taken at five minutes during adenosine infusion. An aliquot of 0.5 ml of blood for each sample was withdrawn into a bubble-free syringe for the assays. Oxygen contents in arterial and venous blood were measured using a LexO₂ Con-K oxygen content analyzer (Lexington Instruments, Waltham, MA). Blood lactate and glucose concentrations were analyzed using lactate and glucose analyzers

(YSI Sports Industrial analyzer, Yellow Springs, Oh). The instruments were calibrated as previously described.

6.2.4 Data calculation

Oxygen consumption and lactate production were calculated by the product of arterial-venous concentration difference and blood flow, normalized for the tissue weight and expressed as $\mu\text{mol}/\text{min}/100\text{g}$ tissue. The superior mesenteric arterial blood flow rate was normalized for the tissue weight and expressed as $\text{ml}/\text{min}/100\text{g}$ tissue.

6.3 RESULTS

6.3.1 Basal condition of the animals

Two groups of animals ($n=7$ and $n=6$) were used in this study. The basal hemodynamics and metabolism of these animals were stabilized within an hour after the surgery. Upon stabilization, the systemic and superior mesenteric arterial pressures were 116 ± 4 and 117 ± 6 mmHg respectively while the superior mesenteric arterial flow was 38.6 ± 4.8 $\text{ml}/\text{min}/100\text{g}$ tissue. The basal glucose and lactate levels were 101 ± 6 mg/dL (5.61 ± 0.34 mmol/L) and 1.95 ± 0.18 mmol/L while the basal oxygen consumption and lactate production across the intestine were 69.2 ± 6.2 and 7.22 ± 3.36 $\mu\text{mol}/\text{min}/100\text{g}$ tissue. The hematocrits at the start of the experiment was 31.4 ± 1.0 % and slightly dropped through the experiment (28.1 ± 1.2 % at the end, $p < 0.05$) due to the blood sampling.

6.3.2 Effects of ADO infusions on oxygen consumption and lactate production

Infusion of adenosine during constant flow perfusion decreased circuit pressure from 122 ± 8 to 50 ± 5 mmHg ($n=7$, $p < 0.001$) (Figure 6-1). Oxygen consumption

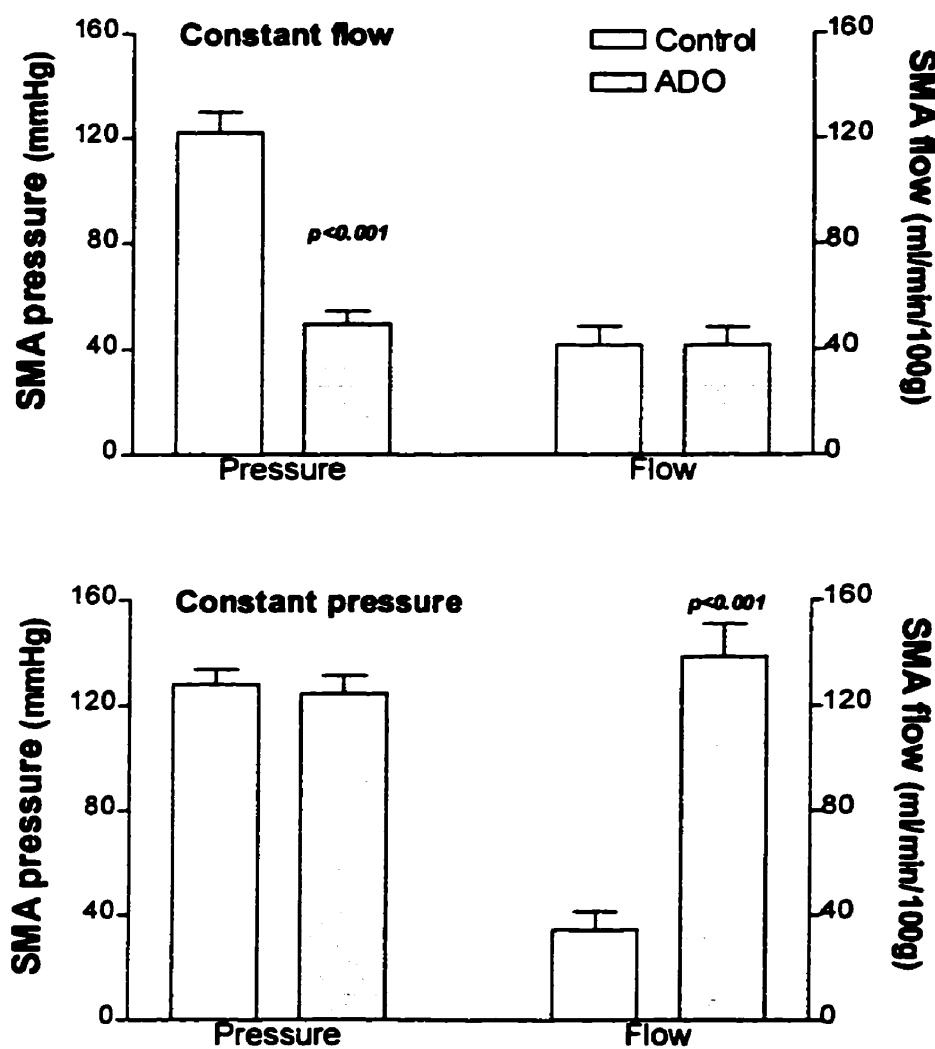


Figure 6-1. Superior mesenteric arterial (SMA) hemodynamics in control and during adenosine (ADO) infusion under constant flow and constant pressure perfusions. Adenosine (0.4 mg/kg/min) was infused intra-arterially. (n=7)

across the perfused tissue was reduced from 73.1 ± 10.8 to 48.0 ± 10.2 $\mu\text{mol}/\text{min}/100\text{g}$ ($p < 0.0001$) whereas the production of lactate was doubled (26.1 ± 3.2 versus 12.8 ± 4.6 $\mu\text{mol}/\text{min}/100\text{g}$ of control, $p < 0.005$) as shown in Figure 6-2. Infusion of adenosine in the constant pressure perfusion increased the superior mesenteric arterial flow from 34.3 ± 7.1 to 139 ± 12 ml/min ($p < 0.001$) while the perfusion pressure did not change (Figure 6-1). However, in response to adenosine, the oxygen consumption (76.1 ± 11.8 versus 72.4 ± 11.6 $\mu\text{mol}/\text{min}/100\text{g}$, NS) and lactate production (20.6 ± 7.9 versus 16.8 ± 5.2 $\mu\text{mol}/\text{min}/100\text{g}$, NS) were not altered when shear stress was elevated by constant pressure perfusion (Figure 6-2). Thus, infusion of adenosine decreased oxygen consumption and increased lactate production in constant flow (shear stress decreased) but not in constant pressure perfusion (shear stress increased).

6.3.3. The effect of NO synthase blockade on ADO effects

Administration of L-NAME, a NO synthase blocker, increased circuit perfusion pressure and tended to increase systemic arterial pressure. The superior mesenteric arterial pressure reached a plateau in 10 minutes after L-NAME infusion and lasted for approximately 3 hours. The systemic pressure before and 10 minutes after L-NAME was 117 ± 7 and 130 ± 7 mmHg respectively (NS). The superior mesenteric arterial pressure was more sensitive to the blockade of NO synthase. The perfusion pressure increased from 113 ± 6 to 162 ± 10 mmHg ($p < 0.01$) after L-NAME at constant flow (37.5 ± 7.6 to 34.8 ± 7.7 ml/min/100g after L-NAME). Basal oxygen consumption (83.6 ± 15.0 before L-NAME and 81.6 ± 17.4 ml/min/100g after, NS) and lactate production (12.5 ± 5.4 before L-NAME and 14.9 ± 5.5 ml/min/100g after, NS) across the tissue were not altered by L-NAME.

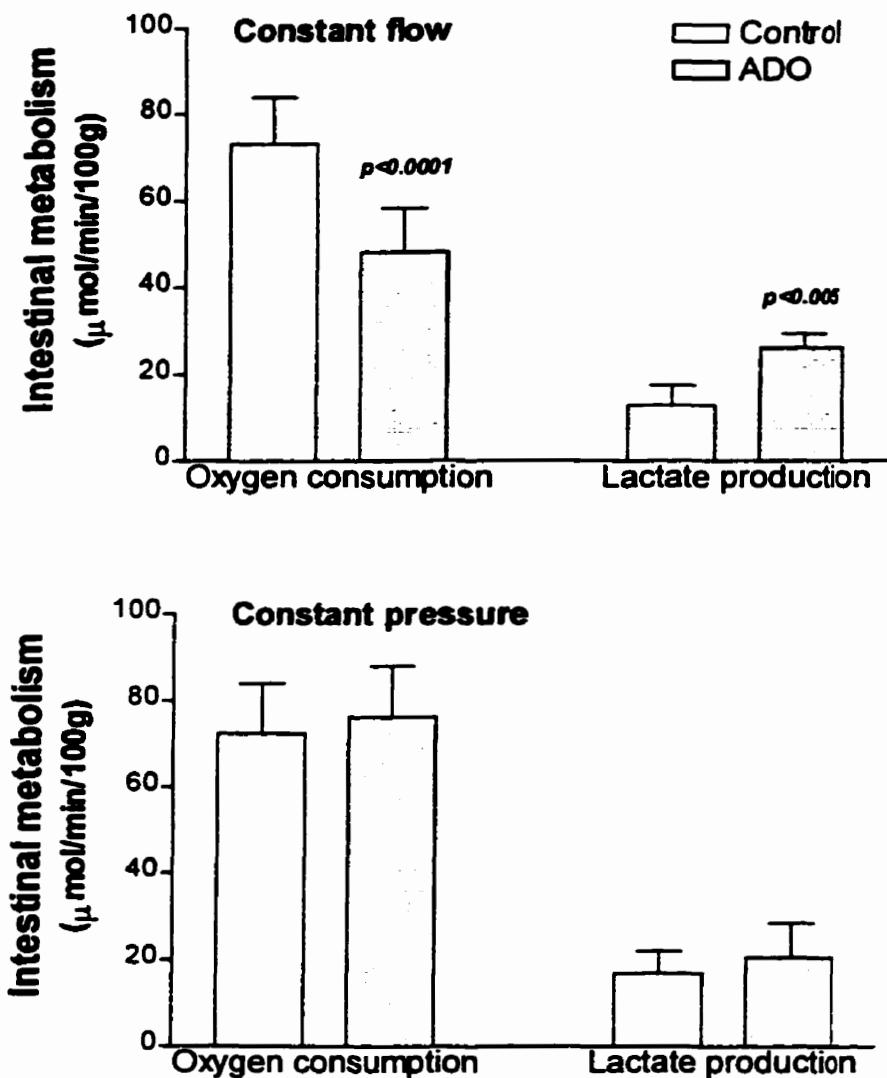


Figure 6-2. The intestinal metabolism in control and during adenosine (0.4 mg/kg/min) intra-superior mesenteric arterial infusion. Data expressed as mean \pm SEM of oxygen consumption or lactate production (n=7). Adenosine infusion decreased oxygen consumption and increased lactate production in the intestine during constant flow perfusion, while adenosine did not change oxygen consumption and lactate production during constant pressure perfusion.

The vasodilator effects of adenosine during both constant flow and constant pressure perfusion were potentiated after L-NAME. Adenosine infusion during constant flow perfusion decreased superior mesenteric arterial pressure by 113 ± 11 from 162 ± 10 mmHg (versus a decrease of 73 ± 11 from 122 ± 8 mmHg before L-NAME, calculated as absolute changes, $p < 0.01$), whereas adenosine increased flow rate by 120 ± 13 from 37.6 ± 9.9 ml/min/100g (versus an increase of 104 ± 15 from 34.3 ± 7.1 ml/min/100g before L-NAME, $p < 0.05$) during constant pressure perfusion (Figure 6-3).

The effects of adenosine on the intestinal metabolism during constant flow perfusion were not altered by the blockade of NO synthase. Oxygen consumption was reduced by 25.1 ± 2.9 before L-NAME and by 24.0 ± 7.2 $\mu\text{mol}/\text{min}/100\text{g}$ after L-NAME (NS, Figure 6-4). Lactate production was increased by 5.47 ± 0.98 $\mu\text{mol}/\text{min}/100\text{g}$ ($p < 0.005$) after L-NAME, which is comparable with that before the blockade of NO synthase. Adenosine infusion during constant pressure perfusion after L-NAME, however, reduced oxygen consumption by 17.5 ± 5.8 $\mu\text{mol}/\text{min}/100\text{g}$, which was significantly different from that before L-NAME (-3.7 ± 6.4 $\mu\text{mol}/\text{min}/100\text{g}$, $p < 0.05$, Figure 6-4) and not different from the results in constant flow adenosine infusion. The lactate production was increased (by 17.10 ± 5.67 $\mu\text{mol}/\text{min}/100\text{g}$, $p < 0.05$) by adenosine during constant pressure perfusion after L-NAME.

Therefore, L-NAME increased basal vascular tone in the superior mesenteric artery and potentiated the vasodilator effects of adenosine in both constant flow and constant pressure settings. Adenosine decreased O_2 uptake and increased lactate output only when shear stress was prevented from rising. If shear stress was allowed to increase (constant pressure perfusion), adenosine metabolic action was prevented but could be restored by

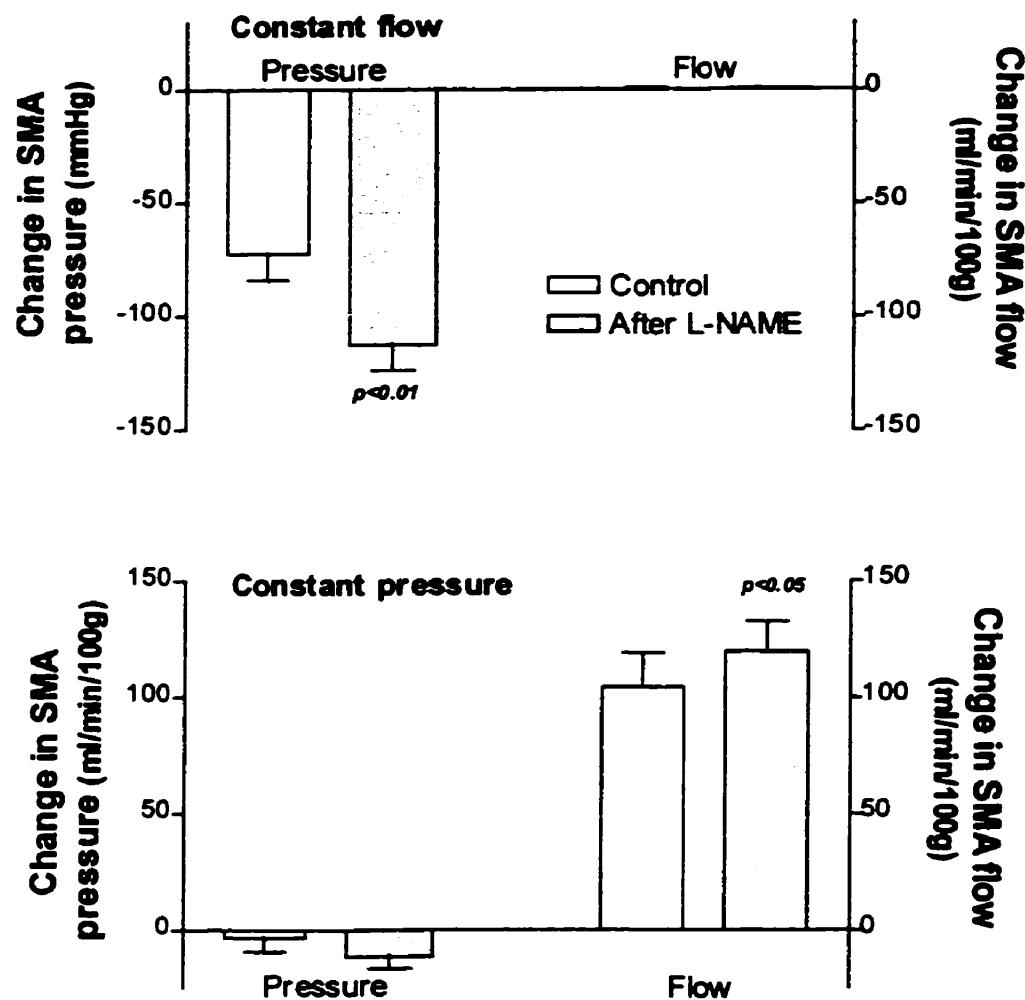


Figure 6-3. Changes in superior mesenteric arterial (SMA) pressure and flow caused by adenosine infusion before and after L-NAME (2.5 mg/kg, i.v.) ($n=7$). The inhibition of NO production by L-NAME potentiated the vasodilator effects of adenosine during both constant pressure and constant flow perfusion.

