ADENOSINE AS A MEDIATOR OF HYPOXIC VASODILATION AND AS A MODULATOR OF NOREPINEPHRINE AND SYMPATHETIC NERVE-INDUCED VASOCONSTRICTION IN THE SUPERIOR MESENTERIC ARTERY

A THESIS PRESENTED TO THE UNIVERSITY OF MANITOBA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE

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ISBN 0-315-51684-4

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ABSTRACT

Two facets of adenosine-mediated blood flow in the feline intestine were examined in this thesis.

First, modulation of sympathetic nerve stimulation (SNS) and norepinephrine (NE) induced vasoconstriction was examined in the presence of exogenous adenosine. Adenosine inhibited the constrictions, regardless of stimuli, in a dose-dependent manner (-9.8%, -24.8% &-54.2% for the low, mid and high doses of adenosine respectively). Adenosine had a larger inhibitory effect on NE-induced constrictions than SNS-induced constrictions

Second, the role of endogenous adenosine in hypoxia-induced vasodilation of the intestine was examined. Vasodilation induced by stepwise decreases in oxygenation of intestinal blood was not altered by adenosine receptor blockade (resistance declined by 23.8% before and 35.6% after receptor blockade).

Exogenous adenosine is a more potent modulator of vasoconstriction of the SMA induced by NE than by SNS. Thus, in the feline intestine, adenosine modulates by a postsynaptic mechanism. However, endogenous adenosine does not mediate hypoxia-induced vasodilation, since blockade of adenosine receptors does not reduce the response.

INTRODUCTION

This thesis deals with some relationships between adenosine and intestinal blood flow, therefore the introduction has been partitioned into three broad categories: adenosine, gastrointestinal circulation, and adenosine involvement in the control of blood flow in various organs.

The first category deals with general aspects of adenosine in a biological system. This includes the adenosine receptors and their second messenger systems. Also, adenosine synthesis and catabolism as well as its transport into and out of cells are examined. Finally, some of the theories as to how adenosine elicits its modulatory effects are presented.

The second section is an overview of the gastrointestinal circulation. The general blood vessels of the splanchnic circulation and their distribution are reviewed and details of the architecture of the vessels supplying the various layers of the intestine are presented. The extrinsic and intrinsic innervation of the intestine and its vasculature, as well as some of the possible neurotransmitters which may affect intestinal function are also presented. Phenomena such as autoregulation and autoregulatory escape are considered, and the myogenic and metabolic theories of blood flow regulation are included here.

The last section of the introduction reviews the relationship between adenosine and blood flow in a number of organs, including the brain, heart, kidney, liver and intestine.

I ADENOSINE - GENERAL

Adenosine Receptors:

Londos and Wolff (1977) divided adenosine receptors into P and R sites based on the structural requirements of agonists at these sites. The P site was named due to the fact that agonists active at this receptor must have an intact purine moiety. The receptor is an intracellular one, with a high affinity for adenosine and is insensitive to methylxanthines. It is thought to be closely associated with the catalytic unit of adenylate cyclase, and receptor occupation inhibits cyclic AMP generation. This P-site is not to be confused with the P-sites designated by Burnstock (Paton,1984). Under his system the P_1 site is sensitive to adenosine and AMP, and is roughly equivilant to the aforementioned P-site, while adenosine triphosphate (ATP) and adenosine diphosphate (ADP) are ligands at the P_2 site.

The extracellular R-sites require an intact ribose moiety in compounds which interact with these receptors. Methylxanthines have an inhibitory effect on R-receptor responses. The R-sites are further subdivided depending

on whether receptor occupation caused inhibition (R_i) or activation (R_a) of adenylate cyclase activity (Londos et al., 1980).

Adenosine receptors characterized in brain cell cultures also inhibited (A_1) or activated (A_2) adenylate cyclase. This terminology has now largely replaced the R_i and R_a classification of Londos. Also, the effects of adenosine on adenylate cyclase activity as a means of identifying adenosine receptor type has now been replaced by rank order of potency of several adenosine analogues in binding assays. A rank order potency of L-PIA>CADO>NECA (L-PIA: L-N^6-phenylisopropyladenosine; CADO: 2-chloroadenosine; NECA: 5'-N-ethylcarboxamide adenosine) is used to identify the A_1 receptor, while NECA>CADO>L-PIA identifies the A_2 receptor (Ribeiro and Sebastiao, 1986).

The P_2 receptor has tentatively been subdivided based on the fact that contraction (via the P_{2X} receptor) is sometimes seen in the presence of ATP or ADP as opposed to relaxation (P_{2Y} receptor). In addition, the alleged P_{2Y} receptor shows steroselectivity for the D-enantiomer analogues of both ATP and ADP, whereas the P_{2X} receptor is indiscriminant. Without appropriate antagonists, subdivision of the P_2 receptor remains speculative. In blood vessels, the effects of P_2 receptor stimulation have been found to be dependent on the presence of intact endothelium and hence the release of endothelium derived

relaxing factor (EDRF) (Furchgott, 1983; Cusack et al., 1988).

Adenosine Second Messengers:

With the use of rank order of agonist potencies it is now evident that cyclic AMP is not the only second messenger utilized by adenosine receptors. Adenosine receptors (probably A_1 receptors) in the heart exert effects on potassium ion (K^+) conductance via guanine regulatory proteins (Stiles, 1986; Fredholm & Dunwiddie,1988). In brain slices adenosine increases the levels of cyclic GMP through a process dependent on extracellular calcium (Fredholm & Hedqvist,1980). Guinea pig cerebral, cortical, and hippocampal vesicular preparations show inhibited calcium dependent K^+-evoked norepinephrine release in response to adenosine application. Increasing the calcium concentration in the medium can prevent this inhibitory effect. In potassium-depolarized synaptosomes adenosine also inhibits ^{45}Ca uptake, a process which is theophylline-sensitive.

There are A_1 receptors identified in a number of tissues whose rank order of potencies of agonists is CHA(N^6-cyclohexyladenosine),L-PIA,NECA>CADO. The effects of these receptors, whose locations are confined to nerve endings and the heart, may also be accounted for by actions through calcium rather than by adenylate cyclase activity (Ribeiro & Sebastiao, 1986). In both the

vesicular and the synaptosomal preparations mentioned above, the rank order of agonist potencies is different from either the A_1 or A_2 receptor. The calcium channel blocker nifedipine has been observed to displace 5'-N-ethylcarboxamide adenosine (NECA) binding from brain membranes as well as to potentiate the depressant actions of adenosine on the rat cerebral cortex. These observations suggest a link between adenosine receptors and some form of a calcium channel (Ribeiro & Sebastiao, 1986). Some authors have proposed that this calcium-mediating adenosine receptor be labelled A_3 and treated as a separate receptor from the A_1 receptor, which mediates cyclic AMP concentrations (Ribeiro & Sebastiao, 1986).

Other authors feel the introduction of another adenosine receptor is not necessary. Their feeling is that the adenosine receptor is linked to GTP binding proteins (or G-proteins) and it is the array of G-proteins, or the permutations and combinations of interaction between these proteins and effector units, that account for the various effects of adenosine receptors (Fredholm et al., 1988).

The evidence supporting the association of the A_1 receptor with G-proteins is quite strong, although circumstantial. The presynaptic inhibitory effects of adenosine are blocked by pertussis toxin. Pertussis toxin is known to ADP-ribosylate the inhibitory G-protein, thus

preventing association with a receptor to effect a response. Therefore, this effect of the A_1 receptor depends on the inhibitory G-protein (Stiles, 1986; Dolphin, 1987). In solubilization studies of the A_1 receptor, the receptor was found to retain all of its pharmacological properties including its sensitivity to guanine nucleotides. In addition, guanine nucleotides do not always eradicate all high affinity agonist binding (Stiles, 1986). N-ethylmalemide (NEM), which binds quite selectively with the inhibitory G-protein, also prevents the prejunctional inhibition of transmitter release. Although NEM also causes a shift from high- to low-affinity binding of adenosine analogues, NEM itself does not bind to the A_1 receptor. Nor does NEM affect adenosine binding or adenylate cyclase activity associated with the A_2 receptors (Fredholm et al., 1987). This sort of experimental data indicate a close association between the A_1 receptor and the inhibitory G-protein.

A definite link between adenosine and calcium movement was established by studying phosphorylation of synapsin I. Synapsin I is confined to nerve terminals and is phosphorylated by both Ca^{+2} and cAMP-dependent protein kinases at distinct sites. In addition, synapsin I and Ca^{+2}/calmodulin-dependent protein kinase II are both involved in controlling transmitter release. The

adenosine agonist, N^6-cyclohexyladenosine (CHA) was able to reduce the Ca^{+2}-dependent phosphorylation at a site on synapsin I not affected by cAMP-dependent protein kinase induced by very brief, but not sustained K^+ depolarization. Furthermore, neither the L-channel (dihydropyridine-sensitive, voltage-sensitive calcium channel) agonist, Bay k 8644, or antagonist, nifedipine, were able to prevent the presynaptic inhibition of neurotransmitter release by adenosine. Thus adenosine is capable of limiting Ca^{+2} entry through N-channels (dihydropyridine-insensitive, voltage-sensitive calcium channels) (Fredholm & Dunwiddie, 1986; Fredholm et al., 1988; Miller, 1987).

The relationship between adenosine and the protein kinase C (PKC) system has begun to be investigated. PKC stimulation has some effects which are opposite to those of A_1 receptor stimulation. PKC stimluation by the phorbol ester, phorbol dibutyrate, caused an increase in stimulus-evoked neuronal Ca^{+2}-influx, and increased norepinephrine release (Fredholm et al., 1988; Schubert, 1988). However, when PKC stimulation and A_1 receptor stimulation were combined, adenosine inhibition of neurotransmitter release was actually increased, indicating the two systems did not detract from each other (Fredholm et al., 1988). On the other hand, the ability of theophylline to increase stimulus-evoked Ca^{+2}-influx

(presumably due to inhibition of endogenous adenosine) was lost upon addition of phorbol dibutyrate (Schubert, 1988). Phorbol esters also blocked A_1-receptor-mediated inhibition of cAMP accumulation (Schubert, 1988). Therefore, PKC stimulation appears to have some antagonistic effects on the actions of adenosine at the A_1 receptor, but the mechanism involved, and consequently which particular adenosine actions are affected, are not well delineated.

Adenosine Biosynthesis:

De novo formation of purines in the body proceeds by the collection of various components from amino acids, formate and carbon dioxide (Phillis & Wu, 1981; Snyder, 1985). It has been shown that glutamine provides N_3 and N_9 of the purine ring and that N_1 and N_7 are derived from aspartate and glycine respectively. Glycine also donates C_4 and C_5, while C_2 and C_8 come from formate and C_6 from carbon dioxide (Phillis & Wu, 1981). The net result of this series of reactions is the formation of inosine monophosphate (IMP), from which 5'-adenosine monophosphate (5'-AMP), and then adenosine can be formed. Since purines formed by this process are normally converted to nucleotides, rather than nucleosides, de novo synthesis does not contribute significantly to cellular adenosine concentrations (Phillis & Wu, 1981).

Alternatively, the formation of adenosine by breakdown of RNA has also been taken into consideration as a source. Direct cleavage of adenosine from RNA terminal groups is a negligible source. Breakdown of RNA to adenosine 3-monophosphate and then dephosphorylation to form adenosine may contribute somewhat to cellular concentrations (Phillis & Wu, 1981).

The formation of adenosine from 5'-AMP is considered to be one of the major sources of adenosine. Conversion from 5'-AMP to adenosine can occur through a number of enzymes, only one of which is considered to be important; the 5'-nucleotidase. Acid or alkaline phosphatases have no specificity for 5'-AMP and at neutral pH their activities are very low. The enzyme purine nucleotide phosphorylase could produce adenosine from adenine through condensation with ribose-1-phosphate. However, low substrate availability and low activity favour the reverse reaction (i.e. adenine formation), again making it an unlikely source of adenosine (Arch and Newsholme, 1978; Phillis & Wu, 1981; Snyder, 1985).

Both the physical properties and locations of the 5'-nucleotidase favour this enzyme as a major producer of adenosine. This enzyme is relatively specific for 5'-AMP (AMP), the main precursor of adenosine, and its optimal pH is close to physiological pH. In addition, the maximal activity of this enzyme is great enough to adequately

account for known rates of adenosine formation (Arch & Newsholme, 1978; Phillis & Wu, 1981; Snyder, 1985; Stone, 1981). Three forms of the enzyme have been isolated from different subcellular fractions: the plasma membrane, the cytosol, and lysosomes. Each enzyme appears to produce adenosine under different conditions.

The enzyme bound to the plasma membrane faces the extracellular space, and therefore is labelled ecto-5'-nucleotidase. It is competitively inhibited by both ATP and ADP and their structural analogues (Collinson et al., 1987; Itoh et al., 1986; Newby et al., 1987). When AMP is used as the substrate the cytosolic enzyme is stimulated by both ATP and ADP. Phosphocreatinine and IMP inhibit the enzyme's activity, but phosphate generated by ATP or ADP hydrolysis has much stronger inhibitory effects. If IMP is used as the substrate by the cytosolic 5'-nucleotidase, low concentrations of AMP are stimulatory and high concentrations inhibitory, to the enzyme's activity. ATP, ADP, phosphocreatinine and phosphate all have the same effect as when AMP is the enzyme substrate. A decrease in the energy-charge value ((ATP + 1/2 ADP)/(ATP + ADP + AMP)) of the cell will also stimulate enzyme activity, even if it is already inhibited by phosphate (Itoh et al., 1986). The lysosomal 5'-nucleotidase is almost completely inhibited at physiological adenine nucleotide concentrations, and is

likely only activated under conditions of severe ATP and ADP depletion (Collinson et al., 1987; Newby et al., 1987).

Adenosine can also be formed through a transmethylation pathway which bypasses ATP catabolism. This involves the transfer of a methyl group from S-adenosylmethionine (SAM) to a methyl acceptor, yielding S-adenosylhomocysteine (SAH). The enzyme SAH hydrolase then hydrolyses SAH to adenosine and homocysteine. The rapid metabolism of adenosine forces the reaction in the direction of adenosine production, rather than SAH production as the equilibrium of the enzyme favours. Experiments in isolated guinea pig hearts indicate that the major portion of adenosine production under normoxic conditions arises through the transmethylation pathway. However, under hypoxic conditions the transmethylation rate only increases slightly compared to the increased rate of adenosine release. Consequently the increased adenosine production probably comes from increased 5'-nucleotidase activity (Lloyd and Schrader, 1987). This balance of enzyme activities has not been validated in other tissues.

Adenosine Catabolism:

Two of the enzymes responsible for adenosine catabolism are adenosine kinase and adenosine deaminase.

Adenosine kinase converts adenosine back to AMP while adenosine deaminase metabolizes adenosine to inosine. Under basal conditions, adenosine kinase may approach substrate saturation with tissue concentrations of adenosine, consequently most adenosine in the cell is cycled back to AMP. However, when the concentration of adenosine increases, the activity of adenosine kinase cannot increase much further, therefore more adenosine must be processed by adenosine deaminase. In the presence of excess adenosine SAH-hydrolase can also use adenosine to form S-adenosylhomocysteine, and in this way reduce the adenosine pool (Arch and Newsholm, 1978; Sparks & Bardenheuer, 1986).

Adenosine Transport:

While the catalytic enzymes function intracellularly by and large most adenosine and adenosine receptors are located extracellularly. Nucleoside transport systems have been identified which efficiently remove adenosine from the vicinity of the extracellular receptors and transport it into the cell. This system links regulatory enzymes for adenosine and its site of action.

In rat cortical synaptosomes two types of uptake systems have been identified (Bender et al., 1981). The first is a very rapid system saturating in 60s, while the second is a slower uptake system which requires

approximately 15 to 30 minutes to saturate. The rapid uptake system is believed to occur through a facilitated diffusion process since the process follows Michaelis-Menten kinetics and is unaffected by addition of cyanide to the incubation medium. In addition, the rapid adenosine uptake process is temperature-dependent, being completely inhibited at temperatures of 0-4°C. It is saturable at very low adenosine concentrations, having an apparent Km of .9uM and a V_{max} of 5.26 pmole/mg prot./30s. Adenosine analogues have been shown to inhibit rapid adenosine uptake, as well as pyrimidine derivatives and other compounds such as dipyridamole, morphine, hexobendine and papaverine. Interestingly, removal of sodium ions from the incubation medium partially inhibited rapid adenosine uptake (35% inhibition). Removal of calcium ions (Ca^{+2}) from the incubation medium had no effect on rapid uptake, however chelation of endogenous Ca^{+2} with ethyleneglycol-bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) or calcium antagonism with cobalt partially inhibited (16%) rapid adenosine uptake. This indicates that extracellular calcium does not affect rapid adenosine uptake, but membrane bound and intracellular calcium may have some role in this uptake process (Bender et al., 1981; Phillis & Wu, 1981; Snyder, 1985).

The slow adenosine uptake process has been further

subdivided into two high affinity uptake systems with Km's of 1uM (high affinity A) and 5uM (high affinity B) (Bender et al., 1981; Phillis & Wu, 1981; Snyder, 1985).

At extracellular adenosine concentrations above 50uM ordinary diffusion into the cell becomes significant when compared to the facilitated diffusion system. In addition, the nucleoside carrier can function to transport adenosine out of the cell if intracellular concentrations become high enough (Bender et al., 1981; Fredholm and Sollevi, 1986; Snyder, 1985).

Adenosine Release:

Since most adenosine receptors are extracellular, then adenosine itself must also occupy the extracellular space to exert its actions. There are several possible sources for this extracellular adenosine. First, adenosine could be generated intracellularly and be transported out of the cell, down its concentration gradient by the same transport system which usually moves adenosine into the cell. Alternatively, adenosine could be released from a cell by a depolarizing stimulus, or co-released with another neurotransmitter upon nerve stimulation. Adenosine could also be generated extracellularly by degradation of circulating precursors or ATP released during nerve stimulation. Most probably all scenarios

occur under different conditions.

There is evidence that under resting conditions intracellular enzymes generate the bulk of adenosine. The cytoplasmic 5'-nucleotidase is stimulated by energy rich adenine nucleotides, and therefore is most active under basal conditions. Despite inhibition by phosphate, this enzyme is very sensitive to the energy charge of the cell. Consequently, a decrease in energy charge (given by the formula: (ATP + 1/2 ADP)/(ATP + ADP + AMP)) causes an increase in the rate of adenosine formation by this enzyme (Fredholm and Sollevi, 1986; Itoh et al., 1986). There is doubt as to whether the ecto-5'-nucleotidase is even involved in adenosine production during ischemia. Antibodies directed against the ecto enzyme failed to prevent adenosine accumulation during global ischemia in pigeon hearts (Newby et al., 1987).

The status of methylation reactions in the cell will also affect intracellular adenosine concentrations. Any activity which increases the number of transmethylation reactions taking place in the cell will increase the production of SAH. Accumulation of SAH causes feedback inhibition of transmethylation reactions, therefore it is quickly degraded to adenosine and homocysteine. This sequence of events may be of major importance in the heart under aerobic conditions (Fredholm & Sollevi, 1986). If these intracellular concentrations reach sufficient levels

then they move down their concentration gradient via the nucleoside transporter, to the extracellular space.

Some authors have even suggested that the ecto-5'-nucleosidase could function as an adenosine transporter by utilizing intracellular AMP as a substrate and the resulting adenosine is deposited on the extracellular side of the membrane (Arch and Newsholm, 1978). Other authors envisage the ecto enzyme as an extracellular adenine nucleotide scavenger closely associated with the nucleoside transporter. Infused AMP appears as intracellular adenosine more rapidly than infused adenosine, hence the proposal for the enzyme's proximity to the carrier (Fredholm & Sollevi, 1986; Sparks & Bardenheur, 1986). Neither proposal is substantiated by formal evidence.

Adenosine release associated with neuronal activity can occur in a number of ways. ATP has been demonstrated to be co-released with a number of transmitters (including NE and ACh) and can be degraded to adenosine in the extracellular space. In addition, adenosine itself can also be co-released with neurotransmitters upon neuronal depolarization. Alternately, adenosine can be released from a postjunctional structure after interaction of neurotransmitters with their receptors (Fredholm & Hedqvist, 1980; Stone, 1981).

It has long been known that ATP is co-released along

with NE and ACh among other neurotransmitters. For example, it is stored with catecholamines in adrenal chromaffin cells, and with acetylcholine in synaptic vesicles isolated from the electric organ of Torpedo marmarata (Fredholm & Hedqvist, 1980; Phillis & Wu, 1981; Stone, 1981). Upon release, ATP can be converted to adenosine or act as a neurotransmitter interacting with its own receptors. Given that extracellular ATP is very rapidly metabolized, it is difficult to assess how much of extracellular adenosine is released as the nucleoside and how much is derived from nucleotide degradation. In studies where rat cerebral cortex slices were pre-incubated with [^{3}H]-adenosine, it was found that 50-70% of the spontaneously released labelled materials were nucleotides. However, other studies show that brain synaptosomes released approximately 75% of their radioactivity in the form of nucleosides in response to hypo-osmotic shock (Phillis & Wu, 1981). Another experiment demonstrated that blockade of the endonucleotidase responsible for converting nucleotides to adenosine failed to prevent the accumulation of cAMP stimulated by veratridine-induced adenosine release (Phillis & Wu, 1981). Consequently, the contribution of co-released ATP to extracellular adenosine levels remains nebulous.

It is clear, however, that ATP stored and released

with other neurotransmitters also functions as a cotransmitter. Studies on the guinea pig vas deferens show response in this smooth muscle to sympathetic nerve stimulation is biphasic. The initial contractile response is antagonized by the P_2-purinoceptor antagonist arylazidoaminopropinoyl ATP ($ANAPP_3$), while prazosin or reserpine antagonizes the more tonic phase of the contraction. Neither compound affects the other contractile response (Burnstock,1985). It is this kind of evidence which indicates that ATP is functioning as a cotransmitter with, in this case, norepinephrine.

Both adenosine and ATP have been observed to be released from postjuctional structures. For example, in the guinea pig taenia coli, both contracting and relaxing stimuli increased purine release. It is not certain, however, whether it is the actual contraction of smooth muscle which causes the purine release, or whether the vasoconstriction leads to a regional hypoxia thus causing purine release (Fredholm & Hedqvist,1980). It appears that the actual contraction itself, be it the membrane depolarization or the disparity in the balance between energy expenditure and production, that is responsible for purine release (Fredholm & Hedqvist,1980). In the cat nictitating membrane NE, ACh, tryamine, and sympathetic nerve stimulation-induced purine release which correlated with the contractile response to the above stimuli. In

addition, exogenous NE and angiotensin II cause purine release. (13).

Neuromodulation:

Modulation of neurotransmission can occur at two sites. It can either affect presynaptic events by altering the rate of neurotransmitter synthesis, its rate of removal from the synaptic cleft or the amount of transmitter released. Alternately, neuromodulation can take place at the postjunctional site and function to alter neurotransmitter binding or the response of the effector to the transmitter. Adenosine has been shown to exert its effects both pre- and postjunctionally.

Presynaptic Neuromodulation

It has become apparent that presynaptic inhibition of neurotransmitter release by adenosine is not as a result of alterations in adenylate cyclase activity. Most presynaptic inhibitory effects by adenosine are mediated by the A_1 receptor, which results in a decrease in cAMP accumulation. However, inhibition of adenylate cyclase activity with 2',5'-dideoxyadenosine failed to produce a concurrent decrease in transmitter release. In addition, increasing intracellular cAMP levels through the use of forskolin and a phosphodiesterase inhibitor failed to overcome the inhibition caused by adenosine analogues

(Fredholm & Dunwiddie, 1986; Fredholm et al., 1988). Some researchers have found that increases in cytoplasmic cAMP levels actually decreased ACh release from motor nerve endings in a manner that paralleled the effects of adenosine. However, these findings are discordant with the bulk of experimental results from other researchers, that is, the A_2 receptor mediates increases in cAMP, but is not the receptor mediating inhibition of neurotransmitter release by adenosine. The method of increasing the cytoplasmic levels of cAMP may be responsible for this discrepancy, since it was delivered by cAMP-loaded liposomes (Silinsky et al., 1987). This same method of loading calcium into the cytoplasm also produces results which conflict with other researchers.

There is overwhelming evidence that adenosine is mediating some form of calcium-dependent effect in order to inhibit neurotransmission presynaptically. Initial observations that adrenergic nerve stimulation and elevated K^+, but not tryamine-induced [^{3}H]NE release from isolated smooth muscle preparations was inhibited by adenosine lead to this proposal, since only calcium dependent transmitter release was affected (Su, 1983).

More substantial evidence has also been collected. Adenosine decreases ^{45}Ca uptake into synaptosomes depolarized by potassium and this can be antagonized by methylxanthines. In guinea pig ileum myenteric plexus

synaptosomes the ^{45}Ca uptake induced by electrical stimulation was completely abolished by adenosine, indicating some possible differences in results obtained by the two methods (Ribeiro & Sebastiao, 1986). Adenosine has been observed to inhibit voltage-sensitive Ca^{+2} currents in dorsal root ganglion cells, superior cervical ganglion cells and in hippocampal pyramidal neurons.

Adenosine receptors also appear to be coupled to the potassium conductance channel by a pertussis toxin-sensitive G protein. Ultimately, changes in potassium conductance could also lead to changes in calcium entry into the cell (Fredholm et al., 1988).

Findings by some authors are difficult to reconcile with the emerging model of presynaptic adenosine neuromodulation. One group (Silinsky et al., 1987)has found that 2-chloroadenosine does not affect nerve terminal action potentials in motor nerve endings. Nor do adenosine analogues affect the rate of clearance of cytoplasmic calcium into storage sites. Also, adenosine does not affect calcium entry into the nerve terminal, a finding in conflict with other researchers. As previously mentioned, the experimental method may be responsible for this difference. They stimulated transmitter release by delivering calcium to the cell using liposomes, thereby circumventing calcium channels. Adenosine analogues were still able to inhibit transmitter release ergo the

conclusion that calcium influx through calcium channels is not involved. The explanation advanced is a decreased affinity for intracellular calcium (Silinsky et al., 1987).

Studies on the phosphorylation state of synapsin I also support the role of calcium in response to A_1 receptor stimulation, as opposed to cAMP changes. Synapsin I is phosphorylated at separate sites by calcium and cAMP-dependent protein kinases. Furthermore, one group (Llinas et al., 1985) has proposed that phosphorylation of synapsin I on site II by calcium/calmodulin-dependent protein kinase II dissociates synapsin I from the neurotransmitter containing vesicle allowing it to participate in neurotransmitter release. The calcium-dependent phosphorylation of synapsin I was decreased by the A_1 agonist CHA during very brief, but not during sustained potassium depolarization (Fredholm & Dunwiddie, 1986).

Postsynaptic Neuromodulation

Far less research has addressed the mechanism of postsynaptic neuromodulation by adenosine. Once again, the mechanism appears to involve the alteration of calcium influx into the postsynaptic neuron. In hippocampal slices 1 um adenosine suppressed postsynaptic calcium

influx, and this effect could be prevented by the A1 selective antagonist 1,3-dipropyl-8-cyclopentylxanthine (Schubert & Kreutzberg, 1987). The addition of the phorbolester phorbol-12,13-dibutyrate (to stimulate protein kinase C) to hippocampal slices paralleled the effect of inhibiting endogenous adenosine action by methylxanthine addition (Schubert, 1988). A patch clamp technique in neuroblastoma cells also found that micromolar concentrations of adenosine abolished barium currents through calcium channels (Ribeiro & Sebastiao, 1986).

II GASTROINTESTINAL CIRCULATION

Gastrointestinal Circulation:

The gastrointestinal circulation is a huge network of vessels supplying a series of organs. Almost one-third of the total cardiac output is divided between three major arteries which, in turn, supply various organs in parallel. The first artery, the celiac, supplies blood to the liver,lower esophagus, stomach, spleen and pancreas. Next, the superior mesenteric artery (SMA) supplies blood to the pancreas, the jejunum, ileum, and the ascending and transverse colon. Finally, the inferior mesenteric artery supplies the descending and sigmoid colon with its blood supply, and sends branches to the rectum. These three arteries also form extensive anastomotic connections amongst themselves and with each other. After passing through various organs, all blood from the splanchnic circulation (except from the liver) empties into the portal vein (Eade, 1976; Jacobson, 1985).

In the intestine, the microcirculation is a complex network supplying all three layers; the muscularis, the submucosa and the mucosa. After the branches of the SMA pass through the mesentery they enter the intestinal serosa and arborize. They then circle around the intestine on both the top and the bottom, like fingers circling around a tube, and join on the antimesenteric

side with other vessels from the opposite direction (Granger et al., 1980). These vessels also give off branches which pierce the muscularis. These small arterioles then give off first order arterioles (1A) which supply the second order submucosa arterioles (2A) which function as collateral channels between adjacent 1A's as well as giving rise to third order arterioles (3A). Branches arising from the 3A's (fourth order arterioles, 4A) returned to the muscularis, while the 3A's continued through the submucosa into the mucosa and finally into the tip of a villus (Bohlen, 1987; Parks and Jacobson, 1987).

This model has been based on studies in the rat and rabbit, and transferred to most animals. However, it has been shown (Greenway & Murthy, 1972) that the cat clearly does not have the same sort of parallel-coupled vessel arrangement in the muscularis and submucosa. In a study using two different sizes of microspheres, each labelled with a different radioactive compound, it was found that microsphere distribution in the muscosa and the submucosa did not follow the expected pattern upon vasodilation. During an initial vasoconstriction period, the larger (17 $\pm$ .16 uM) ^{51}Cr labelled microspheres were found predominantly in the submucosa (79% of injected ^{51}Cr) while the smaller (12 $\pm$ .15 uM) ^{141}Ce labelled spheres were almost evenly distributed between the mucosa (51%) and submucosa (49%). Upon subsequent vasodilation the

percentage of small microspheres in the mucosa increased (63%) as did the larger microspheres (44%). The authors reasoned that if the vascular bed of the submucosa was a parallel coupled one, then vasodilation should not have resulted in microsphere movement into the mucosa. Since very little radioactivity passed into portal blood (<1%) there were no arterio-venous shunts and consequently the microspheres should have remained impacted at resistance sites in the submucosa. Histological evidence confirmed that submucosal vessels were in series with the mucosal capillaries. All submucosal arterioles were 30uM or larger except where they branched at the mucosal-submucosal border and passed on into the mucosa. This accounts for vasodilation causing more microspheres to move into the mucosa (Greenway & Murthy, 1972).