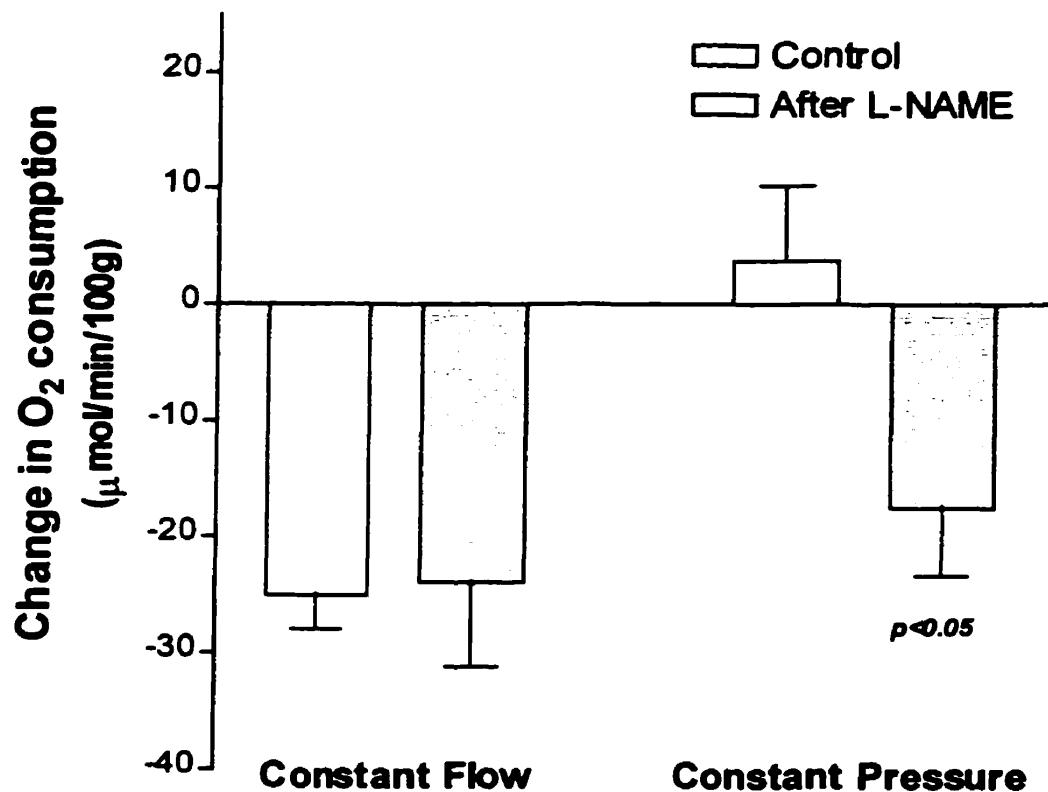


Figure 6-4. Changes in intestinal oxygen consumption caused by infusion of adenosine (0.4 mg/kg/min) intra-arterially during constant flow and constant pressure perfusions before and after L-NAME (2.5 mg/kg, i.v.) (n=7). The administration of L-NAME restored the metabolic effect of adenosine during constant pressure perfusion but did not alter the effect during constant flow perfusion.

blocking NO production. Blockade of NO production did not alter basal metabolism nor did it alter the adenosine-induced metabolic action in the constant flow perfusion where shear stress was not increased.

6.3.4 The effect of SIN-1 on adenosine actions during constant flow perfusion

The effects of the NO donor were evaluated in the constant flow perfusion because in that condition shear stress did not increase during adenosine infusion and endogenous NO effects were absent (see above). Intravenous bolus injection of SIN-1 caused an immediate drop in systemic pressure and superior mesenteric arterial perfusion pressure. The vasodilation response to SIN-1 was less sensitive in the superior mesenteric artery. The systemic arterial pressure dropped from 102 ± 4 to 72 ± 4 mmHg ($n=6$, $p<0.001$) at dose of 0.1 mg/kg and to 64 ± 7 mmHg ($p<0.001$) at dose of 0.2 mg/kg while the superior mesenteric arterial pressure dropped from 109 ± 9 to 103 ± 11 (NS) and to 83 ± 4 mmHg ($p<0.05$), respectively. This comparison was made at the same superior mesenteric arterial flow rates (30.8 ± 3.8 and 29.2 ± 3.9 at the two doses versus 29.7 ± 3.9 ml/min/100g for control). The vasodilator effect of SIN-1 sustained for about 1 hour in the superior mesenteric artery while it lasted up to 3 hours systemically. SIN-1 at either dose had no effect on basal oxygen consumption (64.5 ± 4.2 at 0.1 and 58.9 ± 2.8 at 0.2 mg/kg versus control 60.6 ± 3.8 μ mol/min/100g, NS) and lactate production (4.1 ± 1.6 at 0.1 and 3.5 ± 3.1 at 0.2 mg/kg versus 6.1 ± 2.1 μ mol/min/100g, NS).

The infusion of adenosine decreased superior mesenteric arterial perfusion pressure by 70 ± 11 ($p<0.005$) and 53 ± 4 ($p<0.0001$) mmHg in the presence of 0.1 and 0.2 mg/kg SIN-1 respectively, while adenosine decreased the pressure by 80 ± 9 from 116 ± 8 mmHg ($p<0.0005$) in control. Adenosine-induced maximum vasodilations represented by

superior mesenteric arterial pressure were greater in the presence of SIN-1 (33.1 ± 1.1 , $p<0.05$ and 30.3 ± 0.8 mmHg, $p<0.001$, at dose 0.1 and 0.2 mg/kg respectively, compared to control 36.7 ± 1.2 mmHg), although the absolute decreases in the pressure induced by adenosine were reduced. The effect of adenosine on intestinal oxygen consumption was attenuated dose-dependently by SIN-1. The reductions of oxygen consumption by adenosine infusion were 18.8 ± 4.5 and 9.4 ± 2.2 $\mu\text{mol}/\text{min}/100\text{g}$ at doses of 0.1 and 0.2 mg/kg respectively. It was significantly different at dose 0.2 mg/kg from the control (21.8 ± 4.5 $\mu\text{mol}/\text{min}/100\text{g}$, $p<0.05$) as shown in Figure 6-5. Adenosine-induced increase in lactate production in the presence of SIN-1 was attenuated dose-dependently (Figure 6-5). Thus, SIN-1 produced hypotensive effect in the systemic and superior mesenteric circulation but had no effect on basal metabolism in the intestine. The presence of SIN-1 attenuated the metabolic effects of adenosine on oxygen consumption and lactate production in a dose dependent manner.

6.3.5 Hemodynamic and metabolic effects of isoproterenol

Infusion of isoproterenol in constant flow perfusion caused a decrease in circuit pressure from 110 ± 8 to 46 ± 4 mmHg (Figure 6-6), which is comparable with the response to adenosine. The oxygen consumption across the tissue was reduced by 9.8 ± 2.8 from 64.7 ± 5.3 $\mu\text{mol}/\text{min}/100\text{g}$ by isoproterenol infusion (Figure 6-6). The production of lactate was not altered by isoproterenol infusion.

6.4 DISCUSSION

NO, released either by shear stress or by the exogenous donor, SIN-1, antagonized adenosine effects on intestinal metabolism. Adenosine decreased oxygen consumption

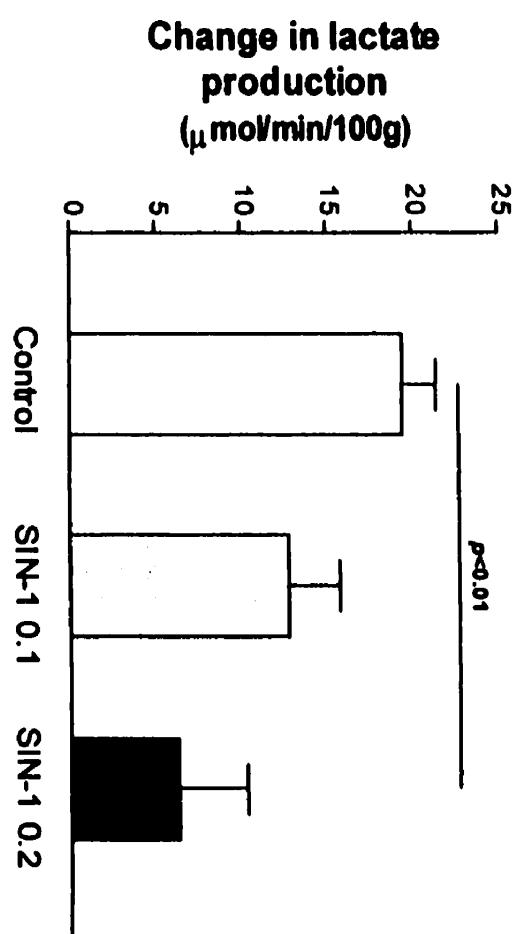
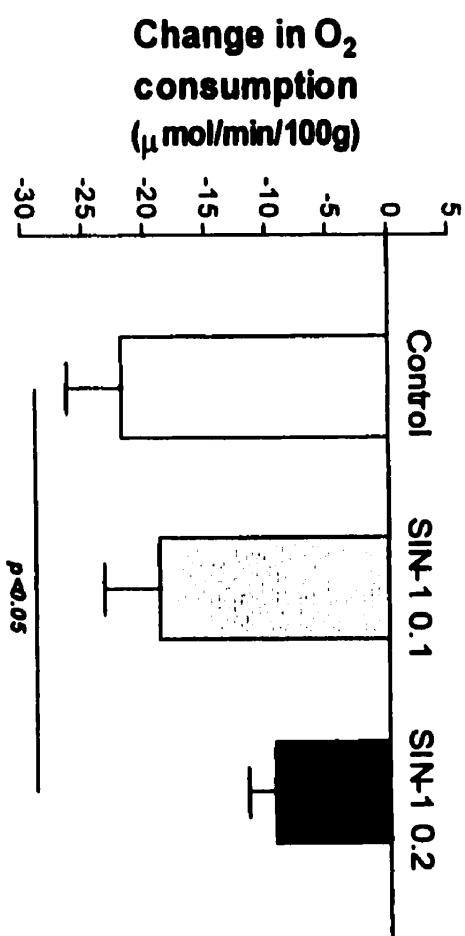


Figure 6-5. Changes in intestinal oxygen consumption and lactate production caused by adenosine infusion (0.4 mg/kg/min) during constant flow perfusion before and after SIN-1 (0.1 and 0.2 mg/kg, i.v.). The NO donor SIN-1 inhibited the metabolic effects of adenosine in the intestine.

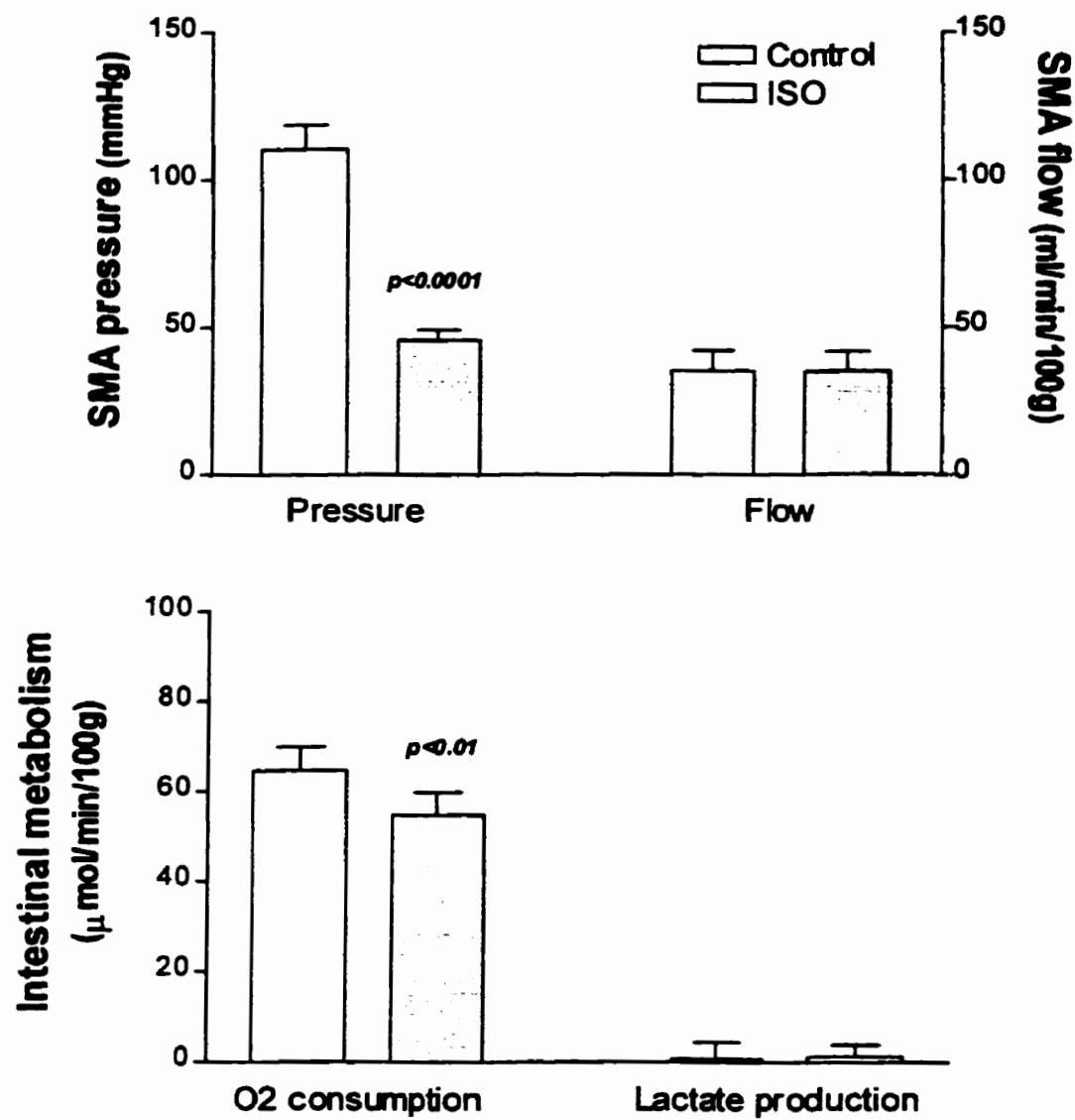


Figure 6-6. Hemodynamic (upper panel) and metabolic (lower panel) effects of isoproterenol infusion ($0.2 \mu\text{g}/\text{kg}/\text{min}$) during constant flow perfusion in the superior mesenteric arterial (SMA) vascular bed. n=7. Isoproterenol caused the similar hemodynamic changes as adenosine did, but did not change lactate production and decreased oxygen consumption only slightly.

and increased lactate production in the small intestine during constant flow perfusion. The results suggested that adenosine suppresses local aerobic metabolism and increases anaerobic metabolism. The effects of adenosine on intestinal metabolism were abolished when shear stress increased (in constant pressure perfusion). The observation that NO synthase blockade by L-NAME restored the effects in this situation strongly suggested an inhibitor effect of NO on the metabolic effects of adenosine. The data are consistent with the hypothesis that adenosine-induced conversion of intestinal metabolism to the anaerobic state is suppressed by shear stress-dependent release of NO but that the suppression of adenosine-induced vasodilation by NO is not shear stress dependent since it occurred in both constant flow and constant pressure conditions. The effects of adenosine on intestinal metabolism in the absence of increased shear stress (in constant flow perfusion) were attenuated by the introduction of exogenous NO into the system using SIN-1. These results further confirmed the antagonism by NO of the metabolic responses to adenosine. Furthermore, the observation that neither the NO synthase blockade by L-NAME nor the administration of SIN-1 changed basal oxygen consumption or lactate production suggested that NO was not involved in the control of basal metabolism in the small intestine. In contrast, NO was involved in maintaining basal vascular tone in the superior mesenteric artery.

6.4.1 Methodology considerations

6.4.1.1 Surgery

The surgical procedure used in this study was modified from a previous study (Macedo and Lautt, 1997). Removal of the large intestine and transection at the duodenum were done to ensure that a defined intestinal segment received blood supply

exclusively from the superior mesenteric artery. The superior mesenteric nerve plexus was surgically ablated to avoid the effect of nerve reflexes on intestinal hemodynamics and metabolism. I used a double aortic cannulation for the source of circuit blood instead of femoral arterial cannulations thereby helping to maintain hind limb perfusion and control of basal lactate level.

6.4.1.2 Control of shear stress states

In order to assess the involvement and effect of NO, the responses to adenosine were evaluated under different shear stress states, which would result in a different endogenous NO production since shear stress is a powerful stimulus of endothelial NO synthase (Moncada et al., 1991). When adenosine was infused during constant flow perfusion, the circuit flow rate was simply unchanged so that a drop in perfusion pressure would be seen in response to vasodilation. Shear rate would decrease with the decrease in perfusion pressure in this condition. On the other hand, in order to maintain the perfusion pressure during adenosine infusion in constant pressure perfusion, the flow rate has to be increased to compensate for the vasodilation response. Shear rate in the constant pressure condition, therefore, would increase when the flow rate increased. The change in shear stress under different perfusion conditions was discussed previously in Chapter 1, section 1.3 (also see Figure 1-1).

The effect of constant flow or pressure on shear stress during vasodilation is different from the situation when a vasoconstrictor is infused. A published paper previously discussed (Macedo and Lautt, 1996b) that the vasoconstriction during constant flow perfusion would increase perfusion pressure and elevate shear rate; whereas, when vasoconstriction was induced under constant pressure, the flow was reduced and shear

rate would not be altered. Further more, when shear stress is increased by a vasoconstrictor during constant flow perfusion, the site where shear rate increases is the site of constriction. When a vasodilator is infused to elevate shear rate in constant pressure perfusion, the site where shear rate increases the most is the site with least dilation either down or up stream of the most dilated site. I would expect that the site where shear stress increased the most in the experiment is more likely the capillary site downstream considering the shear stress dependency of the metabolic effects.

6.4.2 Influence of NO on the basal hemodynamics and metabolism

I used a NO synthase blocker, L-NAME, to block the production of NO. In the previous studies (Macedo and Lautt, 1996b; Macedo and Lautt, 1997) a dose of 2.5 mg/Kg was used to block NO synthase. In the present study, the increase in basal superior mesenteric arterial perfusion pressure after L-NAME indicated a successful blockade of NO synthase. In order to verify my interpretation of the L-NAME data, I used a NO donor, SIN-1, to introduce exogenous NO into the system. SIN-1 is a synthetic compound that degrades chemically to release NO spontaneously (Noack and Feelish, 1989).

The data confirmed that NO contributes to regulation of vascular tone in the superior mesenteric artery (Macedo and Lautt, 1996a; Macedo and Lautt, 1996b; Macedo and Lautt, 1997). The blockade of NO synthase increased basal superior mesenteric arterial perfusion pressure to a greater extent than systemic pressure. The administration of a NO donor, on the other hand, decreased perfusion pressure in the superior mesenteric artery to a lesser extent than that did in the whole system. These might indicate a higher basal level of NO involved in the regulation of basal vascular tone in the superior

mesenteric vascular bed. However, systemic blood pressure effects may simply have been compensated for by efficient baroreflexes (Greenway and Innes, 1981) while the intestine had been denervated. The basal intestinal metabolism was not altered by either the blockade of NO synthase or the administration of SIN-1 although they both changed basal vascular tone in the superior mesenteric artery. These suggested that NO was not primarily involved in the regulation of basal metabolism in the superior mesenteric vascular bed.

NO has been demonstrated *in vitro* to interact with mitochondrial enzymes (Geng et al., 1992; Stadler et al., 1991) and inhibit energy metabolism. It has been reported that NO reduced oxygen consumption in isolated myocardial (Xie et al., 1996) and skeletal muscle slices (Shen et al., 1995). However, the results of *in vivo* studies are very inconsistent. Blockade of NO synthase was reported to increase oxygen consumption in hind limb of dog (King et al., 1994; Shen et al., 1995) and in conscious dog (Shen et al., 1994). In contrast, a study in rat ileum (Bohlen and Lash, 1996) demonstrated that L-NAME did not change oxygen consumption. The basal oxygen consumption of the whole body was not influenced by sodium nitroprusside, a NO donor (Zhang et al., 1995), and was not changed by L-NAME (King et al., 1994; Shen et al., 1994) in anesthetized dogs. The inconsistency of published studies could simply result from the limitations of different preparations such as *in vitro* versus *in vivo* study, the influence of anesthesia (Shen et al., 1994), and the dose of L-NAME used to block NO synthase. A study in dog jejunum (Alemayehu et al., 1994) showed that L-NAME increased oxygen consumption only at doses greater than 10 mg/kg and the effect was time dependent. These doses are much higher than that used in my study (2.5 mg/kg). I infused the drug over a ten-minute

period and started tests in another 10 minutes to avoid any transient effects as reported (Alemayehu et al., 1994).

6.4.3 Consideration of adenosine hemodynamic effects

The metabolic effects of adenosine could possibly be secondary to its vasodilator effects. In this regard, I compared adenosine effects with another vasodilator, isoproterenol. The infusion of isoproterenol at the dose to produce similar hemodynamic effect as adenosine decreased oxygen consumption by only 9.8 $\mu\text{mol}/\text{min}/100\text{g}$ (compared to a reduction of 26.1 $\mu\text{mol}/\text{min}/100\text{g}$ by adenosine). This reduction is possibly due to beta-agonist effect or secondary to the hemodynamic change. There was no change in lactate production by isoproterenol infusion. The possibility of a blood stealing mechanism between mucosa and muscularis was proposed to explain isoproterenol and adenosine effects on oxygen consumption (Shepherd et al., 1984). This explanation, however, seems not acceptable for adenosine. Greenway and Murthy (1972) indicated that the ratio of blood flow to tissue mass in muscularis was only about one tenth of that in mucosa and submucosa. The mucosal layer accounts ~80% of the intestinal weight (Lang et al., 1991). Granger et al demonstrated that adenosine is able to decrease oxygen consumption in both mucosa and muscularis in cat ileum (Granger et al., 1978). The results in this chapter indicated that adenosine decreased oxygen consumption in constant pressure perfusion after L-NAME. The amount of blood flow that could be stolen from the layer in constant flow perfusion should not change during constant pressure perfusion. In the presence of SIN-1, adenosine reduced perfusion pressure to an even lower level but the effects on metabolism were attenuated. The hemodynamic changes were not likely to be the cause of adenosine metabolic effects.

6.4.4 Influence of NO on adenosine effects

The potentiation by L-NAME and the inhibition by SIN-1 of vasodilator effects of adenosine in the superior mesenteric artery are consistent with the previous finding in our laboratory (Macedo and Lautt, 1997) suggesting antagonism by NO of adenosine vasodilator effect.

Adenosine infusion during constant flow perfusion decreased oxygen consumption and increased lactate production in the intestine; however, the infusion did not alter the metabolic profiles during constant pressure perfusion. The antagonism of adenosine metabolic effects by NO was shear stress dependent. The metabolic effects of adenosine were abolished in a shear stress increased state (constant pressure perfusion, Figure 6-2.) where an increase in shear-induced NO production was expected. Blockade of NO synthase by L-NAME in the shear-elevated state restored adenosine metabolic effects suggesting that shear-induced NO had antagonized adenosine metabolic effects. I further tested the effect of NO on adenosine metabolic effects in a shear stress reduced situation (constant flow perfusion) by the introduction of exogenous NO into the intestine. The exogenous NO attenuated the metabolic effects of adenosine in a dose dependent manner (Figure 6-5) confirming my interpretation.

Similar findings that NO antagonizes adenosine effects were reported in other studies. L-N^G-nitro-arginine potentiated coronary autoregulation (Pohl et al., 1994), increased lactate production and decreased oxygen consumption. The authors, however, ascribed these *in vitro* results to a decrease in coronary flow. Woolfson et al. (1995) found that inhibition of NO synthesis potentiated the protection by adenosine in an ischemia-reperfusion model in the heart. In their earlier study (Patel et al., 1993),

pretreatment with L-NAME reduced infarct size while increasing lactate production. They interpreted the increase of lactate production by the administration of L-NAME to indicate a worsened ischemic condition. However, this was not compatible with the observation that the infarct size was actually reduced. If the protection of the heart from ischemia by adenosine was mediated through its metabolic effects, the potentiation of adenosine effects by blockade of NO synthase would be a more likely explanation.

The vasodilator effects of adenosine were suppressed by NO in both constant flow and constant pressure perfusion, while the metabolic effects were suppressed by NO only in shear stress elevated situation. These data and our previous studies (Macedo and Lautt, 1996a; Macedo and Lautt, 1996b; Macedo and Lautt, 1997) are consistent with the rather surprising conclusion that shear stress-induced release of NO in the intestine does not act directly on the vascular smooth muscle but does have metabolic effects; whereas, basal NO production (which is probably shear stress independent) plays a role in maintaining the vascular tone in the superior mesenteric artery but does not play a metabolic role. Shear stress-dependent NO modulates the vascular response to sympathetic nerve stimulation but not to norepinephrine infusion thus suggesting that the modulation by the NO is through a presynaptic mechanism rather than by acting on the superior mesenteric arterial smooth muscle (Macedo and Lautt, 1996b). L-NAME potentiated the vasodilator response to adenosine and isoproterenol (Macedo and Lautt, 1997) in a constant flow situation where shear stress would not be elevated, thus suggesting that non-shear-dependent NO acted on the vascular muscle. I designed constant pressure perfusion in the present study to elevate shear stress but could not demonstrate any additional role for shear-induced NO in the vascular responses. Thus, the suppression of autoregulation in

the superior mesenteric artery by NO (Macedo and Lautt, 1996a) is not likely to be shear stress-dependent. The metabolic effects of adenosine, in contrast, were clearly suppressed by shear stress-dependent NO.

The finding in this chapter that NO also antagonizes the metabolic effect of adenosine is very important in understanding of intestinal physiology. For example, both adenosine and NO are involved in postprandial hyperemia (Alemany et al., 1997; Sawmiller and Chou, 1992). The release of adenosine is necessary to increase blood flow, but the suppression of metabolism by adenosine is inhibited by NO that is produced by increased blood flow-caused elevation of shear stress. The interaction of adenosine with NO at hemodynamic and metabolic levels fully meets the physiological requirement during the postprandial state. In conclusion, adenosine-induced suppression of intestinal metabolism was antagonized by shear-induced NO; whereas, the antagonism by NO of adenosine vasodilator effects was not shear stress dependent. Basal release of NO (which is not likely to be shear stress dependent) is involved in the control of basal tone but not primarily in the control of basal metabolism in the intestine.

7. BLOOD FLOW DEPENDENT PROSTAGLANDIN F_{2α} REGULATES INTESTINAL GLUCOSE UPTAKE FROM THE BLOOD

7.1 INTRODUCTION

Although the gut serves to regulate the absorption of glucose and other nutrients, few studies have addressed how the intestine regulates its own energy metabolism. Substances such as glutamine (Windmueller and Spaeth, 1980) and some digested products including glucose (Nicholls et al., 1983) are important energy sources for intestinal tissues. Increasing evidence suggests that the intestine is not only important for glucose absorption; it may also be important in the disposal of glucose in basal and post-ingestion states. Abumrad et al. (1982) reported that the gut utilized up to 25% of net hepatic glucose output in basal states and up to 15% of the glucose load in the post absorptive state in conscious dogs. The intestinal mucosa was found to be a quantitatively important site of action of metformin, an antihyperglycemic agent, to increase glucose utilization (Bailey et al., 1994).

The mechanism that controls intestinal glucose uptake and metabolism is not known. Kellett et al. (1984) indicated that normal intestinal glucose metabolism is not insulin concentration dependent although the presence of insulin seems necessary. Xie and Lautt (1996) found that insulin has no effect on net glucose balance in extrahepatic splanchnic tissues, mainly the gut, and that a hepatic insulin sensitizing substance released by the liver similarly did not affect the gut. These results are consistent with a previous report that hyperglycemia and hyperinsulinemia had no effect on extrahepatic splanchnic tissue glucose uptake (DeFronzo et al., 1983).

In the previous chapter, I discussed that the inhibitory metabolic actions of adenosine on intestinal oxygen consumption were antagonized by shear stress-induced NO, an effect that was seen only when blood flow was allowed to increase. As an index of the preparation status, glucose uptake at several time points was also monitored. Glucose uptake appeared to increase in the presence of vasodilation but only when blood flow was allowed to rise. Based on this preliminary observation, a hypothesis that increased intestinal glucose uptake is mediated by shear stress-induced release of autacoids such as nitric oxide or prostaglandins during high flow perfusion was formulated and tested in this chapter.

The results demonstrated that intestinal glucose uptake increased when blood flow was raised. The increased intestinal glucose uptake was blocked by a cyclooxygenase inhibitor, indomethacin, but not by NO synthase blocker, L-NAME. In addition, the blockade of increased intestinal glucose uptake by indomethacin could be reversed by PGF_{2α} suggesting that shear stress induced-release of PGF_{2α} mediated the increase in intestinal glucose uptake when blood flow was elevated.

7.2 METHODS AND PROTOCOLS

7.2.1 Surgical preparation

Fifteen cats (either gender, 3.7±0.1 kg) were treated as described previously. Briefly, the animals were anesthetized using sodium pentobarbital. Systemic arterial and central venous pressures were monitored. All pressures were monitored by pressure transducers (Gould and Statham, Gould Inc., Oxnard, CA). General surgical procedure performed was described in the methodology chapter.

The additional surgical approaches used in this study are similar to that in Chapter 6. Simply, after an abdominal midline incision, the spleen and large intestine were surgically removed. The upper end of the intestinal segment was ligated at the duodenum. The superior mesenteric nerve plexus was surgically denervated. The superior mesenteric artery was cannulated. A pump-controlled (Masterflex, Cole Parmer Instrument Co., Barrington, IL) vascular circuit perfused the small intestine through a superior mesenteric arterial cannula. The pump rate was controlled to generate a circuit pressure that was similar to systemic pressure. The flow rate was measured using a flow-through electromagnetic flow probe (EP608, Carolina Medical Electronics, Inc., King, NC) that was incorporated into the circuit. The pressure line was also used for arterial sampling. Two venous catheter units (24G, OptivaTM Medical Inc.) were placed into the portal vein for monitoring portal venous pressure and for blood sampling. At the end of each experiment, the defined intestinal segment was removed from the carcass and cleaned of intestinal contents to obtain a wet tissue weight.

7.2.2 Experimental protocols

7.2.2.1 The influence of blood flow on intestinal glucose uptake

In order to increase intestinal blood flow, a vasodilator, adenosine at a dose to produce maximum vasodilation (0.4 mg/kg/min)(Macedo and Lautt, 1997) was infused into the superior mesenteric artery through an infusion line in the circuit (n=7). A constant superior mesenteric arterial perfusion pressure was maintained using the pump to increase blood flow during the drug infusion. Blood samples for measuring blood glucose, lactate and oxygen content were taken from the circuit and portal vein simultaneously at 5 minutes before the infusion, as control, and after 5 minutes of the

infusion. Adenosine was also infused into the superior mesenteric artery while the blood flow was maintained constant to prevent a rise in shear stress.

7.2.2.2 The involvement of NO in intestinal glucose uptake

The shear rate in the superior mesenteric vascular bed is increased due to the increase in blood flow when a vasodilator is infused during constant pressure perfusion. In order to study the involvement of NO, the animals were treated with L-NAME (2.5 mg/kg, i.v., infused over 10 min), a potent non-selective NO synthase blocker. Adenosine infusions were repeated 10 minutes after the completion of L-NAME administration. The intestinal glucose uptake in basal state and during raised blood flow was compared before and after NO synthase blockade.

7.2.2.3 The influence of indomethacin on intestinal glucose uptake

In order to investigate the role of prostanoids in control of intestinal glucose uptake, the uptake during raised blood flow by adenosine (0.4 mg/kg/min) infusion was compared before and after the inhibition of cyclooxygenase by indomethacin (5 mg/kg, i.v. infusion over 10 min) in the second group of animals (n=5). Adenosine infusion during constant pressure perfusion was performed before and 30 minutes after indomethacin administration.