Each villus is supplied with oxygenated blood by a single arteriole. This vessel usually passes through the centre of the villus and divides into a network of venous capillaries which travel downwards on the outer surface of the villus. This type of arrangement allows for a countercurrent exchange of materials between the arteriole and the venous network (Parks and Jacobson, 1987).

The blood flow distribution between the mucosa-submucosa layer and the muscularis-serosa layer has been determined in a number of animals by a variety of methods. In general the division between the two areas is the same

regardless of the species. Of the total blood flow through the SMA the mucosa and submucosa receive between 50 and 95% while the muscularis and serosa receive 8 to 40% (Granger et al., 1980).

Innervation and Neurotransmitters of the Intestine:

The innervation of the SMA is surprisingly simple. It is predominately innervated with postganglionic sympathetic noradrenergic fibers. There is some contribution from the preganglionic splanchnic nerves normally associated with the celiac artery. However these nerves are not grouped with the splanchnic bundle, therefore severing that bundle will not eliminate their contribution. The sympathetic nerves accompanying the SMA innervate the artery itself, its arterioles and visible mesenteric veins. There are no fibers supplying the precapillary sphincters, capillaries, collecting venules or small veins (Granger et al., 1980; Greenway, 1984a; Parks and Jacobson, 1987).

In the SMA alpha agonists cause vasoconstriction and beta-agonists induce vasodilation. Alpha blockers have been shown to inhibit the response to sympathetic nerve stimulation and norepinephrine infusion while beta receptor antagonists have no effect on these vasoconstrictions. Therefore, the response to norepinephrine infusion or nerve stimulation in the SMA is

mediated by alpha-adrenergic receptors (Greenway, 1984a).

The vagus nerve supplies extrinsic cholinergic innervation to the small intestine, but its stimulation has no effect on SMA blood flow. While acetylcholinesterase activity has been detected in SMA vessels, and acetylcholine infusion causes vasodilation of the SMA, these receptors do not appear to be innervated (Granger et al., 1980; Greenway, 1984a; Parks and Jacobson, 1987).

Serotonin is continuously released into the portal vein and the intestinal lumen. Vagal stimulation increases the rate of release at each site through two separate mechanisms. Atropine pretreatment prevents the enhanced endoluminal 5-HT release, while beta-blockers inhibit portal release. Endoluminal perfusion with physiological levels of 5-HT produce a pronounced hyperemia in the muscularis. Muscarinic receptor blockade prevented the hyperemia, but the serotonin receptor antagonist ketanserin had no effect. Therefore, in this case, 5-HT appears to be inducing a cholinergic-mediated local nervous reflex through 5-HT receptors (Dahlstrom et al., 1988; Gronstad et al., 1987).

Vagal stimulation also enhances the release of substance P into the portal circulation and intestinal lumen. However, this enhanced release was resistant to both cholinergic and adrenergic receptor blockade, as well as adrenergic denervation of the vagal nerves.

Endoluminal perfusion with substance P increases mucosal blood flow (Dahlstrom et al., 1988).

Neuropeptide Y (NPY) is frequently colocalized with norepinephrine in nerve endings. In this situation, depletion of norepinephrine from nerve terminals, with reserpine or 6-hydroxydopamine also depletes NPY stores. This was found to be the case with the adrenergic nerves innervating intestinal vasculature. In the colon, intra-arterial NPY induces a slowly developing vasoconstriction (in comparison to norepinephrine-induced vasoconstriction) which is unaffected by either alpha- or beta-adrenoceptor antagonists. NPY has also been found to presynaptically inhibit norepinephrine release (Dahlstrom et al., 1988). Although not established in the intestine, in the central nervous system this inibition appears to operate through modulation of $alpha_2$-adrenoceptor activity (Heilig et al., 1988).

Vasoactive intestinal polypeptide (VIP) has been indentified in nerves innervating the intestinal mucosa, in particular, those supplying the intestinal villi. VIP causes both vasodilation of the mucosal vasculature and fluid secretion, actions which are opposite to those of norepinephrine. Currently, indirect evidence implicates VIP as a neurotransmitter in the intestinal vasculature (Dahlstrom et al., 1988; Sjoqvist & Fahrenkrug, 1986).

GABAergic (γ-aminobutyric acid) neurons have been

identified in the myenteric plexus of the intestine by immunofluorescence with GABA specific antibodies. The actions of GABA appear to be confined to modulating peristalsis. At the $GABA_A$ receptor, agonists induce a cholinergic-mediated contraction in the ileum, but in all other segments of the intestine a noncholinergic, nonadrenergic relaxation is seen. Stimulation of the $GABA_B$ receptor induces relaxation of the intestine and depresses the contractile response to transmural electrical stimulation (Kerr & Ong, 1986). In rabbit mesenteric arteries GABA has no direct effect nor does it modulate the response to transmural electrical stimulation (Krause, 1986).

There are a number of other substances found in nerve terminals innervating the intestine, and many have unknown functions. Calcitonin gene-related peptide, gastrin releasing-peptide, galanin, and peptide histidine isoleucine are all found in intestinal perivascular fibers, but currently their function in the intestine is not known. Somatostatin also exists in perivascular nerve terminals on submucosal arterioles and causes vasoconstriction after either intra-arterial or intra-venous administration (Dahlstrom et al., 1988).

Theories of Blood Flow Regulation:

There are two theories of blood flow regulation; the myogenic and the metabolic theories. The myogenic theory considers the alteration of pressure in a blood vessel to be the stimulus inducing blood flow changes. In the metabolic theory, the change in blood flow either induces the production of a vasodilating metabolite or changes its local concentration.

Myogenic Control of Intestinal Blood Flow

The myogenic theory supposes that an intrinsic mechanism in smooth muscle cells of arteries and arterioles results in their contraction in the face of increased transmural pressure across a vessel wall. Therefore, an elevation in blood pressure or decreased pressure surrounding an organ would result in constriction of precapillary vessels (arterioles) (Granger et al., 1980; Johnson, 1964; Johnson, 1986).

The mechanism of myogenic responses to changes in intravascular pressure is not yet known. Two hypotheses which attempt to link pressure changes with wall tension development involve 1) the myoendothelial junctions and 2) the dense area on the plasma membrane where the actinomyosin filaments attach to the cell wall (Johnson, 1986).

The first hypothesis proposes that increased

intravascular pressure squeezes endothelial cell contents through fenestrations in the internal elastic lamina, causing the myoendothelial junctions to stretch. Increased membrane conductance for calcium or other ions results in vascular smooth muscle contraction and replacement of the endothelial cells (forming a negative feedback system). However, a myogenic response was still found in rabbit ear arteries from which the endothelium had been removed (Johnson, 1986).

Alternatively, the dense bodies associated with actinomysin filament attachment may function as a tension sensor, deforming the adjacent membrane in a manner reflecting the vessel wall tension. The dense bodies are not the only load bearing elements in a smooth muscle cell, thus the relationship between stretch and tension development cannot be so easily predicted (Johnson, 1986).

It has also been demonstrated that changes in intravascular pressure alter the transmembrane potential (Folkow & Niel, 1971; Johnson, 1986). In isolated cerebral arteries depolarization associated with an increase in pressure was dependant on the external calcium concentration. In the kidney, the calcium blockers nifedipine and verapamil were both shown to abolish autoregulation. The discovery of stretch-activated ion channels, although not specific for calcium, provides a possible mechanism for the transduction of stretch into

contraction in vascular smooth muscle (Johnson, 1986).

Evidence supporting the myogenic theory of blood flow autoregulation in the intestine comes from experiments where the venous pressure is elevated to increase transmural pressure. The resulting increase in resistance is interpreted as a myogenic response. A closer examination of the actual experiments reveals errors in interpretations of results which makes the conclusions questionable, or even refutes them.

One such study (Shepherd, 1977) involved elevating the venous pressure during two different flow rates in the denervated isolated perfused intestinal loop of dogs. Here experiments done at a venous pressure of 0mmHg were designated as the control group, and blood flows for the low flow and high flow groups were 22.6 $\pm$ 2.6 and 34.8 $\pm$ 4.9 $ml.min^{-1}.100g^{-1}$ respectively. These resulted in corresponding arterial pressures of 67.5 $\pm$ 9.4 and 100.3 $\pm$ 11.0 mmHg. Consequently, the high flow group parallels the situation seen in resting conditions in the whole animal where the previously reported range of resting intestinal blood flow in the dog was 35 to 100 $ml.min^{-1}.100g^{-1}$ (Granger et al., 1980). Consequently, although the groups were labelled low and high flow, they are actually low and normal blood flow rates for the dog intestine.

Despite the increase in flow rates, the corresponding

increase in arterial pressure resulted in the resistance remaining the same. Under these conditions there is no evidence for a myogenic response. When the experiment was repeated at an elevated venous pressure of 20mmHg (much higher than normally seen in the whole animal), the values for the low and high rates of blood flow appear to have changed drastically. Although no mean values were reported, one graph shows that the blood flow in the low flow group was 31 $ml.min^{-1}.100g^{-1}$ while the high flow rate was 44.8 $ml.min^{-1}.100g^{-1}$. If these are representative values then the high flow group in the control state corresponds to the low flow group during elevated venous pressure. The paper reported that elevated venous pressure decreased resistance to 86.5% of the control value under low flow conditions, while high flow increased the resistance to 127.6% of control. However, if the only two groups which were acutally matched for flow rate (control high flow and elevated venous pressure low flow) are compared then elevated venous pressure decreased the resistance to 87% of control. Consequently, when the appropriate comparisons are made, there is no evidence for a myogenic mechanism in the regulation of intestinal blood flow in this preparation.

In the same series of experiments (Shepherd, 1977), a constant pressure perfusion preparation was also subjected to an elevated venous pressure of 20mmHg. This resulted

in a decrease in intestinal blood flow from 48.2 ± 2.1 $ml.min^{-1}.100g^{-1}$ to 31 $ml.min^{-1}.100g^{-1}$, as well as a significant increase in resistance (2.0 ± .1 to 2.4 $mmHg.min.ml^{-1}.100g^{-1}$. However, the arterial pressure used to calculate these resistances was monitored from a femoral artery, and may not accurately reflect pressure in the SMA. When the same sort of experiment was performed in a later paper (Shepherd, 1979) and the pressure recorded from a SMA branch then elevation of venous pressure increased resistance by almost 50%. However, in contrast to the previous preparation, this preparation was fully innervated (Granger & Norris, 1980), and the contribution of sympathetic nerves to vascular tone cannot be ruled out. It has been demonstrated that unloading low and high pressure baroreceptors by decreasing venous return to the heart increases resistance in the splanchnic region (Lundgren, 1983).

These studies are typical of the types of experiments involved in establishing a role for the myogenic theory of intestinal blood flow regulation. Since they most often involve the elevation of portal venous pressures to unphysiological levels the relevance of their findings is questionable. This is particularly evident in whole animal experiments in the cat. Here, elevation of portal venous pressure to between 12 and 13mmHg by either occlusion of the portal vein or stimulation of the hepatic

sympathetic nerves, caused a small significant decrease in SMA flow, but no significant decrease in SMA conductance (Lautt, 1986b). These experiments were performed in a denervated gut, but the intestinal loop was left intact, and the control portal venous pressure was the normal resting pressure (7mmHg as compared to 0mmHg in the isolated perfused intestine). In addition, the portal pressure was only raised to a level induced by a physiological stimulus, (i.e. hepatic nerve stimulation). The nerve stimulation itself was not responsible for the difference, since manual occlusion of the portal vein produced the same result.

Metabolic Theory of Intesinal Blood Flow Regulation

The metabolic theory proposes that decreased blood flow is accompanied by relaxation of arterioles and/or precapillary sphincters arising from the accumulation of vasodilator metabolites or a decreased nutrient supply. More subtly, a decrease in the partial pressure of oxygen within the surrounding tissues may either exert a direct effect on the vascular smooth muscle or result in the release of vasodilating metabolites. Thus, this theory may find oxygen delivery to tissues more important than blood flow, although oxygen delivery depends in part on blood flow (Granger et al., 1980; Johnson, 1964; Johnson,

1986).

Two specific metabloic blood flow phenomena seen in the intestine are autoregulatory escape and autoregulation.

Autoregulatory Escape

Autoregulatory escape is defined as the failure of arterioles to maintain their vasoconstriction in the face of continued stimuli. True autoregulatory escape is seen in arteriolar smooth muscle only, and has not been found in venous smooth muscle. In the intestine, it is seen in response to both sympathetic nerve stimulation and norepinephrine infusion, but other vasoconstrictors, such as vasopressin, have only shown escape in vitro. The phenomena occurs at all levels of stimuli, and the proportion of escape from initial constriction is usually the same regardless of the strength of the constrictor stimulus. Escape is seen in constant flow and constant pressure preparations (Greenway, 1984 a&b).

Autoregulatory escape involves the relaxation of the same vessels which were initially constricted. Currently, it is felt that an unidentified vasodilator or cellular mechanism is responsible for the relaxation since a number of other possibilites have been eliminated. For example, escape is not a result of transmitter depletion since the phenomena also occurs during norepinephrine infusion.

Receptor desensitization is also not likely since increasing the rate of norepinephrine infusion or frequency of nerve stimulation can produce a further increase in vasoconstriction, which also shows escape. Blockade of beta-receptors does not affect escape, suggesting it is not an interaction between alpha- and beta-receptors. Redistribution of blood flow is also not a plausible explanation, since arterio-venous shunt vessels do not exist in the submucosa (Greenway, 1984 a&b; Parks and Jacobson, 1987).

In the developing swine autoregulatory escape from exogenous norepinephrine infusion or sympathetic nerve stimulation (10Hz) can be seen as early as two weeks of age. However, in the case of the sympathetic nerve stimulation, escape from a stronger stimulus (12Hz) is not seen until two months of age (Buckley et al., 1987). In older piglets (1 month) destruction of endogenous adenosine by injection of adenosine deaminase (ADA) significantly attenuated escape from vasoconstriction induced by norepinephrine infusion, but does not eradicate it (Crissinger et al., 1988). Despite the apparent evidence for a role for adenosine in autoregulatory escape, these experiments have faults. The pressure in the SMA was not measured, and therefore the sole criteria for assessing escape was blood flow. More appropriately, an increase in vascular conductance should have been

observed to demonstrate escape. The effect of ADA on escape was evaluated by comparing escape blood flow during ADA infusion to that in the control vasoconstriction. However, since ADA infusion also significantly lowered blood flow at the peak of vasoconstriction, one would expect escape blood flow to be decreased as well. Therefore, this apparent attenuation of escape by adenosine destruction could merely be an anomaly of failing to evaluate the escape relative to its own peak vasoconstriction.

Other studies have found that selective blockade of adenosine receptors with 8-phenyltheophylline had no effect on autoregulatory escape. These studies monitored both blood flow and pressure in the intestine, and the conductance changes were expressed as the amount of escape from the peak of vasoconstriction. Therefore, a large vasoconstriction could escape proportionately the same amount as a small one, even though the actual escape conductance would be larger (Lautt et al., 1988b). This study also found that propranolol (a beta-adrenergic receptor blocker) and ouabain (a Na^+-K^+-ATPase pump inhibitor) also did not affect autoregulatory escape.

Since autoregulatory escape occurs in both constant flow and constant pressure preparations, it is unlikely to be a result of metabolite accumulation.