7.2.2.4 The effects of prostaglandins on intestinal glucose uptake

For further confirmation of the results obtained in the protocol above and to identify the role of different prostaglandins in control of intestinal glucose uptake, the effects of prostaglandins (intra superior mesenteric arterial infusion) were tested in the second group of animals and compared before and after the inhibition of cyclooxygenase. The effects on intestinal glucose uptake of PGF_{2α} and PGE₂ (and PGI₂ in some animals)

(41.2 ng/kg/min for all) infusion alone or with adenosine during constant pressure (elevated flow) perfusion were tested before and after the administration of indomethacin. PGF_{2α} was also infused with adenosine (0.4 mg/kg/min) during constant flow perfusion in two animals. One extra dose (8.2 ng/kg/min) of PGF_{2α} was also tested with adenosine infusion after indomethacin. The order of testing was randomly assigned before and after indomethacin. The two different PGF_{2α} doses after indomethacin were tested sequentially but ordered randomly. The infusion lasted for ten minutes in this case, and the dose was changed by switching infusion rate after samples were taken at five minutes for the first dose.

7.2.2.5 The influence of increased shear stress in the liver

In order to study the influence of the liver on intestinal glucose uptake in response to increased blood flow, a special surgical procedure was performed in two animals to bypass portal blood from the liver and shunt it directly into the central vena cava. A loop with a T-connector was placed in the central vena cava by double cannulation. The portal vein was cannulated and connected to the central venous loop. Intestinal glucose uptake in the two animals was studied during increased superior mesenteric arterial blood flow by infusion of adenosine (0.4 mg/kg/min).

7.2.2.6 Sampling and analysis

Blood samples were taken from the arterial circuit and portal vein simultaneously for control or at five minutes during adenosine infusion. The samples were for the analysis of blood glucose and lactate concentration using glucose and lactate analyzers (YSI Sports Industrial analyzer, Yellow Springs, Oh).

7.3 RESULTS

7.3.1 Basal metabolic and hemodynamic conditions

Basal conditions of the animals were stabilized within two hours after the surgery. Upon stabilization, for the first and second group of animals respectively, basal arterial glucose concentrations were 98.1 ± 6.5 and 101.9 ± 2.7 mg/dL (5.44 ± 0.36 and 5.65 ± 0.15 mmol/L); and the superior mesenteric arterial perfusion pressures were 114.9 ± 7.6 and 105.3 ± 3.1 at flow rates of 37.6 ± 8.1 and 35.5 ± 2.0 ml/min/100g. The basic conditions of these two groups were not significantly different. The third group of two animals had similar basal hemodynamic and metabolic conditions.

7.3.2 The influence of superior mesenteric arterial blood flow on intestinal glucose uptake

The infusion of adenosine increased superior mesenteric arterial blood flow by 112 ± 14 from 31.6 ± 7.5 ml/min/100g ($p < 0.0005$, $n=7$, Figure 7-1) while the perfusion pressure was maintained (124 ± 6 versus control 120 ± 4 mmHg, NS). The glucose uptake across the intestine increased by 45.0 ± 18.3 from 25.3 ± 3.8 $\mu\text{mol}/\text{min}/100\text{g}$ ($p < 0.05$, Figure 7-1). The total production of CO_2 and lactate balance across the intestine were not changed (CO_2 : 45.1 ± 25.4 versus control 37.4 ± 12.3 $\mu\text{mol}/\text{min}/100\text{g}$, NS; lactate: 19.0 ± 8.1 versus control 18.0 ± 4.9 $\mu\text{mol}/\text{min}/100\text{g}$, NS). Adenosine was also infused while holding the superior mesenteric arterial blood flow constant. The superior mesenteric arterial perfusion pressure was reduced by 69 ± 11 from 115 ± 8 mmHg ($p < 0.001$, $n=7$) during adenosine infusion while the flow rate was unchanged (36.4 ± 7.7 versus 37.0 ± 7.5 ml/min/100g, NS). Glucose uptake in the constant flow situation, however, was not altered (22.0 ± 3.9 versus control 19.3 ± 4.29 $\mu\text{mol}/\text{min}/100\text{g}$, NS, Figure 7-1).

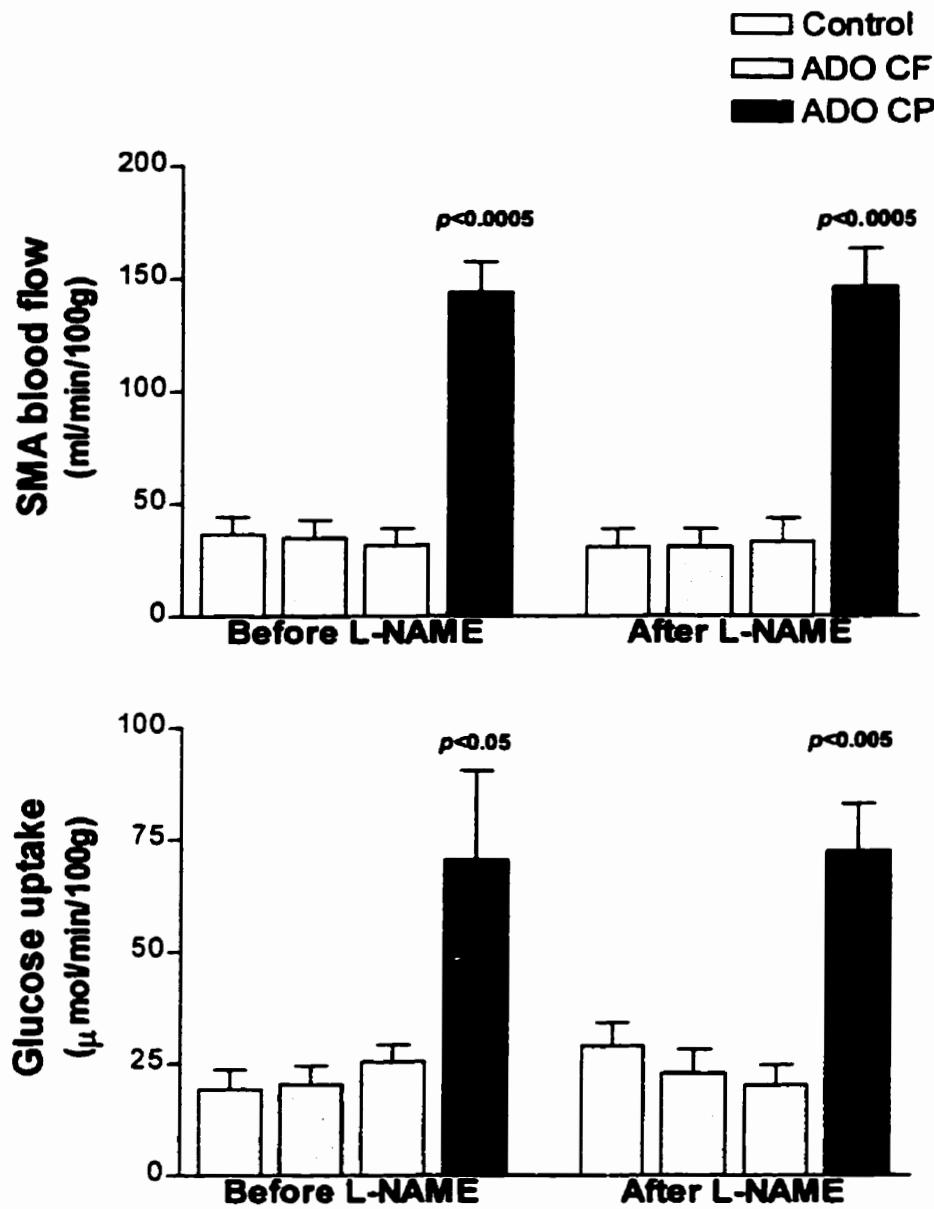


Figure 7-1. The superior mesenteric arterial (SMA) blood flow and intestinal glucose uptake in control and during adenosine (ADO, 0.4 mg/kg/min) infusion during constant flow (CF) and constant pressure (CP) perfusion. The intestinal glucose uptake was increased when blood flow increased. The responses were similar before and after NO synthase antagonism by L-NAME (2.5 mg/kg). (n=7)

Thus, the intestinal glucose uptake increased dramatically when blood flow was increased by adenosine (constant pressure); the glucose uptake was not altered by adenosine infusion during constant flow perfusion.

7.3.3 The influence of NO synthase blockade on intestinal glucose uptake

In order to assess the possible involvement of NO in the regulation of intestinal glucose uptake during constant pressure perfusion where shear stress was elevated, L-NAME (2.5 mg/kg) was used to block endogenous NO production. L-NAME significantly increased the superior mesenteric arterial perfusion pressure from 117 ± 4 to 178 ± 8 mmHg ($p < 0.0005$, $n=7$) while the blood flow was not changed (32.8 ± 8.0 before versus 30.7 ± 8.0 ml/min/100g after L-NAME, NS). The basal glucose uptake across the intestine was not altered by L-NAME. After the administration of L-NAME, the intestinal glucose uptake was increased by 52.0 ± 10.2 from 20.2 ± 4.5 $\mu\text{mol}/\text{min}/100\text{g}$ ($p < 0.005$, $n=7$, Figure 7-1) when the blood flow was increased by 113 ± 15 ml/min/100g ($p < 0.0005$) during adenosine infusion. The increase in blood flow was intentionally controlled as close as possible to that before L-NAME. The increases in the intestinal glucose uptake during increased blood flow were the same before and after L-NAME (increased by 45.0 ± 18.3 before versus 52 ± 10.2 $\mu\text{mol}/\text{min}/100\text{g}$ after L-NAME, NS). Similar to the response before L-NAME, adenosine infusion during constant flow perfusion did not change intestinal glucose uptake (22.8 ± 5.3 versus control after L-NAME 28.8 ± 5.3 $\mu\text{mol}/\text{min}/100\text{g}$ tissue, NS). Thus, the blockade of NO formation had no influence on the basal or blood flow-induced increase in intestinal glucose uptake.

7.3.4 The influence of indomethacin on intestinal glucose uptake

In another group of animals ($n=5$), a cyclooxygenase inhibitor, indomethacin, was

used to block the production of prostanoids during increased blood flow. Before indomethacin, the intestinal glucose uptake was increased by 41.0 ± 4.4 from 18.8 ± 4.1 $\mu\text{mol}/\text{min}/100\text{g}$ ($p < 0.001$) by infusion of adenosine during constant pressure perfusion, which increased superior mesenteric arterial blood flow by 146 ± 10 from 35.5 ± 2.0 $\text{ml}/\text{min}/100\text{g}$ ($p < 0.0001$, Figure 7-2). The blockade of cyclooxygenase by indomethacin did not change basal intestinal glucose uptake (14.1 ± 5.1 before versus 17.5 ± 6.8 $\mu\text{mol}/\text{min}/100\text{g}$ after indomethacin, NS) while the basal superior mesenteric arterial blood flow was slightly increased after indomethacin ($p < 0.05$, by ANOVA). Adenosine infusion during constant pressure perfusion increased superior mesenteric arterial blood flow by 153 ± 24 from 52.0 ± 8.3 $\text{ml}/\text{min}/100\text{g}$ ($p < 0.005$). However, the intestinal glucose uptake after indomethacin was not increased (23.6 ± 11.3 versus control 17.5 ± 6.8 $\mu\text{mol}/\text{min}/100\text{g}$, NS, Figure 7-2).

Therefore, the increase in intestinal glucose uptake caused by the increase in superior mesenteric arterial blood flow was inhibited by the blockade of cyclooxygenase metabolite production.

7.3.5 The role of different prostaglandins in control of intestinal glucose uptake

The infusion of PGE₂ into the superior mesenteric artery decreased the perfusion pressure by 29 ± 4 from 112 ± 4 mmHg ($p < 0.005$, $n=5$) while the blood flow was held steady (40.1 ± 6.0 versus control 35.3 ± 5.1 $\text{ml}/\text{min}/100\text{g}$, NS). Infusion of PGF_{2α} at dose 41.2 ng/kg/min caused a slight and transient increase in the superior mesenteric arterial perfusion pressure followed by a sustained small decrease in the perfusion pressure by 16 ± 4 from 104 ± 3 mmHg ($p < 0.05$) while the blood flow was not changed (32.6 ± 5.9 versus control 32.6 ± 5.8 $\text{ml}/\text{min}/100\text{g}$, NS). After the administration of indomethacin,

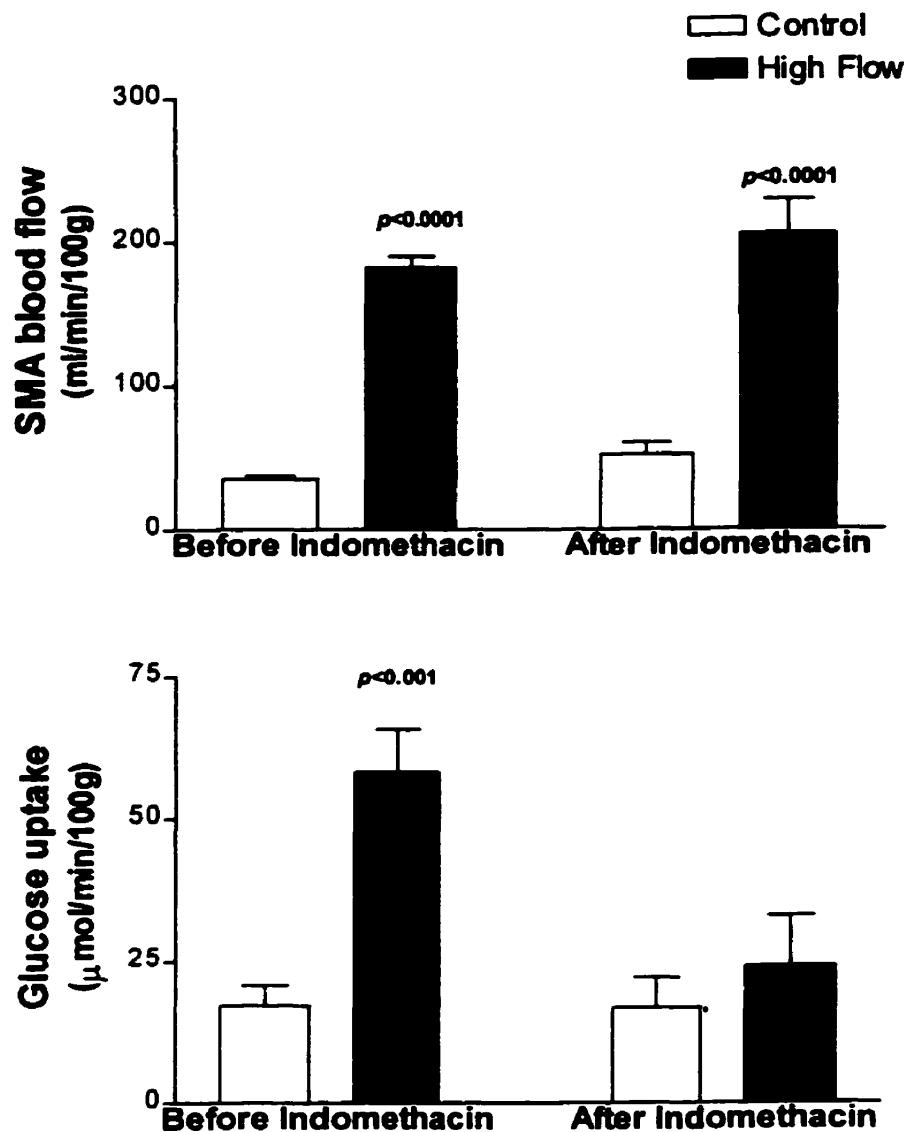


Figure 7-2. The superior mesenteric arterial (SMA) blood flow and intestinal glucose uptake in control and during increased blood flow (High flow) by adenosine infusion during constant pressure perfusion. The blood flow elevation was not altered but the flow-induced increase in glucose uptake was blocked after cyclooxygenase inhibition by indomethacin (5.0 mg/kg). (n=5)

PGE₂ decreased perfusion pressure by 41±4 from 100±4 mmHg (p<0.0005, n=5) while the blood flow was not changed (60.1±9.4 versus control 59.3±9.2 ml/min/100g, NS). The infusion of PGF_{2α} at the same dose after indomethacin did not change perfusion pressure (90±3 versus control 95±1 mmHg, NS). However, neither the infusion of PGE₂ nor PGF_{2α}, before and after the administration of indomethacin, changed intestinal glucose uptake in the absence of elevated blood flow, as shown in Figure 7-3.

The infusion of prostaglandins during increased superior mesenteric arterial blood flow by adenosine during constant pressure perfusion did not change the hemodynamics when compared to the control (Figure 7-4). The intestinal glucose uptake under such conditions increased by 44.9±10.3 and 43.0±10.9 μmol/min/100g during PGF_{2α} and PGE₂ infusion respectively. The increases were not different from that in the situation of adenosine alone (41.0±4.4 μmol/min/100g, NS). Thus, the infusion of prostaglandins during normal superior mesenteric arterial blood flow affected perfusion pressure but did not alter intestinal glucose uptake; the infusion of prostaglandins during increased superior mesenteric arterial blood flow did not have any additional effect on increased glucose uptake.

The infusion of prostaglandins during increased flow by adenosine did not alter blood flow before and after indomethacin (Figure 7-4). However, after indomethacin the infusion of PGF_{2α} during increased blood flow increased intestinal glucose uptake by 24.1±3.9 and 68.2±14.0 (p<0.01, n=5) μmol/min/100g at doses of 8.2 and 41.2 ng/kg/min respectively from their control; whereas, the infusion of adenosine alone during constant pressure perfusion after indomethacin did not change the glucose uptake. The changes in glucose uptake in the presence of PGF_{2α} were dose dependent and were significantly

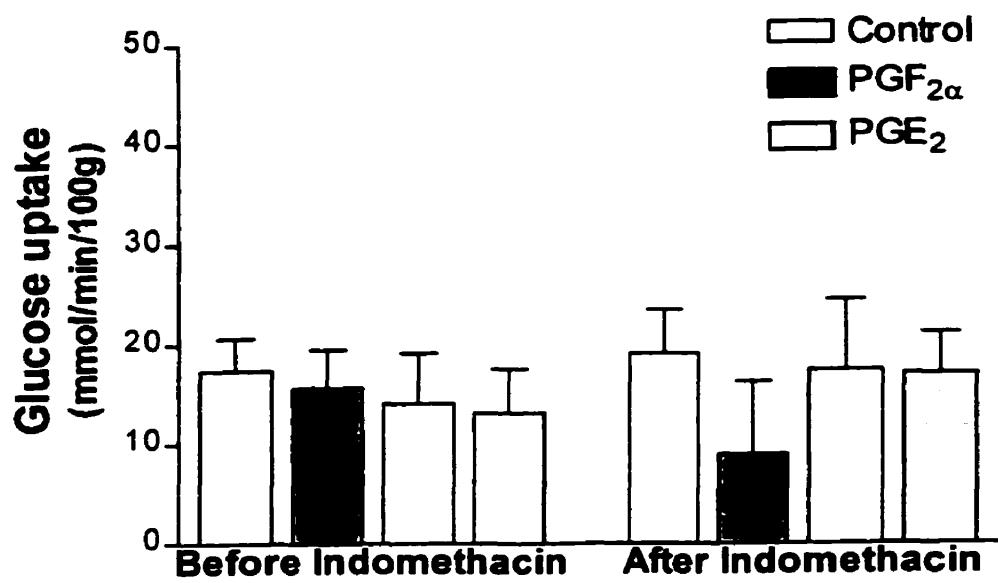


Figure 7-3. The intestinal glucose uptake in control and during the infusion of different prostaglandins under normal blood flow. PGF₂_α and PGE₂ were both at the dose of 41.2 ng/kg/min. There was no effect of prostaglandin infusion and the effects were not different before and after cyclooxygenase inhibition by indomethacin (5.0 mg/kg). (n=5)

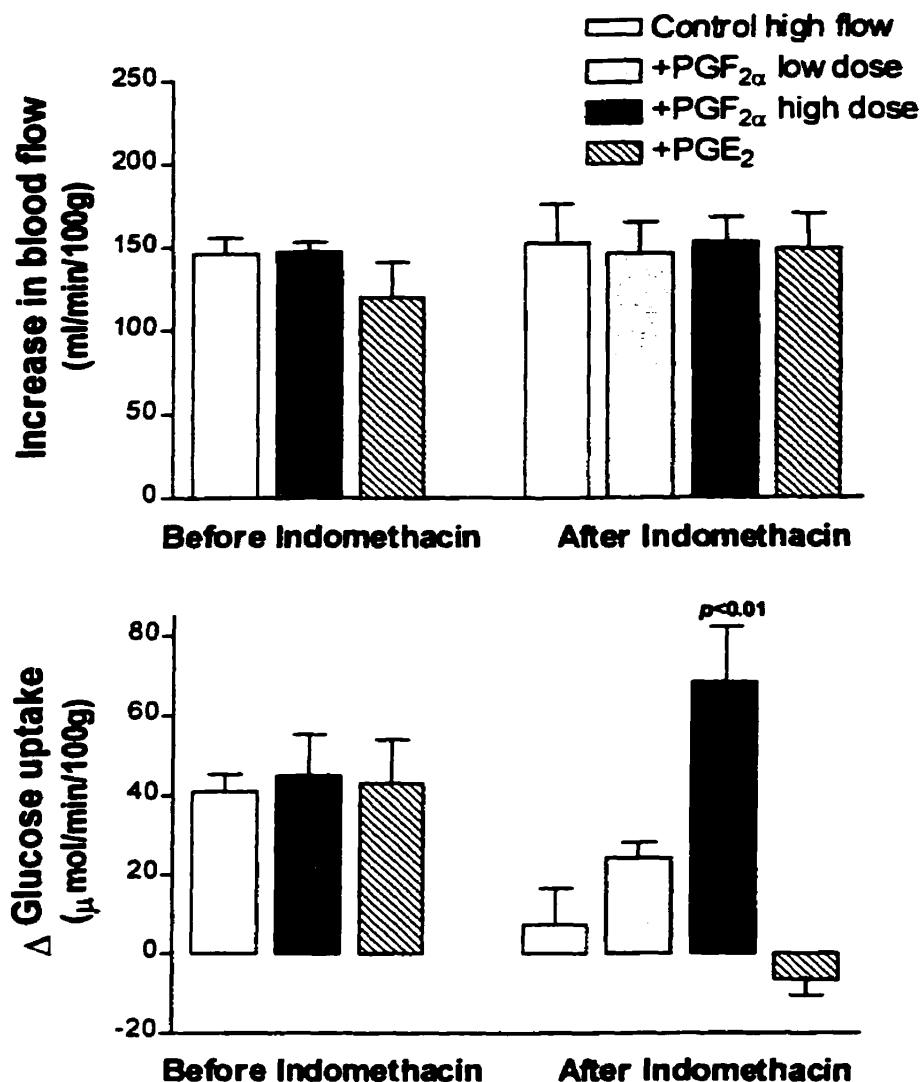


Figure 7-4. The changes in superior mesenteric arterial blood flow and intestinal glucose uptake from control when the blood flow was increased (High flow). The effects of different prostaglandins were compared before and after cyclooxygenase inhibition by indomethacin (5.0 mg/kg). PGF_{2α} and PGE₂ were given at the dose of 41.2 ng/kg/min before indomethacin, and 8.2 ng/kg/min for PGF_{2α} and 41.2 ng/kg/min for PGF_{2α} and PGE₂ after. PGF_{2α} but not PGE₂ (or PGI₂ - data not shown) restored the glucose uptake in the presence of elevated blood flow to similar levels produced before indomethacin. (n=5)

different ($p<0.01$, Figure 7-4) at dose 41.2 ng/kg/min when compared to that during adenosine infusion alone. The infusion of another prostaglandin, PGE₂, during raised blood flow was not able to increase the glucose uptake (12.4±3.4 versus control 18.9±4.2, NS, Figure 7-4) in the presence of indomethacin.

There was a slight increase in arterial glucose level (less than 13 % of control) when blood flow was increased during all adenosine infusions. There was no difference in those increases before and after indomethacin, with and without prostaglandin and with different prostaglandins. The lactate balances across the tissue were not changed in any of the situations before and after indomethacin.

PGF_{2α} was also infused with adenosine during constant flow perfusion before indomethacin in two animals. However, under normal blood flow (27.0 and 22.8 ml/min/100g) the intestinal glucose uptake was not altered (18.8 versus control 20.7, and 6.2 versus control 6.9 $\mu\text{mol}/\text{min}/100\text{g}$) by the infusion. The infusion of PGI₂ with adenosine during constant pressure perfusion after indomethacin was not able to increase the glucose uptake in pilot experiments (data not shown).

Therefore, PGF_{2α}, but not PGE₂ and PGI₂, dose dependently reversed the blockade of increase in intestinal glucose uptake by indomethacin during raised blood flow although the infusion of PGF_{2α} before indomethacin showed no additional effect, and PGF_{2α} under normal blood flow did not affect basal glucose uptake before and after indomethacin.

7.3.6 Intestinal glucose uptake in liver bypassed animals

In two animals, the portal venous blood flow was surgically shunted to the central vena cava. The intestinal glucose uptake increased by 24.0 and 89.0 $\mu\text{mol}/\text{min}/100\text{g}$ when

the blood flow was increased by 80.1 and 172.3 ml/min/100g in response to adenosine in the two animals respectively.

7.4 DISCUSSION

Intestinal glucose uptake from blood increased dramatically when the blood flow increased. Since shear rate was elevated during increased blood flow, I further tested the hypothesis that shear stress-induced release of NO or prostaglandins mediates the increase of intestinal glucose uptake. Indomethacin, a cyclooxygenase inhibitor that blocks the production of prostanoids, eliminated the increase in glucose uptake that was elicited by increased blood flow, suggesting that shear stress-induced release of prostaglandin was involved in mediating increased glucose uptake in the intestine. PGF_{2α}, at a dose that produced only a small vascular response, reversed the inhibitory effect of indomethacin on increased intestinal glucose uptake induced by increased blood flow; in contrast, PGE₂ and PGI₂ had no effect on the glucose uptake. The data suggest that PGF_{2α} was responsible for mediating the increase in intestinal glucose uptake when shear stress was elevated. The involvement of another shear stress-induced autacoid, NO, was ruled out since the blockade of NO synthase did not affect the glucose uptake. In addition, the prostaglandins and NO were not involved in the control of basal intestinal glucose uptake since the blockade of cyclooxygenase or NO synthase did not change the basal glucose uptake. An unusual aspect of the results was that PGF_{2α} only increased glucose uptake when blood flow was increased. To my knowledge, this is the first time that a blood flow elicited increase in intestinal glucose uptake has been reported, and the mechanism that shear stress-induced release of PGF_{2α} was suggested.

7.4.1 Methodology consideration

In this study, we surgically separated a segment of small intestine *in situ*, which is perfused by an arterial circuit through the superior mesenteric artery. This setup provided a model that would be least affected by changes in systemic and hemodynamic reflexes. The surgical procedures, including the removal of the spleen and large intestine and ligation at the duodenum, were to ensure that the portal blood is exclusively from the defined portion of intestine. A sample line in the circuit and a portal catheter provided routes to access arterial and portal blood samples simultaneously.

A pump was incorporated into the circuit to allow the superior mesenteric arterial blood flow to be adjusted. However, it was necessary to use a vasodilator to increase the superior mesenteric arterial blood flow substantially without the need to produce a massive increase in the perfusion pressure. Adenosine is a potent vasodilator and has been suggested to mediate postprandial reactive hyperemia in the intestine (Sawmiller and Chou, 1992). To assess the direct effect of adenosine on glucose uptake in the intestine, I administered the same dose of adenosine during both constant flow and constant pressure perfusion. In contrast to constant pressure perfusion, adenosine did not change basal glucose uptake during constant flow perfusion suggesting that there was no direct effect of adenosine on intestinal glucose balance.

A slight increase in arterial glucose levels was observed when adenosine was infused during constant pressure perfusion. The increases were not different in all tests before and after indomethacin. The increase was probably caused by stimulation of hepatic glycogenolysis by adenosine (see discussions in section 1.5.2.4) escaped from the intestine into the liver during high blood flow perfusion.

7.4.2 The influence of blood flow on the intestinal glucose uptake

I demonstrated that the intestinal glucose uptake from blood increased dramatically when the superior mesenteric arterial blood flow was increased. The active role of the gut in glucose disposal in basal and absorptive states reported by Abumrad et al. (1982) suggested that, in addition to basal utilization of up to 25% of hepatic glucose output by the gut in dogs, the glucose uptake was increased after ingestion; the change in intestinal blood flow was, unfortunately, not reported. The gastrointestinal blood flow is normally increased during ingestion. However, the absorption of glucose from the intestinal lumen makes it difficult to assess the net glucose utilization by the intestine. Direct glucose absorption from the lumen in roughage-fed ruminants is negligible. Bergman et al. demonstrated that the gut of sheep used about 16% of hepatic output of glucose in a "continuously fed" model (Bergman et al., 1974). Blood flow was not reported.

Data from other studies also implied that there is a relationship between increased intestinal blood flow and glucose uptake. Fernández-López et al. reported that the gut consumes a considerable portion of a glucose load in rats where the portal blood flow increased 2 to 3-fold (Fernández-López et al., 1992). In that study, the portal glucose concentration was actually decreased whereas the portal blood flow increased more than two-fold when a small amount of glucose (0.1 mmoles) was gavaged. Intestinal glucose uptake was elevated when blood flow increased during septic shock (Lang et al., 1991), although the authors concluded that the elevated glucose uptake was not dependent on increased blood flow since somatostatin reduced the blood flow but not glucose uptake in the gut. However, somatostatin did not bring the intestinal blood flow to its control level, and somatostatin-induced vasoconstriction could possibly increase shear stress in that

situation.

7.4.3 The influence of shear stress on intestinal glucose uptake

I previously discussed in Chapter 1 and 6 that the shear rate increases as does the shear stress to the endothelium when blood flow increases during constant pressure perfusion. Shear stress stimulates the release from the endothelial cells of autacoids including NO, prostaglandins and endothelium-derived hyperpolarizing factor (Busse and Fleming, 1998). These substances may have direct or indirect effect on the intestinal glucose uptake and metabolism. I showed that the release of NO was increased when blood flow was increased in Chapter 6. In the present study, I used L-NAME to block NO synthase to reduce shear stress-induced release of NO. The dose (2.5 mg/kg) used in the study was previously demonstrated to be sufficient to block NO production (Macedo and Lautt, 1998; Macedo and Lautt, 1997). The increase in superior mesenteric arterial pressure after L-NAME in this study further confirmed the effective blockade. However, neither the basal nor increased intestinal glucose uptake was altered by NO synthase blockade suggesting that NO was not primarily involved in the control of intestinal glucose uptake.

Prostaglandins are arachidonic acid metabolites of cyclooxygenase. Increase in shear stress may stimulate the endothelium to release prostaglandins (Busse and Fleming, 1998; Nollert et al., 1989), which in turn may have direct or indirect effects on intestinal glucose uptake. In order to reduce the release of prostaglandins when shear stress was elevated, indomethacin was used. The slight increase in superior mesenteric arterial blood flow following indomethacin suggested that the basal prostaglandin(s) produced mainly vasoconstriction in cats. The basal intestinal glucose uptake was not affected by

indomethacin, suggesting that the prostaglandin(s) was not involved in the control of basal glucose uptake in the intestine. The increased glucose uptake during raised blood flow was totally blocked in the presence of indomethacin. These results supported our hypothesis that elevated shear stress plays a key role in the control of intestinal glucose uptake, and further suggested that it was shear stress-induced release of prostaglandin that mediated this event.

The increase in superior mesenteric arterial blood flow directly resulted in an increase in the portal blood flow, which may have elevated shear stress in the liver as well. Elevated shear stress in the liver could trigger a hepatic event that had influence on intestinal glucose uptake through hormonal circulation or nerve reflexes. Although the superior mesenteric plexus was denervated, an enterohepatic reflex could pass through the posterior hepatic plexus (Lautt, 1980b) that distributes along the portal vein and bile duct, or hepatic vagal branches (Lee, 1985). In two animals, we surgically diverted portal venous blood from the liver to the central vena cava, which avoided exposure of the liver to raised portal blood flow. The intestinal glucose uptake was stimulated as well when the superior mesenteric arterial flow was increased in this group. The data ruled out the possibility that the liver regulated intestinal glucose uptake in response to elevated portal flow, and provided further evidence that the site of increased shear stress responsible for the regulation of glucose uptake was in the intestine.