Autoregulation

Pressure-flow autoregulation is the ability of a vascular bed to maintain constant blood flow in the face of fluctuating arterial pressure. Autoregulation in the intestinal circulation is relatively weak when compared with other vascular beds since blood flow does not usually return to control levels with pressure decreases. For example, in the feline intestine with intact autoregulating capacity, resistance still decreased by 14% (Lautt,1986a). There is also evidence that the different layers of the intestinal wall have varying autoregulatory capacities, with the mesenteric arterioles and the precapillary sphincters exhibiting the greatest capacity (Parks & Jacobson, 1987). This may be related to the metabolic activity of the adjacent tissues, since it has been found that autoregulation in the canine intestine is greater when the animal is fed as opposed to fasted (Granger & Norris, 1980a,b).

There is continual debate in the literature as to whether autoregulation is a myogenic or metabolic phenomenon. It has been demonstrated that adenosine receptor antagonism abolishes autoregulation in the cat intestine (Lautt, 1986). In addition, adenosine is also responsible for the enhanced autoregulation in the fed dog (Granger & Norris, 1980a). Both histamine H_1-receptor blockade with chlorpheniramine and adenosine receptor

blockade with theophylline attenuated autoregulation in the swine intestine (Buckley et al., 1988). The effects of simultaneous receptor blockade was not investigated in this study.

Given the problems already outlined with attempts to study myogenic responses, and the fact that metabolites such as adenosine have been shown to mediate autoregulation, the mechanism underlying pressure-flow autoregulation is not likely to be myogenic. It remains to be seen whether one or more metabolite is involved.

III ADENOSINE AND BLOOD FLOW

Intestine:

There is evidence that pressure-flow autoregulation in the intestine is adenosine-mediated. In the cat, administration of 8-phenyltheophylline (8-PT) into the superior mesenteric artery (SMA) significantly altered three indices of autoregulation; the autoregulatory index, the slope index and the percent change in vascular resistance (Lautt & Legare, 1985). The autoregulatory index measures the portion of flow change elicited by a pressure change. The slope index reflects the linearity of the pressure-flow curve; the more linear the relationship, the less the vessel is capable of maintaining autoregulation. Therefore, an increase in these two indices reflects a decrease in autoregulatory capacity, since there is less ability to maintain constant flow when the pressure is altered. The percent change in resistance reflected the amount of vasodilation when blood pressure is decreased. During autoregulation, this index is negative due to vasodilation when pressure is decreased. When autoregulation was examined in the absence and presence of adenosine receptor blockade, the autoregulatory and slope indicies increased, and the percent change in resistance decreased, clearly demonstrating that adenosine was mediating autoregulation.

Studies in 1 month old pigs also confirm adenosine involvement in autoregulation (Buckley et al., 1988). Adenosine receptor antagonism with theophylline significantly decreased the autoregulatory range over which 95% of control blood flow was maintained. These studies also demonstrated decreased autoregulation during histamine H_1 receptor blockade with chlorpheniramine. However, this study did not confirm that histamine receptor blockade did not also affect the response to adenosine, and vice versa, leaving the possibility that there is an interaction between adenosine and histamine, rather than both compounds being separately involved in autoregulation.

Even the alleged myogenic vasoconstriction of the intestinal vasculature in response to increased portal venous pressure has been shown to be an adenosine-mediated effect. Hepatic nerve stimulation was used to increase portal pressure over a physiological range from 7 to 12 mmHg. The resultant vasoconstriction of the SMA was a result of the increased mean arterial blood pressure (MABP) and hence pressure-flow autoregulation. This was demonstrated by eradicating the vasoconstriction by returning the MABP to control values or by adenosine receptor blockade. Manually increasing the portal pressure to the same extent as hepatic nerve stimulation did not affect the MABP or the SMA conductance, further

supporting an adenosine mediated autoregulatory phenomena, rather than a myogenic effect (Lautt, 1986b).

As with other vascular beds, the post-occlusive hyperemia is partially adenosine-mediated. Occlusion of the SMA for 60 seconds resulted in a 4-fold increase in venous adenosine concentrations measured at the peak of the hyperemia (Mortillaro & Mustafa, 1978). The reactive hyperemia after a one minute occlusion of the SMA was decreased by adenosine receptor blockade. The percent change in conductance was decreased by 50%, the volume of the hyperemia by 32%, but the duration of the hyperemia remained unchanged (Lautt, 1986b).

The main function of the intestine is to digest and absorb nutrients from ingested food. Adenosine appears to be one of the mediators involved in the postprandial hyperemia. Initial studies indicated that adenosine receptor blockade with theophylline only decreased the enhanced pressure-flow autoregulation seen in dogs with predigested food in the jejunum. Fasted animals did not show this superregulation, nor did theophylline affect their normal autoregulatory responses (Granger & Norris, 1980b). Theophylline did not have any effect on the absorption hyperemia itself. In rat jejunum flaps suffused with an oleic acid, glucose and bile salt solution theophylline and adenosine deaminase both decreased blood flow to preprandial control levels

(Proctor, 1986). In dogs, the sensitivity of the postprandial hyperemia to adenosine receptor blockade was dependent on the level of intestinal motility and the potency of the antagonist (Sawmiller & Chou, 1988). When aminophylline was used as the adenosine antagonist, prevention of the food-induced hyperemia was only seen in those animals in whom gut motility was unaffected by luminal food placement. If 8-PT was used as the antagonist, then the postprandial hyperemia was eradicated regardless of whether the presence of food in the lumen caused an increase in gut motility or not. The authors attribute the difference in the effects of aminophylline and 8-PT to differences in potencies and specificity as adenosine receptor antagonists.

Liver:

The relationship between adenosine and blood flow in the liver has been well-characterized. The liver is an organ which has both an arterial and a venous blood supply, but it has only one exiting conduit for this blood, the hepatic vein. The maintenance of a constant total blood flow through the liver is important to such hepatic functions as drug clearance therefore, blood flow in the portal vein and the hepatic artery must be in some way integrated. Since the blood flow in the portal vein is, for the most part, determined by the intestine and

other splanchnic organs, this is a fixed source of blood, over which the liver is unable to exert any control. Consequently, the hepatic artery must alter its flow in an inverse manner to changes in portal blood flow. Such a relationship has been demonstrated, and adenosine has been identified as the agent mediating the vascular changes.

In experiments conducted in cats it was found that decreasing portal blood flow caused a concomitant increase in the hepatic arterial blood flow. When 8-phenyltheophylline was administered in doses sufficient to eradicate the response to exogenous adenosine infused into the hepatic artery, then changing portal venous flow had no effect on hepatic arterial blood flow (Lautt and Legare, 1985). Studies in humans have shown that caffeine and theophylline, both of which block adenosine receptors, reduce liver plasma flow (Onrot et al., 1986). Consequently, adenosine is functioning as a vasodilator in the hepatic artery in a manner which is dependent on portal blood flow.

The hepatic arterial buffer response explains these observations by hypothesizing that adenosine is released into the hepatic sinusoids at a constant rate and is washed away by the portal blood flow. If the portal blood flow decreases, then adenosine accumulates in the vicinity of the hepatic arterioles and causes vasodilation of the hepatic artery. The increased blood flow would thus

maintain constant total blood flow through the liver (Lautt and Legare, 1984; Lautt, 1985; Lautt et al., 1985). An increase in portal flow would induce the inverse scenario.

The weak pressure-flow autoregulation in the hepatic artery is also mediated by adenosine. Administration of 8(p-sulfophenyl)theophylline (8-So-PT) or 8-phenyltheophylline (both are adenosine receptor antagonists, however 8-So-PT does not cross the blood brain barrier and therefore its effects are confined to the peripheral circulation) into the hepatic artery significantly altered three indices of autoregulation. The mechanism is thought to work on the same basis as the hepatic arterial buffer response, that is, reduced flow results in the local accumulation of adenosine causing vasodilation (Ezzat & Lautt, 1987).

Kidney:

In the kidney adenosine causes vasoconstriction and decreased blood flow; actions which are the opposite to those seen in other organs. These paradoxical effects can be explained in terms of renal blood flow controlling metabolism, rather than the reverse. The bulk of oxygen consumption by the kidney is used in active transepithelial solute transport by tubular epithelial cells. Adenosine generated by ATP hydrolysis during

active transport functions by decreasing blood flow, thus decreasing the glomerular filtration rate and ATP hydrolysis (Osswald, 1988; Spielman et al., 1987).

The affinity of the A_1 receptor in the kidney for adenosine is in the nanomolar range, which is much higher than the affinity at the A_2 receptor (micromolar). Vasoconstrictor response to the A_1 receptor agonist, N^6cyclohexyl adenosine (CHA) was most pronounced in the preglomerular vessels, in particular the distal segment of the afferent arteriole. The A_2 receptor agonist N-ethyl-carboxamide adenosine (NECA) vasodilated predominately preglomerular vessels, but not the segment of the afferent arteriole adjacent to the glomerulus (Holtz and Steinhausen, 1987). NECA, in higher concentrations, also caused dilation of the postglomerular vessels. A non-selective adenosine agonist, 2-chloroadenosine (2-CLA), caused constriction compatible with A_1 receptor occupation as well as slight dilation of the proximal efferent arteriole, in keeping with A_2 receptor occupation. The decrease in glomerular filtration pressure generated by preglomerular vasoconstriction and postglomerular vasodilation also caused a decrease in the glomerular filtration rate (GFR) (Holtz & Steinhausen, 1987).

The actions of adenosine are known to be in part mediated by angiotensin II. It has been demonstrated that adenosine inhibits renin release, a substrate necessary to

convert angiotensin to its active form. Pretreatment with pertussis toxin blocked the ability of CHA to inhibit renin secretion, indicating a possible role for the G_i-protein. Other studies in cultured rabbit cortical collecting tubule cells demonstrated that adenosine analogues produced a dose-dependent reduction in cyclic AMP accumulation. Adenosine analogues also inhibited vasopressin stimulated cyclic AMP production, an effect which was also blocked by pertussis toxin treatment. Thus, it is likely that adenosine exerts these actions in the kidney through interaction with a G_i-protein (Spielman et al., 1987; Osswald, 1988).

Angiotensin antagonists and converting enzyme inhibitors both prevent the transient vasoconstriction and decrease in GFR caused by adenosine. In addition, the afferent arteriole, which is not normally constricted by angiotensin II, shows sensitivity to angiotensin II in the presence of adenosine (Holtz & Steinhausen, 1987).

Adipose Tissue:

Adenosine has been shown to mediate blood flow in canine adipose tissue. Under basal conditions, administration of adenosine deaminase and consequently reduction of endogenous adenosine levels, caused an increase in resistance. Likewise, the vasodilation normally associated with intravenous norepinephrine

infusion was also eradicated by adenosine deaminase. There was also a strong correlation between lipolysis and adenosine tissue content. Since norepinephrine-induced lipolysis proceeded in the presence of adenosine deaminase, thus it is likely that norepinephrine stimulates lipolysis, but the increased metabolic activity generates adenosine, which in turn mediates blood flow (Martin & Bockman, 1986).

The removal of lipolytic byproducts (free fatty acids) which are involved in feedback inhibition, is dependent on albumin delivery, and hence on blood flow. Therefore, adenosine forms a metabolic feedback system between the rate of lipolytic activity and blood flow in adipose tissue. That is, the greater the rate of lipolysis, the greater the rate of adenosine production, leading to increased blood flow and albumin supply, preventing an accumulation of intracellular free fatty acids (Martin & Bockman, 1986).

Skeletal Muscle:

In skeletal muscle there is evidence for adenosine involvement in the hyperemia during contraction, after arterial occlusion, and possibly during autoregulation. During sustained muscle contraction adenosine levels in venous plasma increased significantly without a concomitant increase in the arterial levels of adenosine.

This was observed in spite of the fact that the preparation was perfused at a high flow rate and high arterial PO_2. It was also shown that 90% of intra-arterially infused adenosine was eliminated from the plasma by the time it was measured on the venous side of the preparation (Ballard et al., 1987). Thus the levels of adenosine measured in this type of protocol are not likely to accurately reflect adenosine production during muscle contraction, but rather indicate whether the levels increased or decreased and some idea of the magnitude of change.

The reactive hyperemia in the human forearm caused by arterial occlusion also showed sensitivity to adenosine receptor antagonism by theophylline. Intravenous infusion of theophylline reduced the reactive hyperemia by approximately 35%.

Interestingly, prostaglandin synthesis inhibition with ibuprofen also reduced the reactive hyperemia by approximately 70%, but theophylline and ibuprofen administered together did not produce an additive effect (Carlsson et al., 1987). This suggests there may be a link between prostaglandin and adenosine synthesis during reactive hyperemia in skeletal muscle.

Although adenosine has a role in autoregulation of skeletal muscle blood flow, it does not appear to be the major contributor. Autoregulation curves were obtained

from isolated creamaster muscles by gradually occluding the sacral aorta. The muscle itself was in a bath with a buffer having PO_2 of either 70mmHg (high oxygen) or 10mmHg (low oxygen). Theophylline added to the high oxygen bath had little effect on the autoregulatory curves. In the low oxygen bath theophylline abolished the superregulation seen in the control experiments and diminished but not eliminated normal autoregulation (Morff & Granger, 1983). Thus, in skeletal muscle, adenosine is involved in autoregulation when oxygen supply is compromised.

Heart:

Two approaches have been taken to determine the relationship between adenosine and coronary blood flow. One is to try determining the concentration of adenosine at the coronary adenosine receptor, and the other is to attempt to block the effects of endogenous adenosine.

The first approach is plagued with technical problems. Measuring adenosine levels in cardiac tissue is complicated by the fact that most of the adenosine is bound up intracellularly in S-adenosylhomocysteine (SAH), thus measured levels would not reflect those in the interstitial fluid. Measuring coronary arteriovenous adenosine or cardiac lymph levels is also hampered by the fact that these fluids are in contact with epithelial cells and erythrocytes, both of which are capable of

taking up and metabolizing adenosine as well as producing and releasing it. One method which might circumvent these problems is the use of epicardial chambers. These are small fluid-filled plastic chambers which are placed on the epicardial surface of the heart. The extent to which the epicardial mesothelium metabolizes adenosine is not known, but in cases where it is removed, the chamber communicates directly with the interstitial space. It has not been assessed whether removal of the mesothelium alters adenosine metabolism (Gidday et al., 1988; Olsson & Bunger, 1987). Some authors have used the levels of S-adenosylhomocysteine (SAH) as a measure of adenosine concentrations. Infusion of L-homocysteine thiolactone ensures excess substrate to combine with free cytosolic adenosine forming SAH. Since the SAH pool is more stable than the adenosine pool, a change in the rate of SAH accumulation is thought to parallel adenosine production (Deussen et al., 1988).

However, regardless of the method used to measure adenosine levels, it is clear that there is a strong correlation between changes in cardiac blood flow and adenosine concentrations. For example, using an epicardial chamber it was found that the functional hyperemia accompanying catecholamine stimulation also saw an increase in interstitial adenosine concentrations (Gidday et al., 1988).

When attempts to block the effects of adenosine are made by using adenosine receptor antagonists or degradative enzymes, a different picture of adenosine's relationship to coronary blood flow emerges. In microsphere studies in whole animals, infusion of adenosine deaminase into the left anterior descending coronary artery did not alter transmural flow in the distal zone. Although transmural flow in the circumflex zone was significantly reduced, the difference was small. Neither adenosine deaminase administration (Gewirtz et al., 1986) or adenosine receptor blockade with 8-phenyltheophylline (Bache et al., 1988) were found to affect coronary blood flow at rest. Therefore it would seem adenosine does not have a role in basal coronary blood flow.

In isolated perfused hearts adenosine was only responsible for one-third the increased coronary flow induced by metabolic stimulation with either norepineprhine or isoproterenol infusions. In these experiments both adenosine receptor blockade with 8-phenyltheophylline and adenosine destruction with adenosine deaminase decreased coronary flow to the same extent (Headrick & Willis, 1988). However, in conscious dogs, increased coronary blood flow stimulated by treadmill exercise was not affected by intracoronary administration of adenosine deaminase or systemic

administration of 8-phenyltheophylline (Bache et al., 1988). In the same experiments, these compounds reduced the reactive hyperemia after 5 to 20 seconds of coronary occlusion by 33 and 40% for adenosine deaminase and 8-phenyltheophylline respectively. These values correspond to the contribution of adenosine to increased coronary flow in metabolically stimulated isolated heart preparations. Therefore, compensatory mechanisms may be elicited in the whole animal. Thus, adenosine may contribute somewhat to increased coronary blood flow caused by metabolic stimulation, but is it not the major factor involved, and its absence can be compensated for.

Brain:

Adenosine has been accepted as the major chemical mediator of cerebral blood flow during hypoxia, anoxia, and ischemia. Using a freeze-blow technique adenosine tissue levels increased sevenfold after five minutes of severe hypoxia (partial pressure of oxygen (PaO_2) of 30mmHg). During moderate hypoxia (PaO_2 = 50mmHg) adenosine levels doubled, but this difference was not significantly higher than control levels (Winn et al., 1981). Because measuring adenosine levels in whole tissue samples may not accurately reflect the concentrations in the interstitial fluid seen by the blood vessels, other methods of measuring adenosine levels have also been

employed with the same results. The cerebral cortical cup technique uses artifical cerebrospinal fluid to perfuse the surface of the brain. This method detected a significant increase in adenosine levels when the PaO_2 was reduced to 64mmHg. However, both the partial pressure of carbon dioxide ($PaCO_2$) and the mean arterial blood pressure (MABP) decreased significantly, therefore the effect of hemorrhagic hypotension on adenosine levels was examined, while holding both the PaO_2 and $PaCO_2$ constant. Decreasing the MABP from 107 to 46mmHg did not affect cortical perfusate adenosine levels (Phillis et al., 1987). This still does not deal with the involvement of carbon dioxide levels in adenosine production. The levels of adenosine measured by this method (basal adenosine levels of 10-70mM) are also one to two orders of magnitude lower than estimates reported in dialysis experiments.

Experiments in which dialysis cannulas were implanted into rat brains and perfused with artifical cerebrospinal fluid also showed increased adenosine levels in response to reduced inspired oxygen. These experiments also had a concomitant decrease in MABP and $PaCO_2$, neither of which were controlled in additional experiments. Estimated interstitial fluid adenosine concentrations under resting conditions by this method is approximately 1uM (Van Wylen et al., 1986).

The adenosine receptor blocker, theophylline,

administered intraperitoneally, also attenuated the increased blood flow during both short term and sustained hypoxia. In these experiments both the MABP and $PaCO_2$ remained constant over the course of the hypoxic intervention (Morii et al., 1987), firmly establishing adenosine involvement in the hyperemia caused by just hypoxia.

Anoxia, induced by replacement of oxygen with nitrogen in the inspired gas mixture, also caused an increase in adenosine concentrations measured by the cortical cup and dialysis methods (Phillis et al., 1987; Van Wylen et al., 1986). The hyperemia during anoxia could also be attenuated by caffeine (Phillis et al., 1984).

The involvement of adenosine in the effects of carbon dioxide on cerebral blood flow is not clear cut. When cerebral blood flow was measured using microspheres, hypercarbia caused a significant increase in flow. Pretreatment with intraperitoneal theophylline 30 minutes before hypercarbia was induced had no effect on cerebral blood flow, despite attenuating hypoxic hyperemia in the same experimental paradigm (Morii et al., 1987). However, when venous outflow from the brain was measured by cannulating the left retroglenoid vein, pretreatment with theophylline 15 minutes prior to the hypercapnic challenge did significantly decrease the hyperemia (Phillis & DeLong, 1987). Measuring blood flow through the

retroglenoid vein only represents 30 to 60% of the total cerebral blood flow (Phillis et al., 1984), and if the percentage of total flow does not remain constant during the experimental intervention, this may account for the different results from two apparently similar protocols.

The decrease in cerebral blood flow during hypocarbia is counterbalanced by increased levels of adenosine. When theophylline was administered during hypocarbia, vasoconstriction (measured through a closed cranial window) was more pronounced, whereas it was attenuated by the adenosine uptake inhibitor dipyridamole (Ibayashi et al., 1987).

Evidence concerning autoregulation of cerebral blood flow is also conflicting. In studies where adenosine levels were measured by the freeze-blow technique decreasing MABP from 135mmHg to 107mmHg caused no significant change in the adenosine levels. However, a further decrease in pressure to 72mmHg resulted in a twofold increase in adenosine levels, which led the authors to conclude that adenosine was responsible for cerebral autoregulation (Winn et al., 1985). It should be noted that adenosine levels were not obtained for any other pressures between 107 and 72mmHg. Other researchers used brain dialysis to examine adenosine levels at multiple points between MABP's of 100 to 28mmHg. In this case adenosine levels did not increase until the MABP

decreased to 50mmHg, out of the autoregulatory range in these experiments (Van Wylen et al., 1987). Therefore, at present, it is not likely that adenosine is involved in cerebral blood flow autoregulation, until MABP drops to the lower end of the autoregulatory range.

In light of the ubiquitous nature of adenosine in the body and its multifaceted roles such as neuromodulation and blood flow regulation, this thesis examined aspects of adenosine's function in the feline intestine. The initial intention of this thesis was to examine modulation of vasoconstriction by both exogenous and endogenous sources of adenosine. The inhibition of intestinal vasoconstriction by exogenous adenosine was examined and found to be through a postsynatpic, non-competitive mechanism. However, under resting conditions endogenous adenosine did not appear to modulate vasoconstriction (Lautt et al., 1988a). In an attempt to find a situation where the intestine produced sufficient quantities of adenosine to modulate vasoconstriction, the relationship between adneosine and hypoxia induced vasodilation was examined. In this situation it also appears that endogenous adenosine is not mediating the changes in vascular resistance.

METHODS

Animal Preparation:

Fasted cats (18 hrs) were anesthetized by injecting 32.5 mg/Kg of sodium pentobarbital (Somnotol, M.T.C. Pharmaceuticals) intraperitoneally. They were then placed on a temperature-controlled table maintained at 37.5°C through a rectal probe and thermal control unit (Yellow Springs Instruments, Model 72) operating heating rods under the table.

The brachial vein was cannulated (PE90) for administration of additional anesthetic in bolus doses of 6 mg as judged necessary by eye reflexes. A tracheotomy was performed in order to assist breathing should the animal cease to breath spontaneously.

Neuromodulation Series:

In this experimental series the mean arterial pressure was monitored through a cannula (PE 205 with a pulled down end) inserted into a femoral artery.

The intestines were exposed through a midline abdominal incision and small cannulas passed into a cecal artery (PE50) and vein (PE90 with side holes). The superior mesenteric arterial (SMA) pressure was measured through the arterial cannula. The portal venous pressure was measured through the venous cannula after it had been passed into the portal vein as far as 1 cm from the hilum

of the liver. A multiline cannula was attached to the SMA cannula to allow simultaneous pressure monitoring and drug infusion (Harvard Apparatus infusion pump). The inferior mesenteric artery was then ligated to ensure all blood supplying the intestines came from the SMA.

The nerves were cleared from around the SMA, tied and cut. The peripheral end was placed in a circular bipolar stimulating electrode. The stimulating electrode was controlled by a Grass S9 stimulator set at 15V, 1 ms duration at a frequency of 1, 3 or 9 Hz.

An electromagnetic flowprobe (Carolina Medical Electronics EP408) and an inflatable vascular occluder were placed on the section of the SMA cleared of nerves. The occluder was placed upstream from the flowprobe in order to set a zero baseline.

The flowprobe was calibrated by cannulating both the aorta (at a point below the SMA) and the SMA, leaving enough room to accommodate the probe. The aorta was tied off just above the SMA and any small vessels coming off the loop created by the cannulas were ligated, creating a circuit involving the aorta and the SMA. Blood from a reservoir (collected from the animal at the termination of the experiment and heparinized) was pumped through the aorta and out the SMA cannula into a calibrated receptacle, which in turn emptied back into the reservoir. Flow was calculated by measuring the time for a given

volume of blood to fill the receptacle. This process was repeated several times at several pump speeds to obtain an average value and correlate it with the chart recording.

Hypoxia Series:

In this experimental series the mean arterial blood pressure was monitored from a catheterized (PE90) carotid artery. The central venous pressure was measured by passing a catheter (PE90) through a femoral vein and advancing it into the central vein. At this time both femoral arteries were prepared for later cannulation.

The intestines were exposed through a midline incision. Both the SMA and portal venous pressures were monitored as in the neuromodulation series. In addition, the portal vein was punctured just below the splenic vein with an IV catheter (Jelco 24g, Critikon Canada) in order to sample venous blood leaving the intestines. Both the gastroduodenal and inferior mesenteric arteries were ligated. The nerve plexus was cleared from around the SMA, tied, cut and the peripheral end placed in a circular bipolar stimulator.

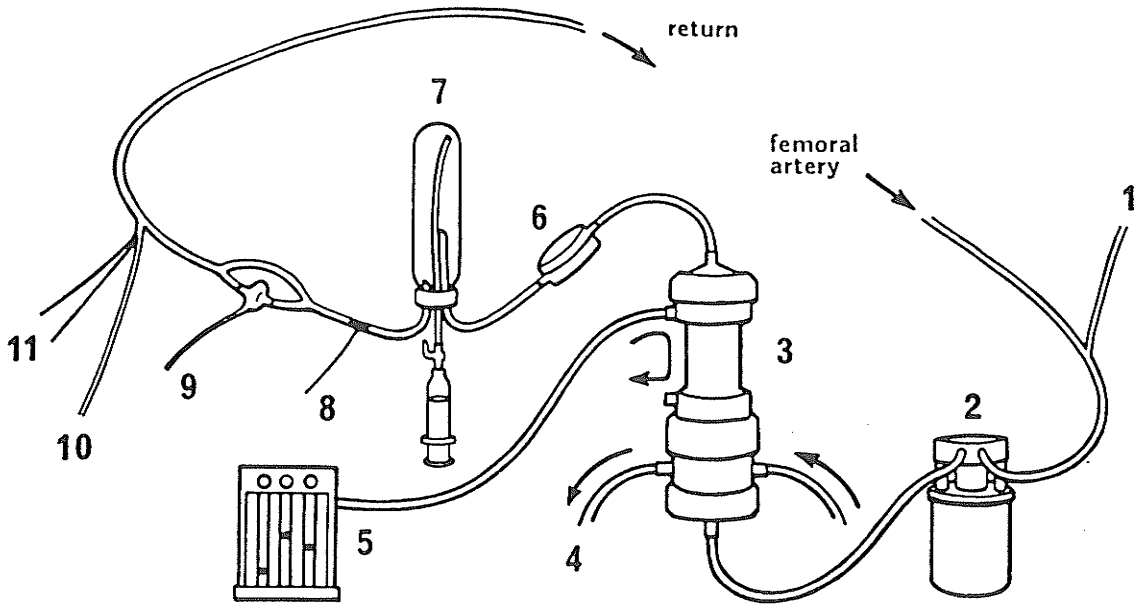
An incision was made through the abdominal cavity just below the ribs to facilitate access to the SMA. The animal was then allowed to stabilize (approximately 30 minutes) while the arterial long circuit was positioned on the surgical table. The circuit was first rinsed with

saline and then circulated with heparinized blood from a donor cat (100 to 120 ml). The flow probe was calibrated in situ by recording the time for collection of a fixed volume of blood and correlating it with the chart recording. 800 UI of heparin in Ringer's solution was administered to the cat and then the circuit was connected. This was accomplished by advancing cannulas (PE 90 or PE205 with side holes) through the femoral arteries into the aorta. The cannula (PE205 or PE240) on the distal end of the circuit was then inserted into the base of the SMA just after it branched off the aorta. The process of connecting the long circuit was done in the shortest time possible, preferably under three minutes.

The pressure was monitored as the blood entered the circuit. At this point, a positive pressure would indicate that the aortic blood flow was higher than the demand by the circuit. Conversely, a negative pressure indicated that blood was being drawn out by the action of the pump and there existed a danger of collapsing the aorta. In addition, the pressure in the circuit before it entered the animal was also monitored. An increase in this pressure indicated obstruction to flow in the animal, either through kinking of the vessel or blood clots in the intestinal vasculature. Once the circuit was in place and a reasonable flow established, the abdominal incision was clamped shut and covered with gauze soaked in Ringer's

solution to prevent moisture and heat loss.

The arterial long circuit (Fig. 1) consisted of a variable speed pump which controlled the blood flow rate through the rest of the circuit. The blood moved through a hollow fiber oxygenator with integrated heat exchanger (Capiox II 08, Terumo Corp.). Connected to the oxygenator was a gas flow meter which controlled the relative concentrations of oxygen, carbon dioxide and nitrogen. This gas mixture passed over the hollow fibers containing blood, countercurrent to the direction of blood flow. Next, the blood flow went through a filter and a Windkessel chamber consisting of a test tube stoppered by a cork with inlet and outlet tubes. These devices protected the animal from clots and air bubbles as well as buffering pressure fluctations generated by the pump. This was followed by an electrical ground for the flow probe, and a Y-branch with an electromagnetic flow probe (Carolina Medical Electronics EP408) on one side and polyethylene tubing on the other side. This functioned as a shunt when the flow probe was clamped off for zeroing the baseline. Next, there was a multiline infusion cannula and cannulas for monitoring the pressure in the circuit and taking blood samples. Finally there was the last cannula which connected to the animal. All parts of the circuit were connected with the shortest possible pieces of polyethylene tubing to minimize the volume of



1. inlet pressure
2. roller pump
3. gas exchanger
4. water heater
5. gas regulator
6. filter
7. windkessel chamber - bubble trap
8. ground
9. em flow probe
10. circuit pressure
11. infusion lines

FIGURE 1 Arterial Long Circuit

blood required by the circuit.

On the day prior to the experiment the circuit was assembled and chemically sterilized. This procedure consisted of pumping a 3.7% solution of formaldehyde through the circuit for approximately an hour and then a thorough water rinse. Then a 5% solution of strong ammonia was passed through the circuit followed by an extensive water rise, changing the rinse with fresh water six times.

Once the circuit was hooked up, the pump speed was adjusted so that the pressure recorded in the SMA was 100 mmHg.

All pressures were monitored by Gould and Statham pressure transducers which had been set to zero at the level of the thoracic vena cava. Both the pressures and flows were recorded on a Sensor Medics R611 Dynograph chart recorder.

PROTOCOLS

Neuromodulation series:

The first step of this experimental series was to determine an intra-arterial adenosine dose-response curve and select a low, mid and high dose from it. Low dose gave a small vasodilation, high dose produced near maximal vasodilation, and the mid dose was between the two.