7.4.4 The effect of prostaglandin F_{2α}

PGI₂, PGE₂ and PGF_{2α} are released from the endothelial cells in response to shear stress (Busse and Fleming, 1998; Charo et al., 1984; Nollert et al., 1989). PGE₂ and PGI₂ had no influence on glucose uptake in this study. PGF_{2α} stimulated glucose metabolism

in rat uterus (Gonzalez et al., 1989). PGF_{2α} is believed to be a vasoconstrictor which causes increase in superior mesenteric arterial pressure (Lippton et al., 1987) or decrease in the blood flow (Pawlik et al., 1975). However, PGF_{2α} at the dose used in the present study, which was about twelve times lower than that used in dogs (Pawlik et al., 1975) and much lower than that in the cat study (Lippton et al., 1987), produced only a minor and transient increase in superior mesenteric arterial pressure followed by a sustained small vasodilation. In this chapter, the indomethacin-inhibited increase in intestinal glucose uptake was reversed by PGF_{2α} in a dose dependent manner but not by PGE₂ and PGI₂ at a similar molar dose. My data suggest that PGF_{2α} is the specific prostaglandin that is responsible for mediating shear stress-induced increase in intestinal glucose uptake.

7.4.5 Unresolved issues

It seems that the increased shear stress or blood flow per se was a necessary, or permissive factor, since the infusion of PGF_{2α} under normal blood flow did not alter the glucose uptake. An example of permissive control of prostaglandin effects is previously reported. 17-beta estradiol is necessary for PGF_{2α} and PGE₁ to have their effect on uterus glucose metabolism although the hormone per se did not have any effect on glucose metabolism (Gonzalez et al., 1989). Adenosine did not serve this role since the infusion of PGF_{2α} with adenosine during constant flow perfusion did not change the glucose uptake. Shear stress-induced release of other endothelium-derived factor(s) could possibly serve as a permissive factor for PGF_{2α} action. It was reported that NO had a permissive effect on endothelial arachidonic acid metabolism and the arteriole dilation caused by the metabolite(s) (Bakker and Sipkema, 1998). This possibility, however, was

ruled out by the observation that L-NAME did not block the increased intestinal glucose uptake.

Rather than shear stress, the blood flow itself could possibly be the important factor due to the complex structure of intestinal villus and its microcirculation. A shunt between the central arteriole and the venule that drains the blood in the opposite direction in the intestinal villus was modeled by Shepherd and Kiel (1992) who suggested that the tip of the microvilli could be in a hypoxic or subhypoxic state during normal blood flow. Raised blood flow might be necessary for oxygenation and activation of the tissue to respond to PGF_{2α}. Further studies are required to elucidate the factor behind the permissive effect of blood flow on the prostaglandin action in control of intestinal glucose uptake.

During raised superior mesenteric arterial blood flow, the glucose uptake increased about 3-fold, but there was no evidence of increase in glucose oxidation since the CO₂ production and lactate balance were not different from the baseline. Whether the glucose taken up into the intestinal tissue was used for glycogen synthesis, for mucus synthesis (polysaccharide as a major component) or for other purposes are questions that need to be addressed in further studies.

It is interesting that the intestinal tissue is not sensitive to insulin. Even though the intestine could use other substrates, glucose is still one of the most important energy sources for basal intestinal metabolism and activities such as absorption and motility. The results from the present study suggest that raised superior mesenteric arterial blood flow increases intestinal glucose uptake secondary to the release of PGF_{2α}. Prostaglandins may

play a role in the initiation and maintenance of postprandial intestinal hyperemia (Gallavan and Chou, 1985). Increased blood glucose concentration due to post-ingestion absorption normally occurs at the same time as the hyperemia, and this is also the time that the intestine needs energy for absorption and increased motility. The extent of low-flow-induced intestinal mucosal lesions, including epithelial disruption and damage of the villi, is flow dependent (Chiu et al., 1970a). This could be due to the deprivation of oxygen and energy supply to the intestinal villi. Protection against the low-flow-induced mucosal lesion by intraluminal glucose (Chiu et al., 1970b) strongly suggested that the deprivation of energy source was the main cause of the damage. The absence of PGF_{2α} and other essential factor(s) in the low-flow state, however, could be the primary mechanism of the cause. The findings in this chapter may also explain, in part, the side effect on the gut of indomethacin and other non-steroidal anti-inflammatory drugs. In conclusion, the intestinal glucose uptake is regulated by blood flow dependent release of prostaglandin F_{2α} and other factors, which may be important for the prostaglandin action, however the character of this factor needs to be identified.

8. UNFINISHED STUDY AND PRELIMINARY OBSERVATIONS

8.1 HEPATIC-INTESTINAL NERVE REFLEX

8.1.1 Preliminary observations

8.1.1.1 Experimental setup

The experimental setup was similar to that used in Chapter 4. Briefly, the anterior hepatic nerve and superior mesenteric nerve were surgical denervated. A flow probe was placed on the celiac artery to monitor hepatic arterial blood flow. The superior mesenteric arterial blood flow was controlled using an arterial circuit that acquired blood from an abdominal aortic loop. The flow rate was controlled using a pump and was not changed throughout the experiment. In addition, the inferior mesenteric artery was ligated, and the spleen was removed. Two intravenous catheters were used for monitoring portal pressure and infusion of drugs into the portal vein. Systemic arterial pressure and central venous pressure were monitored as described previously.

8.1.1.2 The observations

In such an experimental setup, I noticed that the superior mesenteric arterial perfusion pressure was increased when the anterior hepatic nerve bundle was electrically stimulated. During 2 Hz stimulation of the hepatic nerve, the superior mesenteric pressure was increased by 28% (from 132 to 169 mmHg) while the systemic arterial and hepatic arterial pressure increased about 20%, portal pressure increased about 44% (from 9 to 13 mmHg) and the hepatic arterial flow was decreased. During 10 Hz stimulation, the superior mesenteric arterial pressure increased by about 30% while the systemic and hepatic arterial pressure increased by 60%, and portal pressure increased by about 70%. The changes in pressure and flow are shown in Figure 8-1. Although the amplitudes of

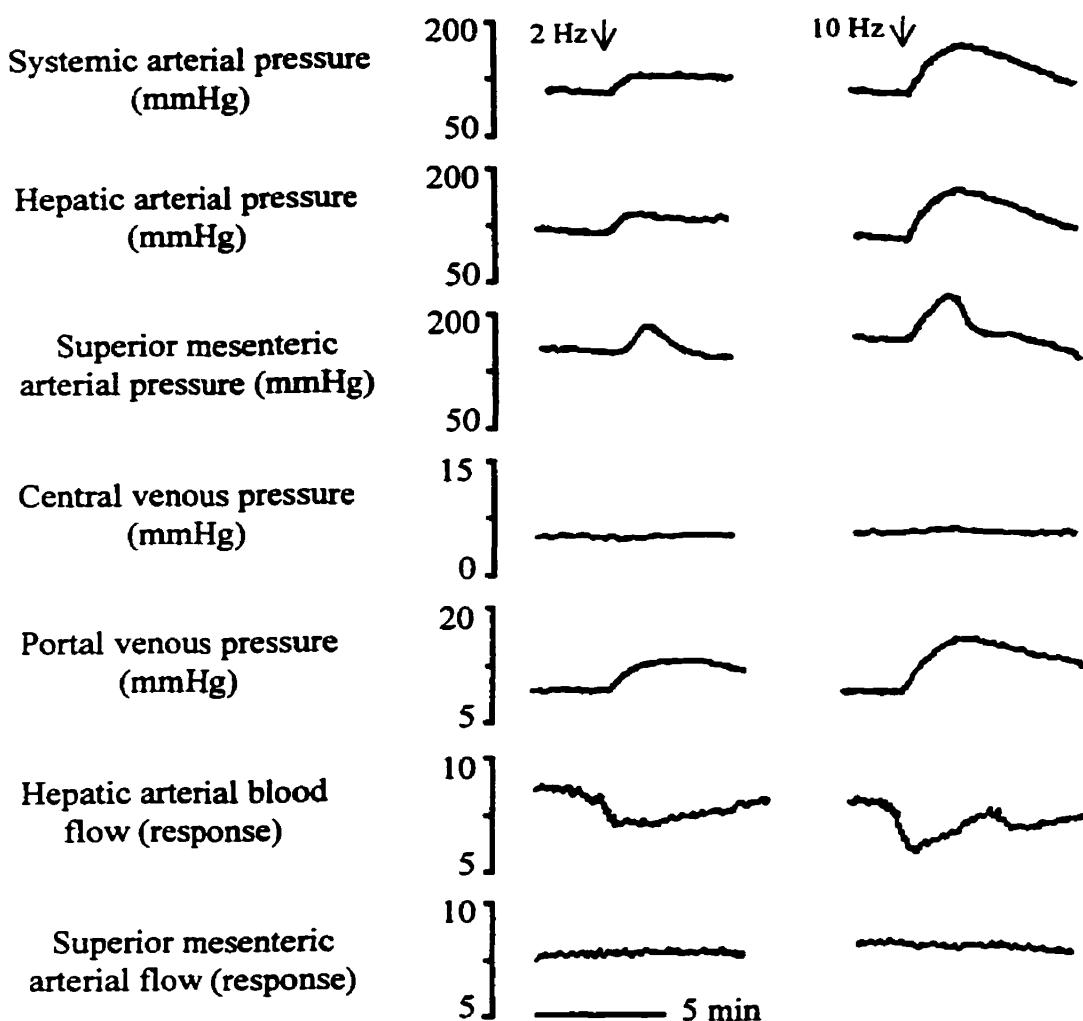


Figure 8-1. In an anterior hepatic and superior mesenteric nerve-denervated animal, electric nerve stimulation (\downarrow) of the anterior hepatic bundle caused an increase in arterial and hepatic arterial pressure and a decrease in hepatic arterial blood flow. Interestingly, superior mesenteric arterial perfusion pressure (circuit pressure) was increased too. The increase was Hz dependent. The superior mesenteric arterial blood flow was maintained using a pump.

the increases in superior mesenteric arterial perfusion pressure were variable in different experiments, the phenomenon were seen in most experiments with this setup.

It is an interesting observation since the superior mesenteric and hepatic nerves were cut. The stimulation was supposed to be towards the liver unidirectional. Any systemic effects of the stimulation should not affect the intestine. Although norepinephrine overflow might cause vasoconstriction in the intestine, the pressure in the superior mesenteric artery increased earlier than recirculation could reach the organ through the circuit.

With this thought in mind, I injected norepinephrine ($10\mu\text{g}/\text{kg}$) directly into the portal vein. A small increase (18%) in superior mesenteric arterial pressure was seen right after the injection (about 16 seconds). The pressure then started to drop in the next ten seconds followed by a big increase that was about a fifteen-second delay from the increase in systemic arterial pressure (Figure 8-2). The time delayed is about the time to cover the length of the arterial circuit at the flow rate. The first increase in the superior mesenteric arterial pressure was not likely caused by recirculation of injected norepinephrine since the increase appeared even earlier than the increase in systemic arterial pressure. Intra-venous injection of the same dose of norepinephrine caused similar increases in systemic arterial pressure and superior mesenteric arterial perfusion pressure but not the early small increase.

8.1.2 First hypothesis

The observation in the intestine suggested that a vasoconstrictor disturbance in the liver caused changes in vascular resistance of the intestine. Direct injection of norepinephrine into the portal vein ruled out the possibility of short-circuit electric

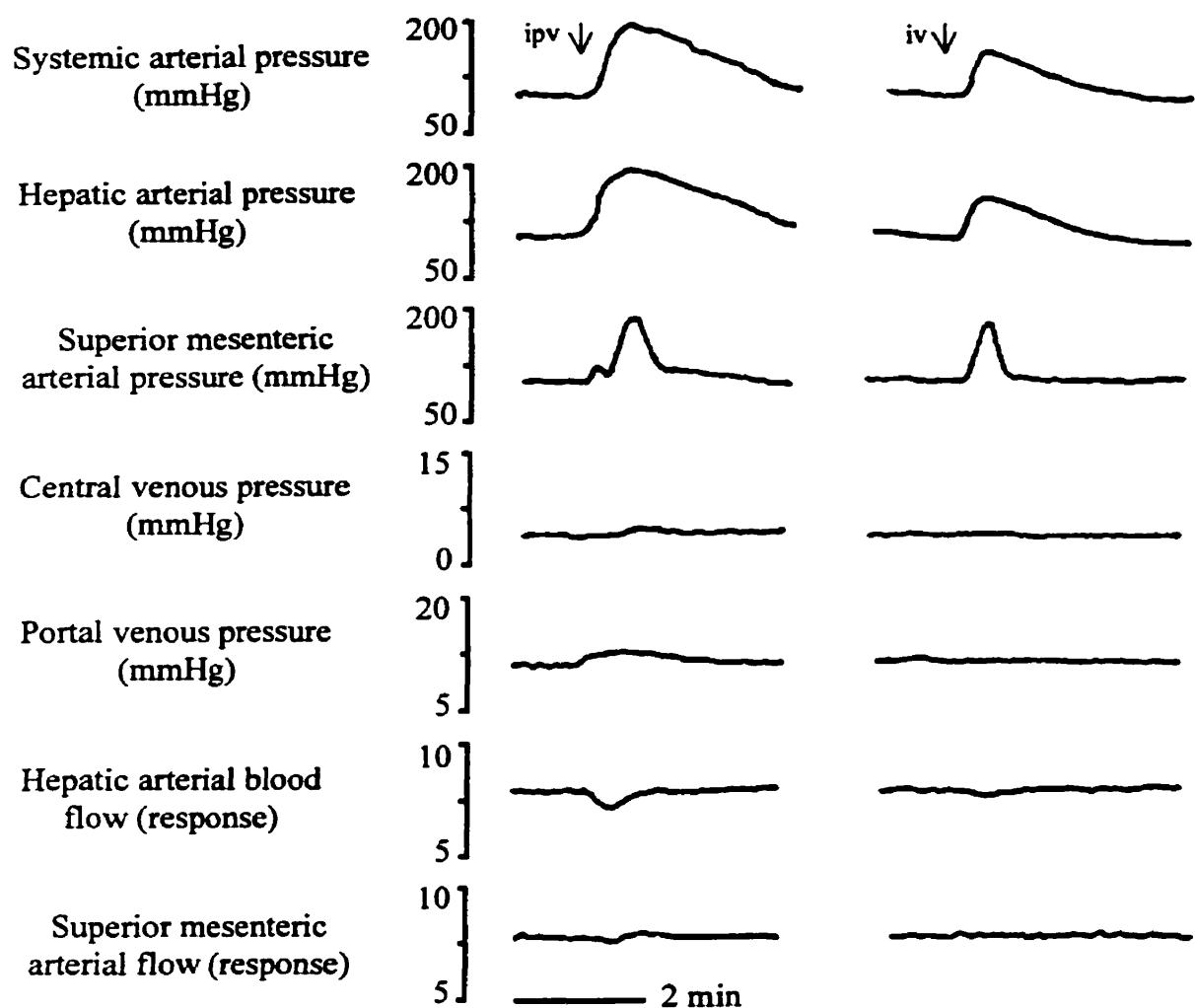


Figure 8-2. Intra-portal injection of norepinephrine ($10 \mu\text{g}/\text{kg}$, \downarrow) caused a small brief initial increase in superior mesenteric arterial pressure followed by a decreasing phase and then a large increase while intravenous injection did not cause the initial increase. Norepinephrine increased arterial and hepatic arterial pressure and decreased hepatic arterial blood flow.

stimulation of the gut. Nerve stimulation and intra-portal injection of norepinephrine increased portal pressure; intravenous injection of norepinephrine did not increase portal pressure. Elevation in portal pressure may increase shear stress in the portal vein.

8.1.2.1 The hypothesis

I hypothesized that there is an intra-hepatic sensor regulating portal flow through controlling intestinal vascular resistance. The sensor is triggered by nitric oxide that is released by increased portal venous shear stress.

8.1.2.2 Test the hypothesis

Intra-portal venous injection of a NO donor, nitroglycerin (1mg/kg), caused an initial increase in the superior mesenteric arterial perfusion pressure following a decrease associated with systemic arterial pressure (Figure 8-3), while intravenous injection of the same dose of nitroglycerin only decreased pressures. The results were supporting the hypothesis. However, L-NAME (2.5 mg/kg) and indomethacin (3 mg/kg) did not block nerve stimulation induced increase and norepinephrine induced initial small increase in superior mesenteric arterial pressure.