For each frequency of nerve stimulation, and each norepinephrine (NE) concentration, a data set was obtained as follows: Control nerve or norepinephrine vasoconstriction (Control 1); a nerve or NE response during infusion of the low dose of adenosine; Control 2; nerve or NE response during mid dose adenosine infusion; Control 3; nerve or NE response during high dose adenosine infusion; Control 4. For each experiment the nerve or norepinephrine order was altered randomly. Both the adenosine (1.94 $mg.Kg^{-1}.ml^{-1}$) and the norepinephrine (mean low dose of 0.181 and mean high dose of 0.406 $ug.kg^{-1}.ml^{-1}$) were infused into the cecal artery through the multi-infusion line attached to the cecal artery.

Hypoxia series:

In this experimental series a hypoxia curve was induced by decreasing the proportion of oxygen in the gas mixture supplied to the oxygenator. This resulted in a decrease in the partial pressure of oxygen in the blood. The decreases were made in steps corresponding to 100%, 70%, 50%, 30%, and 10% of the flow rate of the oxygen in the gas mixture. After each oxygen decrease there was a five minute stabilization period, then 0.5 ml of blood was drawn from the circuit and analyzed for pH and partial pressures of oxygen and carbon dioxide (Instrumention Laboratory System 1302 pH/blood gas analyzer, Instrumentation Laboratory, Inc., Lexington, MA). In some experiments the total oxygen content was also measured using a LexO_2 Con-K OC-60 total oxygen content analyzer (Lexington Instruments Co., Waltham, MA). After the last step of the hypoxia curve the oxygen level was returned to 100% and the animal was allowed to stabilize. If necessary, the blood flow was adjusted after the hypoxia curve, but not during the oxygen manipulations, to maintain the pressure in the SMA around 100 mm Hg.

Blockade of exogenous adenosine was accomplished by injecting 1 mg/kg of 8-phenyltheophylline (8-PT) into the portal vein and then testing for eradication of the response to adenosine infused into the SMA. The dose of 8-PT was doubled until there was virtually no response to

infused adenosine. The response to infused isoproterenol was also tested to ensure that 8-PT was specific for adenosine blockade. With selective adenosine blockade having been achieved, the hypoxia curve was repeated.

The vasodilation obtained with .04 mg/kg/min adenosine, and .2 ug/kg/min isoproterenol was measured between each step of the protocol and at the beginning and end of the experiment to ensure that the preparation was not deteriorating. The infusion line was also flushed with warm Ringer's solution between administration each vasodilator.

Adenosine (Sigma Chemical Company) was dissolved in warm Ringer's solution to a concentration of .194 mg/kg/ml. Norepinephrine and isoproterenol (Sigma Chemical Company) were prepared from stock solution of 1 mg/ml and diluted in warm Ringer's solution to a concentration of .735 and 1.47 ug/kg/ml respectively. The stock solutions were made in saline containing 0.3 mg/ml ascorbic acid which functioned as a preservative. 8-phenyltheophylline (8-PT) was used in a 2 mg/kg/ml solution by dissolving the powder in warm saline (pH 11.6) which was adjusted to approximately pH 12.3 with 1M NaOH at which point the 8-PT was completely dissolved.

Calculations:

In the modulation series all data points were expressed as SMA conductance (SMAC), calculated from the flow divided by the arterial-venous pressure gradient ($ml.min^{-1}.Kg$ body $weight^{-1}$ /mmHg). The effects of experimental manipulations were then expressed as the percent change in conductance (% SMAC) and calculated as [(SMAC peak response - SMAC baseline)/SMAC baseline] x 100.

The percent inhibition for each vasoconstriction during adenosine infusion was calculated as [(% SMAC control - % SMAC test)/% SMAC control] x 100. In this expression % SMAC control represents the average of the two control responses bracketing the response obtained during the adenosine infusion (% SMAC test).

In the hypoxia series data points were reported as resistance, the inverse of conductance, or the arterial-venous pressure difference divided by the flow ($mmHg/ml.min^{-1}.Kg$ body $weight^{-1}$). The percent change in resistance (% SMAR) was calculated for each experimental manipulation in the same fashion as for % SMAC: [(SMAR peak response - SMAR baseline)/SMAR baseline] x 100.

All values are reported $\pm$ standard error. Comparisons were by blocked ANOVA followed by the least significant difference test (LSD), or by t-tests.

RESULTS

NEUROMODULATION SERIES

Adenosine Dose Response Curve:

The low, mid and high doses of adenosine were selected from a dose-response curve conducted for each animal. The curve was obtained by stepwise infusions into the superior mesenteric artery. The low dose, producing a small measurable vasodilation was 0.009 ± 0.002 (range 0.001-0.02) $mg.kg^{-1}.min^{-1}$ and produced an increase in SMAC of $27.7 \pm 8.0\%$. The mid dose of 0.045 ± 0.017 (range 0.004-0.2) $mg.kg^{-1}.min^{-1}$ produced a change in SMAC of $90.6 \pm 12.8\%$. The highest dose causing near maximal vasodilation without systemic effects was 0.386 ± 0.172 (range 0.01-2.0) $mg.kg^{-1}.min^{-1}$ producing $178.7 \pm 33.3\%$ change in SMAC (Fig. 2).

Peak Vasoconstriction:

For each vasoconstrictor stimulus the vasoconstrictor response (% SMAC) for controls 1 through 4, and during the low (LLA), mid (MLA) and high (HLA) levels of adenosine were pooled and compared by blocked ANOVA. The first control constriction at each level of nerve stimulation was significantly greater ($P<0.01$) than the other three controls. The remaining 1Hz control nerve stimulations were not significantly different. Of the remaining 3Hz

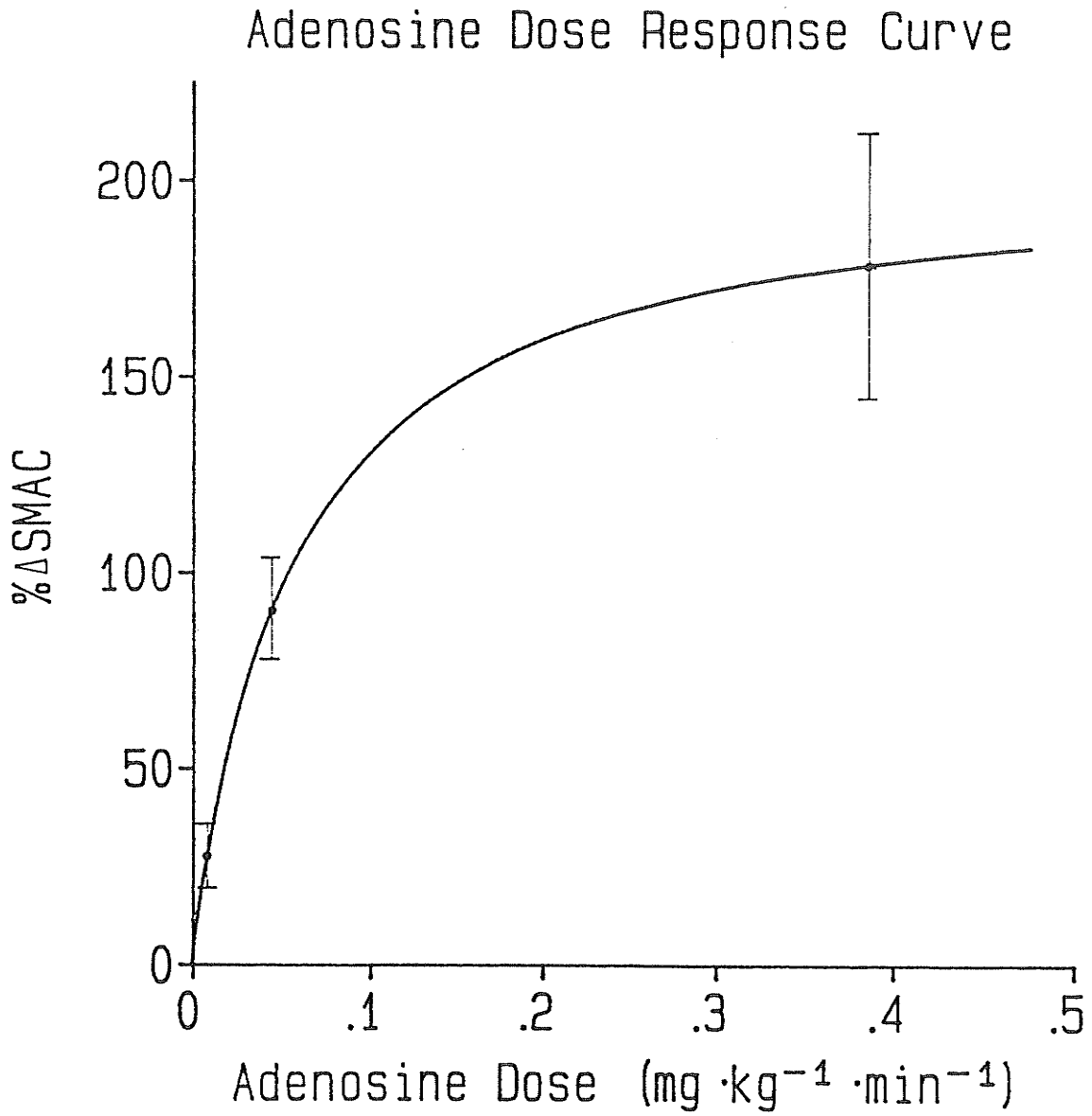


FIGURE 2 Adenosine dose-response curve.

control vasoconstriction, the second was significantly larger than the third but not the fourth control vasoconstriction, and these latter two were the same. In the 9Hz nerve stimulation group, the third and fourth constrictions were also equivalent and both were smaller than the second control.

The controls for the high dose norepinephrine constrictions were all equivalent, as were the first two low dose norepinephrine control vasoconstrictions. The third control of this latter group was significantly different from the first, but not the second control vasoconstriction. The fourth low dose norepinephrine constriction was smaller than the first three (Fig. 3).

Consequently, the control vasoconstrictions showed a statistically significant trend to decrease with time. This effect was less pronounced with the norepinephrine infusions, and, in fact, was not observed with the high level norepinephrine infusion.

The low level of adenosine (LLA) had varying effects on the different levels of vasoconstrictor stimuli (Table 1). In the case of the 1Hz nerve stimulation and the low level norepinephrine infusion, it significantly decreased the vasoconstriction ($P<0.01$). With the other nerve stimulations (3 and 9 Hz) LLA had variable effects with respect to its bracketing controls, but compared to an

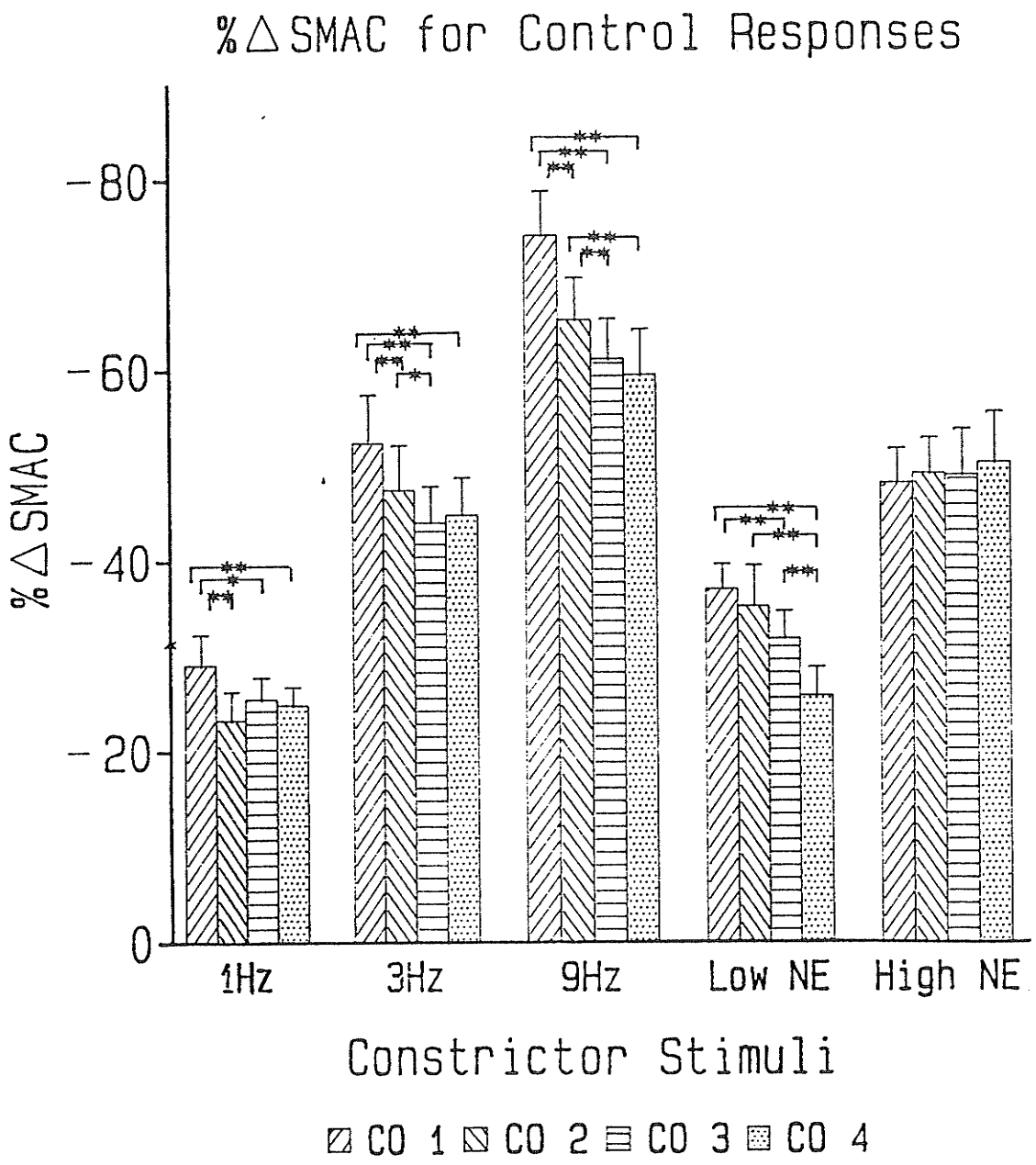


FIGURE 3
Control peak vasoconstrictions. Each constrictor stimulus was analyzed by a blocked ANOVA and compared by LSD. ANOVA included control as well as adenosine modulated (not shown) vasoconstrictions. *P<0.05; **P<0.01.

average of the two controls the effects were not significant. The high dose of norepinephrine during LLA was not significantly different from either control. Overall, the only distinct effect of LLA was on the weakest vasoconstrictor stimuli, 1Hz nerve stimulation and the low dose of norepinephrine.

In all cases MLA and HLA significantly decreased vasoconstriction when compared to their respective bracketing controls ($P<0.01$) (Table 1). In addition, the response during LLA, MLA and HLA were each progressively significantly lower than each other ($P<0.01$).

The increasing dose of adenosine significantly decreased the vasoconstrictor response to a range of stimuli. In comparison with controls, the low level of adenosine only had a significant inhibitory effect on weaker vasoconstrictions. This inhibition became ambiguous, or was eradicated with stronger vasoconstrictor stimuli, whereas larger doses of adenosine maintained their effects.

Extent of adenosine modulation:

The extent to which adenosine modulated the vasoconstrictor responses, regardless of the magnitude of the vasoconstriction, was examined by expressing the results as % inhibition. A square root transformation was performed on each value to reduce the heterogeneity of

Table 1 Peak Vasoconstrictions

Vasoconstrictions obtained in response to sympathetic nerve stimulation and norepinephrine (NE) infusion. All responses were obtained during infusion of three levels of adenosine; low (LLA), mid (MLA) and high (HLA). Control constrictions (CONT) bracket each constriction during adenosine.

TABLE 1

Peak Vasoconstriction

TREATMENT		%^SMAR
1 Hz	CONT 1	-29.08± 3.2
	LLA	-16.82± 4.2 ** ++
	CONT 2	-23.33± 3.0
	MLA	-21.07± 2.5 ** ++
	CONT 3	-25.50± 2.3
	HLA	-10.44± 2.8 ** ++
	CONT 4	-24.86± 1.9
3 Hz	CONT 1	-52.44±5.1
	LLA	-48.03±4.0 **
	CONT 2	-47.46±4.7
	MLA	-39.20±4.6 ** ++
	CONT 3	-44.05±3.8
	HLA	-27.75±4.8 ** ++
	CONT 4	-44.91±3.9
9 Hz	CONT 1	-74.20±4.6
	LLA	-68.19±5.2 ** +
	CONT 2	-65.27±4.4
	MLA	-53.17±4.5 ** ++
	CONT 3	-61.20±4.2
	HLA	-39.05±5.0 ** ++
	CONT 4	-59.55±4.8
LOW NE	CONT 1	-37.07±2.6
	LLA	-30.87±4.7 ** ++
	CONT 2	-35.26±4.3
	MLA	-17.21±5.7 ** ++
	CONT 3	-31.91±2.9
	HLA	- 7.77±3.5 ** ++
	CONT 4	-25.88±3.0
HIGH NE	CONT 1	-48.17±3.6
	LLA	-46.62±3.7
	CONT 2	-49.21±3.7
	MLA	-33.91±6.4 ** ++
	CONT 3	-48.99±4.8
	HLA	-16.11±6.6 ** ++
	CONT 4	-50.27±5.3

* P<0.05 COMPARED WITH PREVIOUS CONTROL
** P<0.01 COMPARED WITH PREVIOUS CONTROL
\+ P<0.05 COMPARED WITH FOLLOWING CONTROL
++ P<0.01 COMPARED WITH FOLLOWING CONTROL

variance and these were analyzed by repeated measures ANOVA according to vasoconstrictor stimuli and adenosine dose. This analysis indicated a significant effect of adenosine dose, but no effect of the vasoconstrictor stimuli (Table 2). Therefore the transformed values were then pooled by adenosine dose and compared by Tukey's HSD test. The inhibitory response to the high dose of adenosine was significantly higher than both the mid ($P<.06$) and low ($P<.01$) doses of adenosine. The mid and low doses of adenosine were not significantly different.

In an attempt to determine whether the effects of adenosine on nerve stimulation differed from those on norepinephrine infusion, the data for the two vasoconstrictor stimuli were separated and pooled by adenosine dose (Table 3). The low dose of adenosine equally inhibited nerve and norepinephrine-induced constrictions by 10.3% and 9.1% respectively. However, both the mid and the high dose of adenosine had a greater inhibitory effect on norepinephrine-induced vasoconstrictions (mid: 39.3%; high: 66.9%) than on nerve-induced constriction (mid: 15.9%; high: 45.7%). Thus, adenosine affected the norepinephrine constrictions to a greater extent than the nerve-induced vasoconstrictions.

Table 2 % Inhibition of Vasoconstriction

	ADENOSINE DOSE		
	LOW	MID	HIGH
NERVE STIMULATION			
1 Hz	22.35± 2.8	7.69±11.8	59.12±11.8
3 Hz	4.04± 3.8	19.69± 4.4	40.77± 9.0
9 Hz	4.57± 2.8	17.89± 3.5	37.30± 7.0
NOREPINEPHRINE INFUSION			
LOW	12.67±13.5	40.72±19.1	67.90±15.6
HIGH	5.57± 6.9	35.80±11.5	65.91± 8.7

Vasoconstriction in response to nerve stimulation (1, 3, 9 Hz) and norepinephrine infusion (low: 0.18 $ug.kg^{-1}.min^{-1}$; high: 0.41 $ug.kg^{-1}.min^{-1}$) during 3 doses of adenosine (low:0.009 $mg.kg^{-1}.min^{-1}$; mid: 0.045 $mg.kg^{-1}.min^{-1}$; high: 0.386 $mg.kg^{-1}.min^{-1}$). The extent to which adenosine modulated the vasoconstriction was expressed as %inhibition $((\%\hat{}SMAC_{CO}-\%\hat{}SMAC_{TEST})/\%\hat{}SMAC_{CO})$. The $\%\hat{}SMAC_{CO}$ represents the mean of the two control percent change in SMAC responses bracketing the adenosine-modulated response. Analyzed by repeated measures ANOVA. Significant effect of adenosine ($P<.001$), no effect of vasoconstrictor stimuli. All treatments in all animals.N=11.

Table 3 Inhibition of Vasoconstriction

ADENOSINE DOSE	NERVE STIMULATION	NOREPINEPHRINE INFUSION
LOW	10.32± 2.4	9.12± 7.8
MID	15.09± 4.5	39.26± 11.4
HIGH	45.73± 5.8	66.90± 9.1

% Inhibition values were pooled by adenosine dose and vasoconstrictor stimulus (nerve stimulation and norepinephrine infusion) to allow comparison of adenosine effect. Low adenosine had equal effects on nerve and norepinephrine-induced constrictions. Mid and high adenosine inhibited norepinephrine vasoconstrictions more than nerve stimulated constrictions. N=33 in each nerve stimulation cell; N=22 in each norepinephrine cell.

Competitive vs Non-Competitive Inhibition:

In order to determine whether adenosine antagonism of nerve-stimulation-induced vasoconstriction was competitive or non-competitive in nature the K_r and R_{max}'s were determined during the mid and high dose and with no adenosine (control) infusion. The K_r $r_e$presented the frequency which produced half-maximal vasoconstriction and R_{max} was the maximum vasoconstriction. These values were determined by fitting a rectangular hyperbolic curve (Graphpad, ISI Software) to a plot of the nerve stimulation frequency against the %^SMAC. The same information was also obtained from an Eadie-Hofstee plot of the %^SMAC against %^SMAC/stimulation frequency. The negative slope of this plot gave K_r and the Y-intercept R_{max} (Fig. 4).

The low level of adenosine was omitted from this manipulation because of its variable effects, but a consistent pattern emerged from the other adenosine doses. From the frequency response curve the K_r was calculated to be 2.1Hz in both the control state and during infusion of the mid dose of adenosine. These curves also had a R_{max} of -80.5% and -65.6% respectively. The Eadie-Hofstee plot also gave K_r's of 2.1 for both control and mid dose of adenosine. The R_{max}'s were -78.5% for control and -66% for the mid dose of adenosine. The 1Hz data during the high dose of adenosine was also extremely variable, and

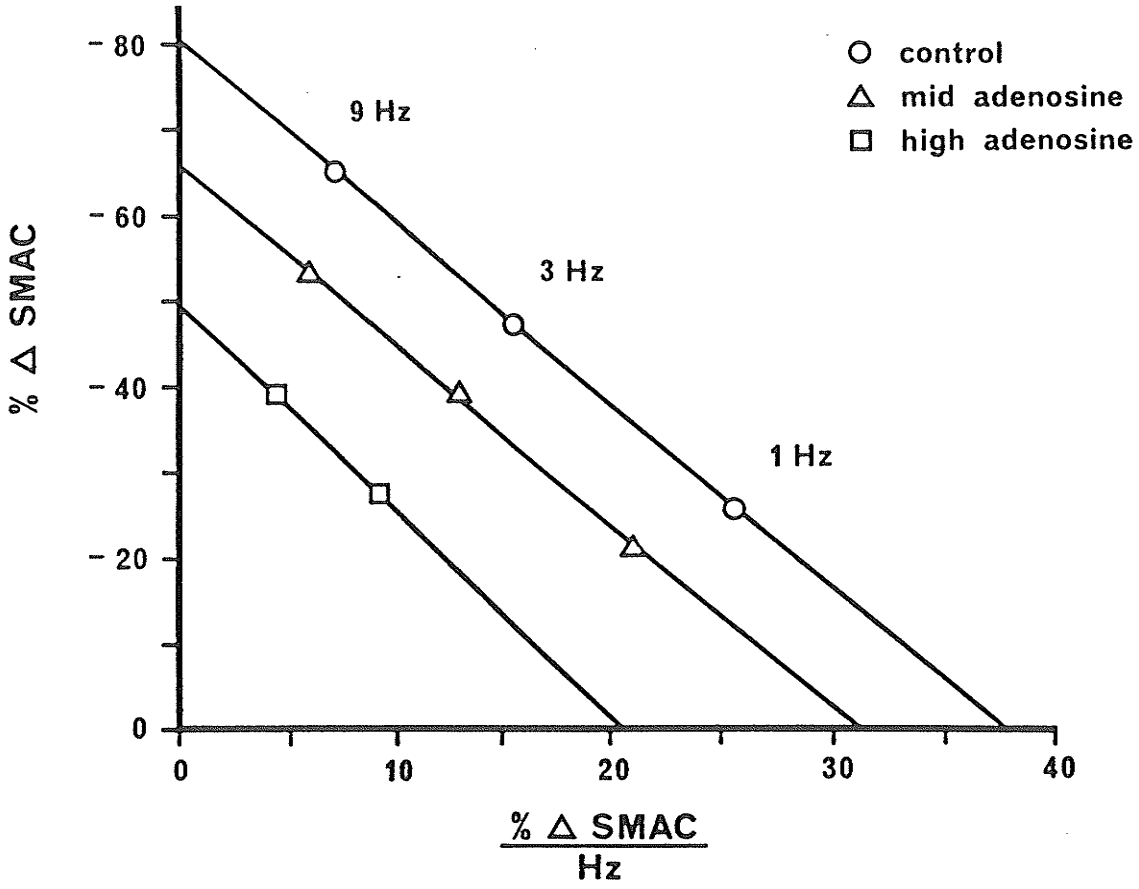


FIGURE 4 Eadie-Hofstee Plot

The peak vasoconstriction for the nerve stimulations only was plotted against the peak vasoconstriction divided by the stimulation frequency. The responses obtained during infusion of the low dose of adenosine were omitted due to their extreme variability. The data obtained with the high dose of adenosine and 1Hz nerve stimulation were also omitted for the same reason. K_r was obtained from the negative slope of each line: control 2.1Hz; mid 2.1Hz; high 2.3Hz. V_{max} was read from the Y-intercept: control -78.5%; mid -66%; high -49%.

consequently was omitted. Thus it was only possible to calculate K_r (2.3 Hz) and R_{max} (-49%) from the Eadie-Hofstee plot. Regardless of the method used to calculate K_r, it remained essentially constant during the control state and the mid and high doses of adenosine. Both methods also gave similar approximations of R_{max}.

Conductance vs Resistance:

The modulation series of experiments utilized a preparation where the animal regulated both the pressure and the flow. Consequently, the flow varied to a much greater extent than the pressure. In the conductance term, the variable which undergoes the largest changes, flow, is then in the numerator and the more constant variable, pressure, is in the denominator. However, the hypoxia experiments utilized a constant flow preparation and, therefore, the results were expressed as resistance in order to keep the constant term (flow) in the denominator.

The importance of having the more constant term in the denominator is obvious from a plot of flow against both conductance and resistance. In the first hypoxia experiment each data point was expressed as both conductance and resistance and plotted against flow (Fig. 5). The resulting graph shows resistance to have a linear

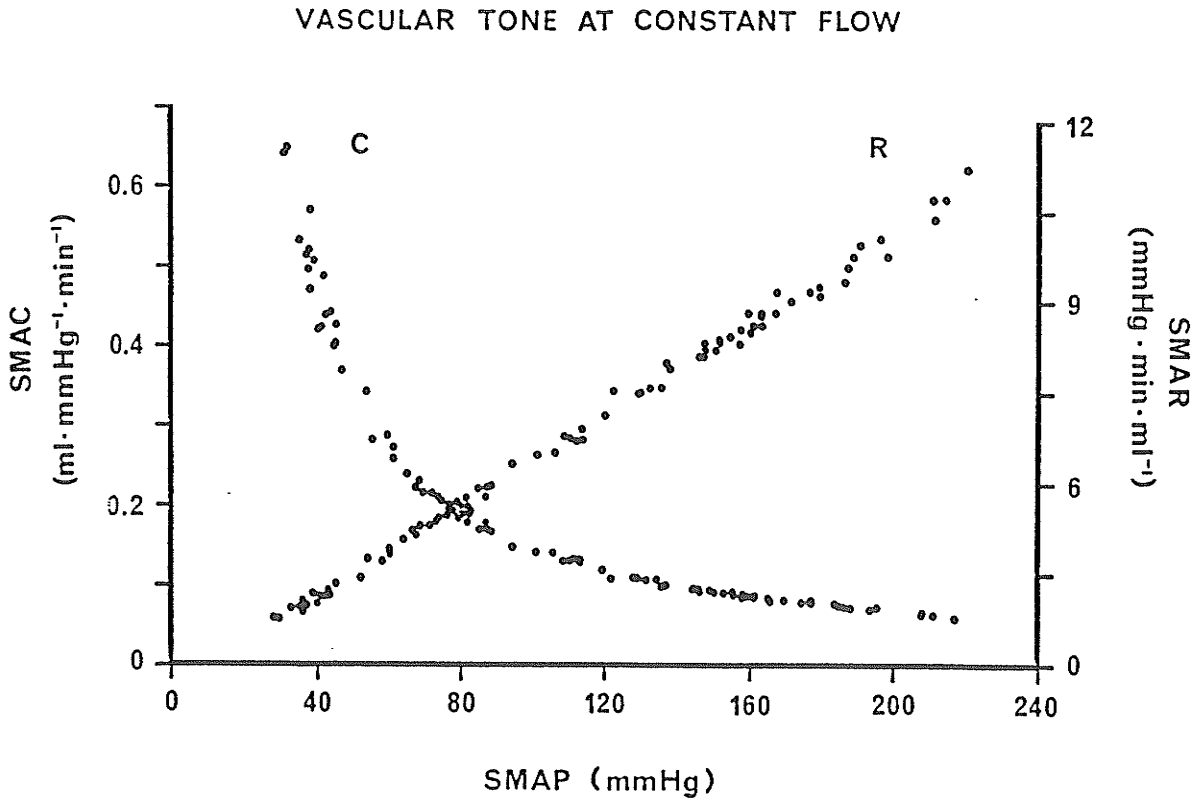


FIGURE 5 Conductance vs Resistance

All data points from a constant flow experiment were calculated as both resistance and conductance and plotted against pressure. The coefficient of determination for the resistance slope was 0.997 ± 0.0004. N=97.

relationship with flow, whereas the conductance curve resembles an exponential function. This difference becomes important when simple mathematical functions (i.e. addition and subtraction) are performed on these values.

HYPOXIA SERIES

Stabilization Time:

Before experiments were performed on cats, the entire long circuit was set up and saline was pumped through it in lieu of blood. From this it was determined that alterations in the gas supply to the hollow fiber gas exchange cannister resulted in stable changes in the partial pressures of carbon dioxide (CO_2) and oxygen (O_2) within two minutes. Consequently, in the animal experiment five minutes was judged to be a sufficient stabilization period. In the first experiment this assumption was shown to be correct as pressure changes and blood gases produced five minutes after alterations in gas supply to the oxygenator were unchanged after an additional five minutes of stabilization.