In addition, I found that, in another couple of experiments, denervation of posterior hepatic nerve bundle around the portal vein and bile duct did not block the response to hepatic nerve stimulation and intra-portal norepinephrine of the intestine. A stronger response in the intestine vasculature was more frequently observed when the intestinal and anterior hepatic nerve was kept intact. A similar response as seen in the intestine was also seen in the splenic artery in an experiment in which the splenic blood flow was controlled using a circuit.

The data could not all be explained by the above hypothesis.

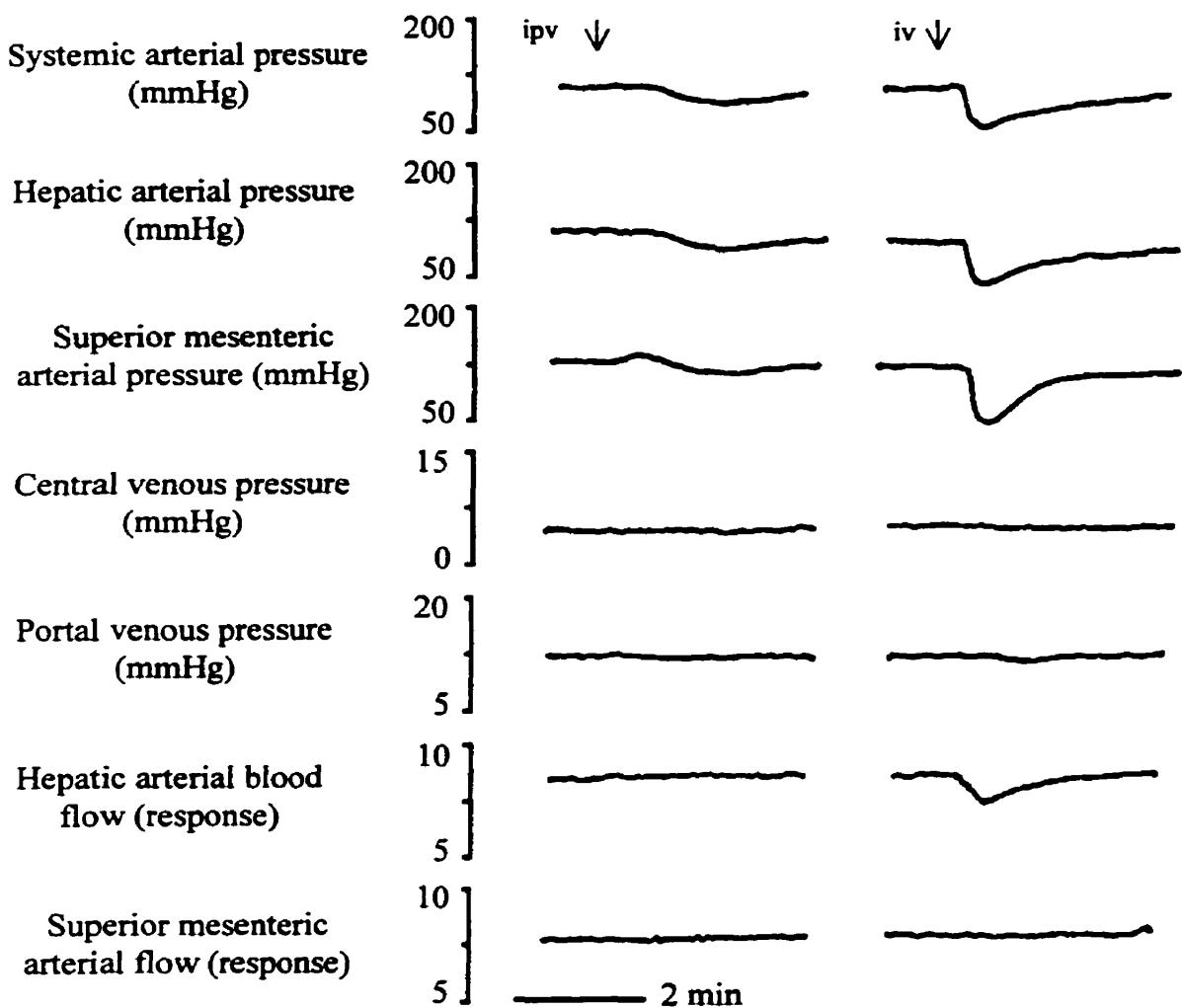


Figure 8-3. Intra-portal injection of nitroglycerin (1 mg/kg, \downarrow) caused an initial increase in superior mesenteric arterial pressure followed by a decrease while intravenous injection cause only decrease in the pressure. Nitroglycerin decreased arterial and hepatic arterial pressure, and intravenous injection decreased hepatic arterial blood flow.

8.1.3 An alternative hypothesis

Shear stress and NO seem not to be involved in the "sensor-reflex" event in the liver and intestine. Another factor left is the portal pressure and the intra-hepatic pressure since the phenomenon was seen with elevated portal pressure and during nitroglycerin injection which did not change portal pressure but might increase intra-hepatic pressure. The results from an early study also showed that raised hepatic venous pressure decreased gut conductance and portal flow (Lautt, 1977b). An alternative hypothesis can thus be formulated.

8.1.3.1 The alternative hypothesis

Pressoreceptors or pressure sensors exist in the liver to sense the change of intra-hepatic pressure. A local nerve reflex conducts the signal from the sensor to the intestine to regulate splanchnic vascular resistance resulting in a relatively constant portal and therefore intra-hepatic pressure.

8.1.3.2 More experiments

8.1.3.2.1 Splenic-intestinal double circuit. In an experiment, two arterial circuits were used. They both drew blood from the aortic loop; one circuit controlled superior mesenteric arterial blood flow while the other controlled splenic arterial blood flow. In such an experimental setup, I could adjust the flow to the intestine during nerve stimulation so that the portal blood pressure was maintained. Splenic arterial resistance did not increase if the portal pressure was not allowed to increase.

8.1.3.2.2 Vessel occlusion at different sites. In one experiment, I occluded the central vena cava at the diaphragm, which increased abdominal central venous and portal venous pressure, resulting in an increase in superior mesenteric arterial perfusion

pressure. But a portal occlusion increasing portal pressure to an even higher level did not alter the vascular resistance in the intestine. The experiment excluded myogenic effect of increased portal pressure on the arterial side. An unilateral occlusion of the carotid artery elevated systemic arterial pressure, to a similar extent as found in other experiments during nerve stimulation or changing circuit flow rate, but had no influence on intestinal blood flow ruling out the effect of systemic reflex.

8.1.3.3 A double balloon catheter and venous circuit model

In order to manipulate intra-hepatic pressure with least influence on systemic circulation, I used a surgical model in which extensive surgery allowed to drain renal and lower body venous blood separately into a reservoir. A pump controlled venous circuit returned the blood through a jugular venous cannulation to the heart. A double balloon catheter (Figure 8-4) was inserted into the central vena cava, and the hepatic vein was located between the two balloons. Inflation of the rear balloon blocked the central vena cava between the renal vein and hepatic vein. Inflation of the front balloon allowed adjusting hepatic venous pressure. Two clamp-type flow probes monitored splenic and superior mesenteric arterial blood flow.

Increase in hepatic venous pressure decreased splenic and intestinal blood flow without change in systemic arterial pressure (Figure 8-5); while the occlusion of portal vein increased portal pressure to a similar level but did not change splenic and intestinal blood flow (data not shown). However, phentolamine (3 mg/kg, blocked response to nerve stimulation) did not block the response in the spleen and intestine to elevated intra-hepatic pressure. The administration of atropine (3 mg/kg) attenuated the response in one experiment.

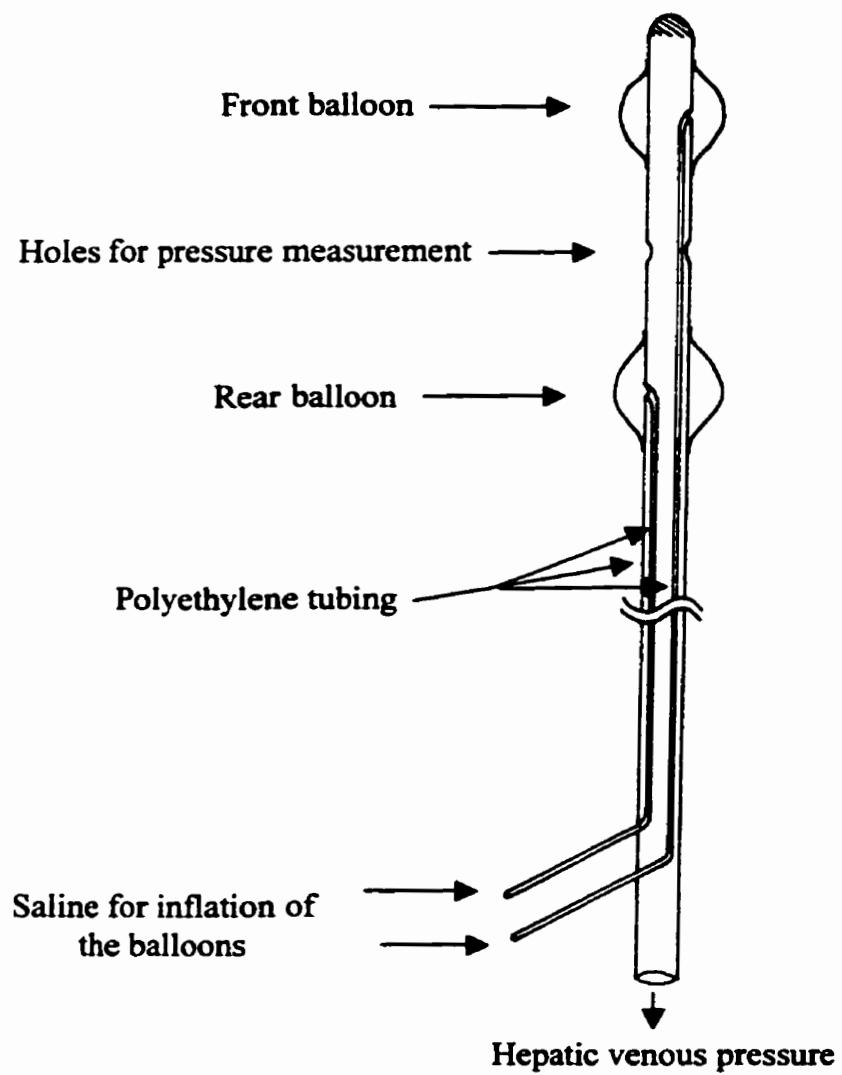


Figure 8-4. Illustration of the balloon catheter. The catheter was inserted into the central vena cava, and the hepatic vein was located between the two balloons. Inflation of the rear balloon blocked vena cava, inflation of the front one could adjust hepatic venous pressure. The balloon was made of plastic film.

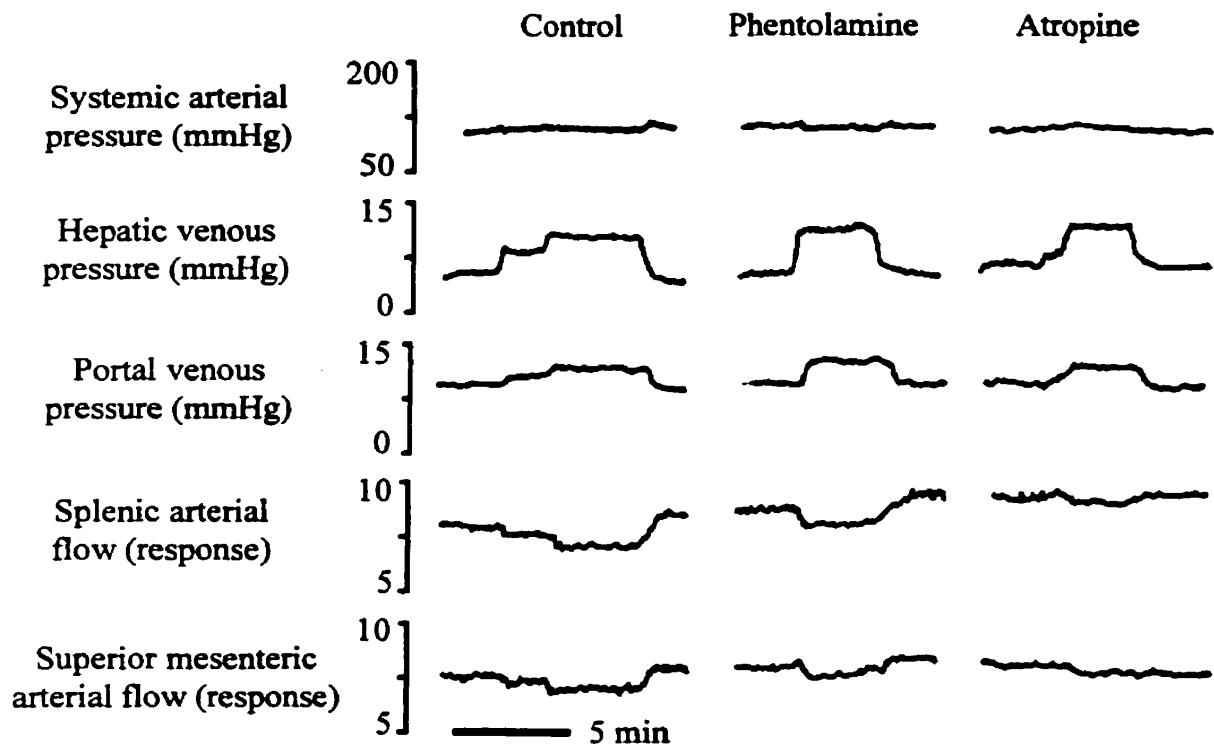


Figure 8-5. In an animal with venous circuit, the hepatic venous pressure was controlled using a double balloon catheter. A stepwise increase in hepatic venous pressure caused a stepwise decrease in splenic and superior mesenteric arterial blood flow. The response in blood flow was not blocked by phentolamine and atropine. Portal occlusion increased portal pressure to a similar level but did not alter splenic and mesenteric blood flow (data not shown).

In one experiment, an increase in renal venous pressure suggested an increase in the flow during elevated hepatic venous pressure.

8.1.4 Summary of the preliminary experiments

The data from these preliminary experiments suggested that the increase in intra-hepatic pressure triggered an increase in splanchnic vascular resistance. The time of response was in favor of local nerve reflex. If nitroglycerin caused vasodilation more in presinusoidal sphincters and norepinephrine and sympathetic nerve caused more constriction in postsinusoidal sphincters, then, both vasodilator and vasoconstrictor could increase intra-hepatic pressure that was proposed to be the trigger. Besides, phentolamine did not block the response excluded sympathetic nerves in the reflex.

Different sensory responses in the liver resulting in discharge of the afferent nerves have long attracted researchers (Lautt, 1983a; Sawchenko and Frideman, 1979). The functional consequence of the sensors or their afferent nervous discharge, however, was rarely discovered. Niijima observed afferent discharges caused by venous pressure stimuli in the liver (Niijima, 1977). The author suggested that pressoreceptors are present in the portal venous system and send information about portal pressure to the central nervous system. Studies demonstrated the relationship between hepatic afferent and renal efferent nerve activities (DiBona and Sawin, 1995; Kostreva et al., 1980), suggesting that a reflex system may play a role in the regulation of plasma volume. Nevertheless, little functional evidence was provided. My experiments provided some preliminary data that the hepatic pressoreceptor reflex regulates splanchnic blood flow. In one of the experiments, the data suggested an increase in renal venous blood flow when intra-hepatic pressure was elevated. These data supported the postulated mechanism that a

hepatic venous pressoreceptor reflex controlling plasma volume or venous pressure through the regulation of intestinal and renal blood flow (decrease in water absorption and increase in urine excretion) although the observation is still very preliminary and lacking many steps. The nervous pathway and type of the nerves need to be discovered. My data were not in favor of adrenergic nerves mediating the reflex.

One of the difficulties in the study is to change intra-hepatic pressure without disturbing the systemic circulation. I used a venous circuit with double balloon catheter to achieve this goal. The model worked successfully. Renal venous flow could be monitored simultaneously with the splanchnic flow if enough channels on the recorder and flow meters were available. The catheter worked although it was a hand made one. Nevertheless, I did feel it necessary to develop a better model. Besides, clamp-type electromagnetic flow probe could not fully meet the need, and the hand-made catheter was a little too rough.

Thus, although the data are suggestive, the mechanism behind hepatic regulation of gut, spleen and kidney circulation remains unknown.

8.2 BETA RECEPTOR AGONISM AND INTESTINAL GLUCOSE UPTAKE

8.2.1 Intestinal glucose uptake

8.2.1.1 Background

The mechanism for the intestine to regulate its glucose utilization is not well understood. I discussed that the intestinal tissue is not sensitive to insulin and the hepatic insulin sensitizing substance (Xie and Lautt, 1996). The involvement of PGF_{2α} in increased intestinal glucose uptake from the blood was discovered and studied in

different aspects in comparison with other prostaglandins in Chapter 7. The reason why increased blood flow was necessary for PGF_{2α} action remained to be elucidated.

In some experiments using the similar model as used in Chapter 6 and 7, I found that isoproterenol stimulated intestinal glucose uptake. The mechanism is not clear at this point, but it seems to be prostaglandin-independent.

8.2.1.2 Observations

In an *in situ* perfused intestinal model as described in Chapter 6 and 7, isoproterenol (0.2 µg/min/kg) was infused into the superior mesenteric artery during constant pressure or constant flow perfusion. The infusion of isoproterenol decreased superior mesenteric arterial perfusion pressure by 65±6 from 110±8 mmHg (n=6, p<0.0001, Figure 8-6) during constant flow perfusion while the flow rate was not changed (35±7 ml/min/100 tissue). Isoproterenol increased intestinal glucose uptake by 28.7±5 from 0.4±3.0 µmol/min/100g tissue (p<0.01, Figure 8-7).

During constant pressure perfusion, isoproterenol increased superior mesenteric arterial blood flow by 91±20 from 31±5 ml/min/100g tissue (p<0.005, n=6, Figure 8-6) while the perfusion pressure was not changed (107±10 mmHg). The infusion of isoproterenol under this condition increased intestinal glucose uptake by 78.3±16.0 from 2.9±5.3 µmol/min/100g tissue (p<0.005, Figure 8-7). The intestinal glucose uptake increased by isoproterenol during constant pressure perfusion is more than that during constant flow perfusion (p<0.01).

Isoproterenol stimulated intestinal glucose uptake from the blood during both constant pressure and constant flow perfusion. The amplitude of the increase in glucose uptake was higher during constant pressure perfusion where the flow rate was increased

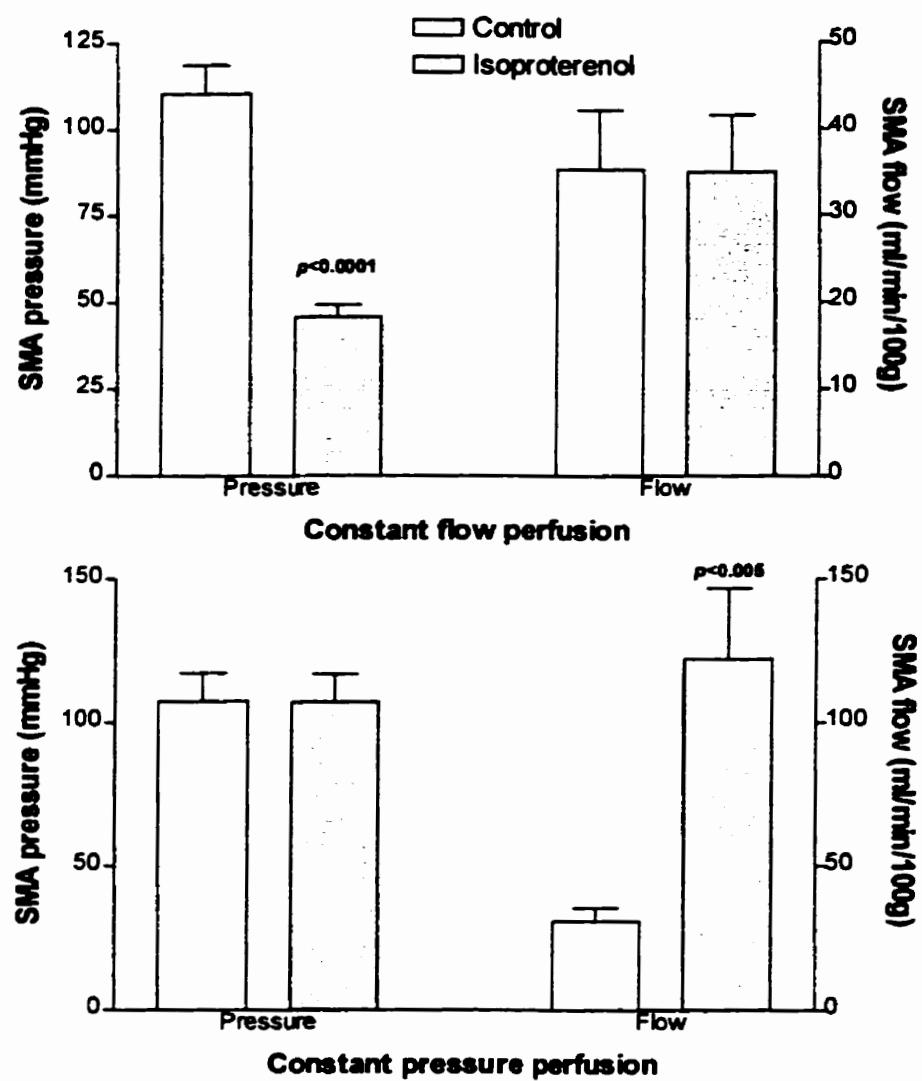


Figure 8-6. Changes in superior mesenteric arterial (SMA) perfusion pressure and blood flow caused by isoproterenol (0.2 mg/min/kg) intra-arterial infusion. The perfusion pressure decreased by isoproterenol during constant flow perfusion while blood flow increased during constant pressure perfusion. n=6

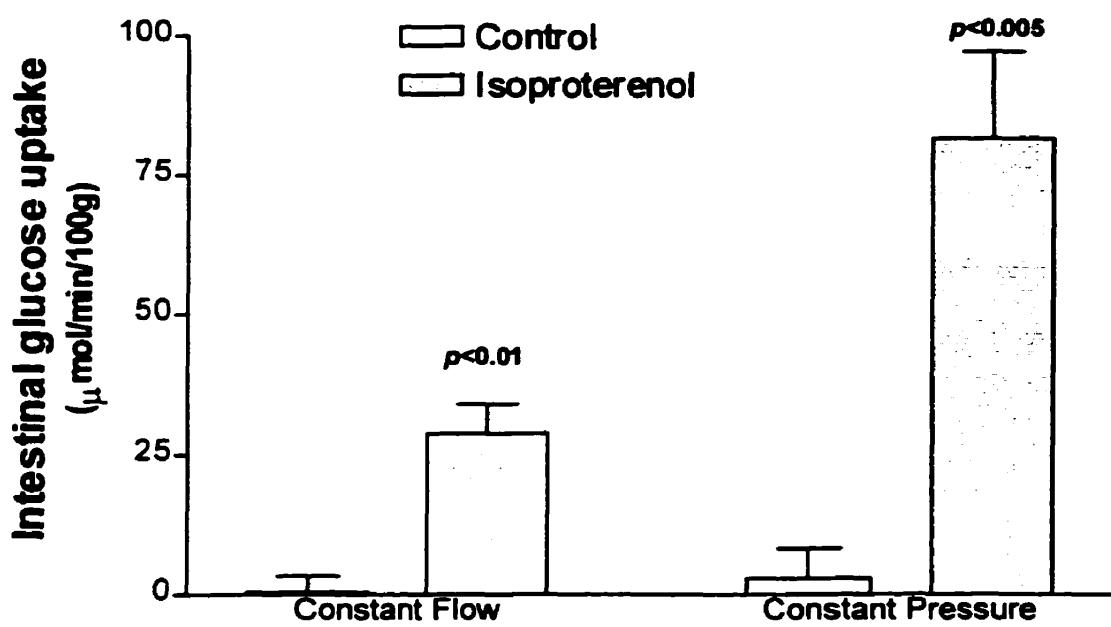


Figure 8-7. Intestinal glucose uptake from the blood during control and isoproterenol intra-arterial infusion (0.2 mg/min/kg). Isoproterenol increased the glucose uptake during both constant flow and constant pressure perfusion. The glucose uptake increased more during constant pressure perfusion ($p < 0.01$). $n=6$

due to the vasodilator effect of isoproterenol.

8.2.2 Discussion

Isoproterenol increased intestinal glucose uptake during constant flow perfusion suggesting that isoproterenol has direct effect on intestinal glucose metabolism. These results, to my knowledge, were not reported before. The effect of isoproterenol on intestinal glucose uptake seems not likely to be secondary to its vasodilator effect since the infusion of adenosine during constant flow perfusion decreased perfusion pressure to a similar level but did not change glucose uptake. Isoproterenol is a beta agonist. Its vasodilator effect is generally suggested to be mediated through agonism of beta-2 receptors. However, all three types of beta-receptors were found in the intestine (Taneja and Clarke, 1992), and their functions are not fully clear. Whether the effect of isoproterenol on intestinal glucose uptake is mediated via beta-receptors and which subtype of the receptors is involved are not clear at this point.

During constant pressure perfusion where the blood flow was increased, isoproterenol increased glucose uptake to a greater extent. The first explanation would be that shear stress-induced production of prostaglandin mediated an additional increase in intestinal glucose uptake when blood flow was elevated (see the results and discussion in Chapter 7). The difference in glucose uptake induced by isoproterenol between constant flow and constant pressure perfusion should be the portion of prostaglandin-induced increase in uptake. However, in a preliminary experiment, isoproterenol-induced increase in intestinal glucose uptake was not altered by indomethacin during either constant flow or constant pressure perfusion (data not shown). Blood flow, again, seems to play an important role in intestinal glucose uptake stimulated by isoproterenol when the flow rate

was elevated. The mechanisms behind increased blood flow are to be further investigated.

9. SUMMARY AND FUTURE DIRECTIONS

9.1 SUMMARY OF THE MAJOR FINDINGS

This thesis addressed several aspects in splanchnic hemodynamics and hemodynamics-related regulation of oxygen and glucose metabolism. The results demonstrated the interaction of different endogenous vasoactive substances and vascular factors at hemodynamic and metabolic levels in whole animal preparations.

Adenosine is a very important vasodilator in the liver. NO is another endogenous vasodilator, which was found not to be involved in control of basal hepatic vascular tone in previous studies. Results from previous studies also indicated that adenosine and NO inhibit sympathetic activities in the liver. In addition, NO was found to antagonize the vasodilator effect of adenosine in the intestine but not in the liver. My results demonstrated, in the hepatic artery, that the inhibition of NO release potentiated the vasoconstrictor effect of norepinephrine. Norepinephrine, at a dose to produce maximum vasoconstriction in the hepatic artery, only suppressed the vasodilator effects of adenosine and isoproterenol slightly. However, the suppression of the vasodilators by norepinephrine was substantially potentiated after the blockade of NO synthase. These results indicate that NO inhibits the vasoconstrictor effect of norepinephrine, and furthermore, that NO strongly inhibits the effects of norepinephrine in its interaction with other vasodilators. The results imply that impaired NO function in pathological situations could result in a potentiation of the vasoconstrictor effect of norepinephrine and an exaggeration of the suppression by norepinephrine of other vasodilators.

Sympathetic nerve stimulation or norepinephrine infusion stimulates hepatic

glycogenolysis and increases hepatic glucose output. Having shown that NO inhibited the vascular effect of norepinephrine, I further investigated the influence of NO on norepinephrine-induced hepatic glycogenolysis. NO potentiated norepinephrine-induced increase in hepatic glucose output while it inhibited the vasoconstrictor effect. This finding suggested that the action of NO fully met the physiological requirement in both hemodynamics and metabolism in that post-synaptic action of NO in the liver permits NO to protect the vasculature against elevated shear stress but maintains and potentiates the glycogenolysis that leads to increased hepatic glucose output for extra-hepatic tissues.

In the intestine, NO antagonizes the vasodilator effects of adenosine and isoproterenol. Adenosine inhibits intestinal oxidative metabolism. I hypothesized that NO also antagonizes adenosine effects in control of intestinal metabolism. The results demonstrated that adenosine decreased oxygen consumption and increased lactate production in the small intestines. Shear stress-induced release of NO inhibited the metabolic effect of adenosine. Blockade of NO synthase restored the adenosine effects on oxygen consumption and lactate production when shear stress was elevated. Introduction of exogenous NO inhibited the metabolic effects of adenosine when shear stress was not elevated. The finding supported the hypothesis that NO antagonizes adenosine effects in both hemodynamics and metabolism in the intestine, and advanced the studies on the interaction of adenosine and NO. The finding may also lead to a better understanding of intestinal physiology in the situation such as postprandial hyperemia.

The mechanism for the regulation of intestinal glucose utilization is not clear. It is known that the intestinal tissues are not sensitive to insulin and the hepatic insulin sensitizing substance. For the first time, I reported, based on the observations made in

this thesis, that increased blood flow elicited an increase in intestinal glucose uptake from the blood. I hypothesized that the release of autacoids by elevated shear stress, secondary to increased blood flow, mediated increased intestinal glucose uptake. I found that prostaglandin F_{2α} was the mediator of increased intestinal glucose uptake when blood flow was increased, supporting the hypothesis. Nevertheless, elevated blood flow was another necessary factor for the increase in intestinal glucose uptake. The mechanism behind this factor needs further study to elucidate. The findings in this study open a new area of research and emphasize the importance of the intestine not only as an absorptive organ but also as an organ being important for whole body glucose homeostasis. The findings may also lead to a therapeutic approach towards the protection against the injury of intestinal mucosa caused by non-steroidal anti-inflammatory drugs or during low-flow states.

The results from the studies in this thesis demonstrated intimate relationships of hemodynamics and metabolism, and provided a body of evidence that the interaction between the major vasoregulators occurred at both hemodynamic and metabolic levels, which is important in maintaining functional homeostasis of an organ. Changes in hemodynamics and the factors secondary to the changes are also intimately related to the regulation of metabolism.

9.2 FUTURE DIRECTIONS

In the first chapter of the thesis, I discussed the tangled relationship of the mechanisms in control of splanchnic blood flow and metabolism. There are still many pieces of this puzzle missing. NO antagonizes the vasodilator effect of adenosine in the

intestine but not in the liver. I found that the antagonism of adenosine by NO happens at the metabolic level in the intestine. NO suppresses the norepinephrine effect in the inhibition of vasodilator effects of adenosine and isoproterenol in the hepatic artery. I also found that NO potentiated norepinephrine-induced glycogenolysis while it inhibited the vascular effects in the liver. One interesting future area will be to determine if there is any interaction between NO and adenosine in control of hepatic metabolism.

NO inhibits the vasodilator effect of adenosine while it can fully shut down the metabolic effect of adenosine in the intestine. I would like to hypothesize that the interaction of these two compounds, reliant on changes in blood flow, regulates the hyperemia and hypermetabolic activities in the postprandial state. Further experiments are needed to test the hypothesis.

Increased blood flow was found necessary for mediating increased intestinal glucose uptake in the presence of PGF_{2α}. The mechanism for the permissive effect of increased blood flow is not clear. Blood flow-associated oxygen tension in the intestinal mucosa may be necessary for glucose uptake. What happens to glucose taken up by intestine tissue? A preliminary hypothesis was formulated that the glucose is used for mucus synthesis, and the mucus excreted into the lumen is partially reabsorbed in the large intestine, which forms an important circle and plays a role in control of glucose homeostasis. A future project could be designed to test the hypothesis.

I have mentioned that PGF_{2α} may be the missing factor that causes cyclooxygenase inhibitors-related damage of gastrointestinal mucosa. If proven, this may lead to a therapeutic approach in gastrointestinal protection against damage caused by non-steroidal anti-inflammatory drugs. The findings in my current study are only the

beginning of the story.

I have provided evidence for the influence of blood flow on intestinal metabolism. Published data suggested that portal blood flow may have influence on liver metabolism. Portal venous blood flow is mainly controlled by the mesenteric vascular bed. Under an integrative consideration, the intestinal blood flow should have influence on both the intestine and liver. We have seen some aspects, for example, the influence on intestinal glucose metabolism and shear stress-induced release of NO in the liver. Further experiments are needed to incorporate the blood flow-related regulations of intestinal and hepatic metabolisms, which are linked by the portal flow. In addition, I would like to investigate if there is any feedback mechanism of the liver to control intestinal vascular resistance. All these will be incorporated into my long term research interests.

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