Basal Parameters:

As a means of assessing the basal conditions over the course of the experiment, the mean arterial (MAP), superior mesenteric arterial (SMA), portal venous (PVP) and central venous (CVP) pressures, and the SMA flow (SMAF) and resistance (SMAR), the partial pressure of carbon dioxide (PCO_2) and pH were tabulated at the beginning and end of the experiment as well as before and after adenosine receptor blockade with 8-PT (Table 4).

TABLE 4

BASAL PARAMETERS

	Beginning	Pre 8-PT	Post 8-PT	End
MABP	117.9 ± 9.5	86.9 ± 10.1	84.6 ± 7.7	66.4 ± 9.5**+
SMAP	107.8 ± 3.3	103.9 ± 5.1	99.1 ± 4.6	85.9 ± 17.2
SMAF	11.5 ± 2.1	11.9 ± 1.9	14.7 ± 2.7	19.8 ± 3.8**++
SMAR	10.9 ± 2.8	9.2 ± 1.8	7.9 ± 2.1	4.5 ± 1.3**+
PVP	10.6 ± 1.0	9.7 ± 0.8	11.7 ± 2.9	10.4 ± 1.0
CVP	5.7 ± 6.8	5.5 ± 0.9	5.8 ± 0.7	6.3 ± 0.8
PCO_2	27.5 ± 2.0	27.5 ± 1.7	27.8 ± 1.4	27.8 ± 0.9
pH	7.31 ± 0.02	7.26 ± 0.02	7.27 ± 0.02	7.24 ± 0.02**

Tabulated basal parameters for hypoxia experiments. 8-PT: 8-phenyltheophylline; HABP: mean arterial blood pressure (mmHg); SMAP: superior mesenteric arterial pressure (mmHg); SMAF: superior mesenteric arterial flow ($ml.min^{-1}.Kg^{-1}$); SMAR: superior mesenteric arterial resistance ($mmHg.Kg.min.ml^{-1}$); PVP: portal venous pressure (mmHg); CVP: central venous pressure (mmHg); PCO_2: partial pressure of carbon dioxide (mmHg). All values represent mean ± standard error. N=7.

** $P<0.01$ compared to beginning of experiment.
+ $P<0.05$, ++ $P<0.01$ when compared to pre 8-PT.

These were then pooled, assessed by blocked ANOVA and compared by the least significant difference test.

Over the course of the experiment the PVP, CVP, SMAP and PCO_2 did not change significantly. The SMAF at the end of the experiment was significantly higher than the beginning of the experiment and before 8-PT administration ($P<0.01$). Consequently, the SMA resistance at the end of the experiment was significantly lower than at the beginning of the experiment ($P<0.01$) and before 8-PT administration ($P<0.05$). In addition, the mean arterial pressure at the end was significantly lower than the beginning of the experiment, before 8-PT administration ($P<0.01$) and after 8-PT administration ($P<0.05$). The pH decreased significantly by the end of the experiment, but this was due to the tightness of the data and hence the very small errors. The actual magnitude of the change was less than 1% by the end of the experimental course.

The %^SMAR in response to adenosine and isoproterenol infusion at the beginning and the end of each experiment were pooled and compared by a t-test. This served a dual function. First, in the case of the isoproterenol infusions, to demonstrate that the preparation had not deteriorated over the course of the protocol. Second, to establish the selective blockade of adenosine response by 8-PT.

The pooled %^SMAR for 0.2 $ug.Kg^{-1}.min^{-1}$ isoproterenol

at the beginning of the experiment was -61.3 ± 1.8%. This value was not significantly different from -51.9 ± 7.2% during adenosine receptor blockade at the end of the experiment. Consequently, the preparation was capable of the same responses at the beginning of the experiment as at the end. In addition, 8-PT administration did not affect the isoproterenol response.

The decrease in SMA resistance in response to 0.04 $mg.Kg^{-1}.min^{-1}$ adenosine was 51.9 ± 3.4%. An average dose of 4.7 ± 0.9$mg.Kg^{-1}$ 8-PT was needed to effectively block the adenosine response reducing the %^SMAR to -4.4 ± 1.9%.

The effect of the 8-PT administration into either the SMA or the portal vein was to decrease the SMA resistance from 9.2 ± 1.8 to 7.9 ± 2.1 $mmHg.Kg.min.ml^{-1}$. As already stated, this difference in resistance was not significant. This represents an average %^SMAR of -19.8 ± 7.4% (obtained from the pooled %^SMAR from each experiment).

In one experiment the carrier was administered in bolus doses of 0.5ml, 1ml, 2ml, 4ml and 8ml in the same fashion as progressively larger doses of 8-PT were delivered. The %^SMAR for 2ml and 4ml of carrier (which corresponds to 4 and 8mg/Kg 8-PT respectively) was -12.7% and -21.3%. After the last dose of 8ml had been delivered the total %^SMAR was -33.2%. Consequently, it is difficult to determine whether the observed change in resistance is a result of 8-PT or its carrier solution.

More experiments would have to be performed to answer this question.

To ensure that the carrier was also not affecting the vasodilator responses, adenosine and isoproterenol infusions were repeated after each bolus of carrier. The %^SMAR for isoproterenol was -66.6% before carrier and dropped to -56.3% after the last dose of 8ml of carrier. For adenosine %^SMAR was -55.8% before and -48.1% after the 8ml dose of carrier. Subsequent administration of 4mg/Kg 8-PT changed the %^SMAR of adenosine to 0.23% and isoproterenol to -53.7%. Thus, the carrier had little effect on the vasodilator responses, and was not likely responsible for the selective eradication of the adenosine response by 8-PT.

Oxygen Delivery and Resistance:

In the first three experiments the total oxygen content (TOC) was measured and used to calculate oxygen delivery (TOC x SMA flow). The oxygen delivery in the normoxic state was significantly different from the oxygen delivery taken at the lowest point of hypoxia, both in the control state and during adenosine receptor blockade (blocked ANOVA). However, hypoxia did not produce a similar difference in the corresponding resistances (blocked ANOVA).

The values for both oxygen delivery and resistance

from each point on the hypoxia curve were pooled and analyzed by linear regression. The resulting slopes for the control and adenosine blocked states were not significantly different, however their coefficients of determination were also very poor. In order to standardize the starting values among experiments the percent change in oxygen delivery ([(hypoxia-normoxia)/normoxia] x 100) was calculated, pooled and plotted against the pooled %^SMAR. The control slope of 0.18 $\pm$ 0.15 was not significantly different from the adenosine blocked slope of 0.58 $\pm$ 0.14 ($P<0.07$).

When the same analyses were performed with the partial pressures of oxygen (PO_2's) instead of oxygen delivery the results were similar. Consequently, PO_2 and %^PO_2 values were used in the remainder of the analyses.

Hypoxia and Resistance:

In both the control and adenosine receptor blocked states the PO_2's during normoxia were significantly different from those at the lowest point of the hypoxia curve. In addition, adenosine receptor blockade had no effect on the PO_2's for normoxia or hypoxia. In the control state, decreasing PO_2 from 109 to 38 mmHg resulted in the resistance significantly decreasing from 10.2 to 7.5 $mmHg.kg.min.ml^{-1}$ ($P<0.05$). During adenosine receptor blockade the same decrease in PO_2 (from 109 to 40 mmHg)

also decreased the resistance significantly from 5.7 to 3.4 mmHg.kg.min.ml^{-1}($P<0.05$). In both cases, returning PO_2 to normoxic values also caused resistance to return to statistically equivalent values.

To ensure that the hypoxia had not induced maximum vasodilation, the resistance resulting from adenosine and isoproterenol vasodilation in the control state and during adenosine receptor blockade were compared to the resistance during maximum hypoxia (Table 5). In the control state, both adenosine and isoproterenol (3.9 $\pm$ 0.8 and 3.7 $\pm$ 0.7 mmHg.kg.min.ml^{-1} respectively) were capable of inducing significantly greater vasodilation than was produced by hypoxia (7.5 $\pm$ 1.4 mmHg.kg.min. ml^{-1}). During adenosine receptor blockade, isoproterenol induced a larger decrease in resistance than did hypoxia (2.9 $\pm$ 0.6 and 3.4 $\pm$ 0.6 mmHg.kg.min.ml^{-1} respectively), although the difference was not significant.

The resistances obtained during the control normoxic states were significantly higher than those values seen during adenosine receptor blockade, therefore the responses to hypoxia, adenosine and isoproterenol were expressed in terms of %^SMAR and compared by a blocked ANOVA (Table 6). The response to hypoxia, both in the control state and during 8-PT, was significantly less than the response to either adenosine or isoproterenol ($P<0.01$). The hypoxic response during adenosine receptor

TABLE 5

RESISTANCES DURING INTERVENTIONS

	Control SMAR ($mmHg.Kg.min.ml^{-1}$)	8-PT SMAR ($mmHg.Kg.min.ml^{-1}$)
Normoxia 1	10.2 ± 2.0	5.7 ± 1.0
Hypoxia	7.5 ± 1.4 ** +	3.4 ± 0.6 **
Normoxia 2	11.7 ± 1.6	4.9 ± 0.8
Adenosine	3.9 ± 0.8 ##	5.6 ± 0.3 ##
Isoproterenol	3.7 ± 0.7 ##	2.9 ± 0.6

The resistances before (control) and after (8-PT) adenosine receptor blockade with 8-phenyltheophylline. Normoxia 1 was obtained before hypoxia, and normoxia 2 after. All values mean ± SE. Analyzed by blocked ANOVA and LSD. N=7.

** $P<0.01$ when compared to normoxia 1.
+ $P<0.05$ when compared to normoxia 2.
$P<0.01$ when compared to hypoxia.

TABLE 6

%^SMAR DURING INTERVENTIONS

	Control	8-PT
Hypoxia	-23.8 ± 7.3	-35.6 ± 10.8
Isoproterenol	-64.8 ± 3.6**	-64.7 ± 4.5**
Adenosine	-61.1 ± 3.1**	-6.3 ± 0.8**

Relative changes in resistance before (control) and after adenosine receptor blockade with 8-phenyltheophylline (8-PT). Mean ± SE compared by blocked ANOVA and LSD. N=7.

** $P<0.01$ when compared to hypoxia.

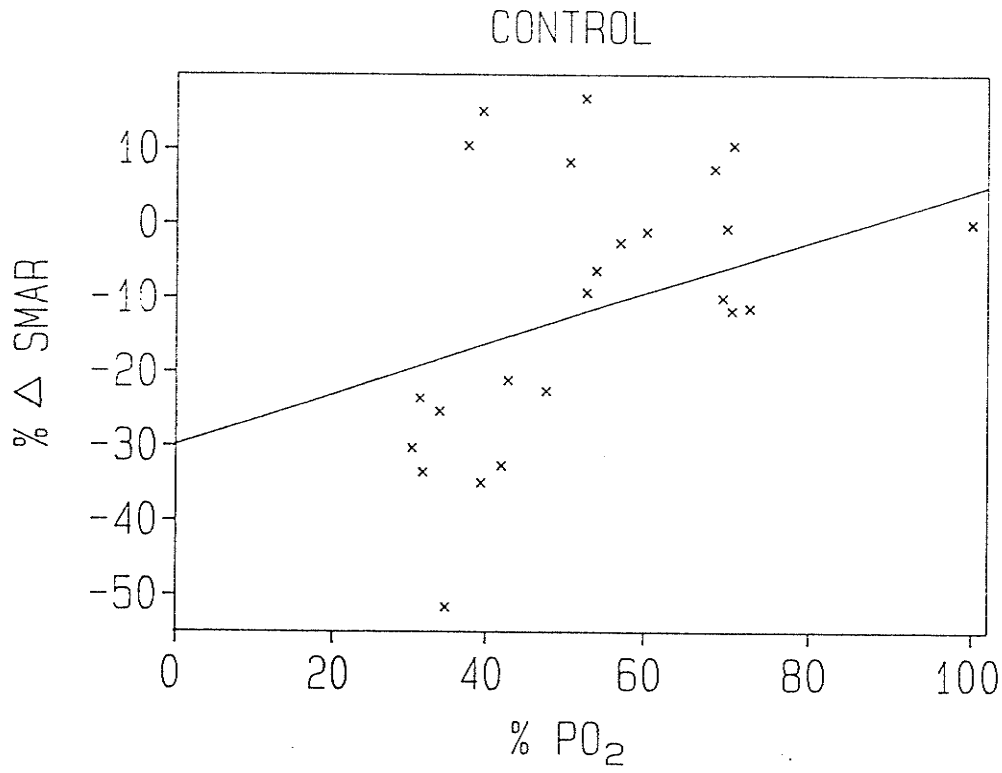
blockade was also larger than during the control state, although this difference was not significant.

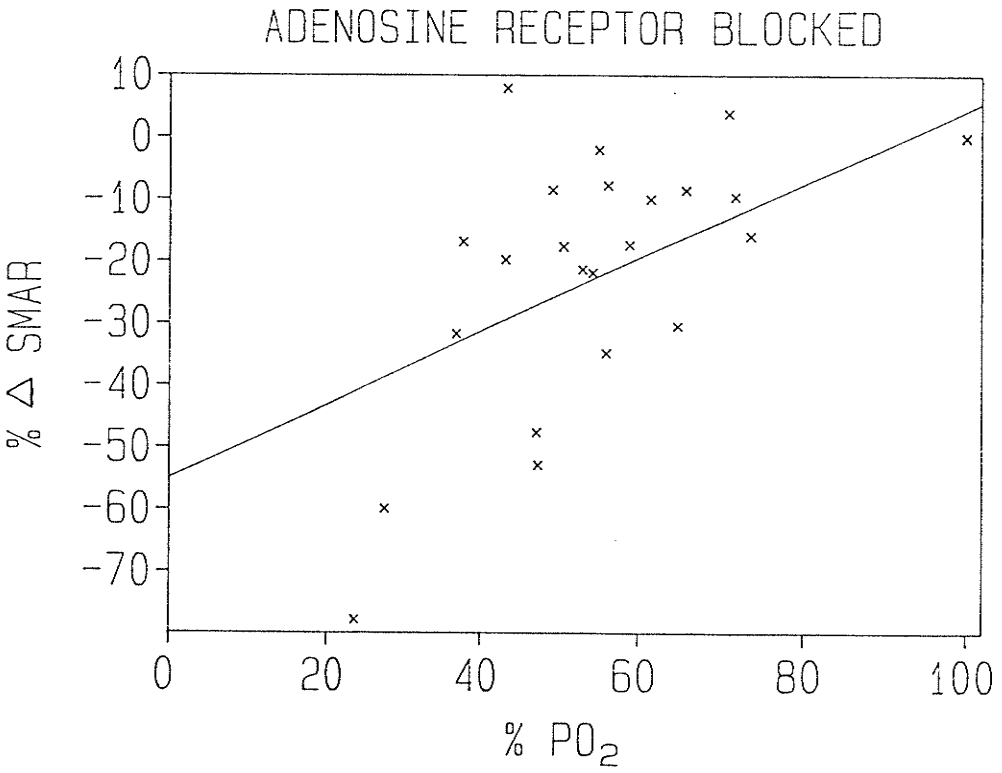
Since adenosine receptor blockade did not alter the net effect of hypoxia, the hypoxia curves themselves were examined. For each experiment, the partial pressure of oxygen was expressed as a percentage of the first normoxic value ($\%PO_2$) and plotted against the %^SMAR. In all but one experiment, the individual slopes of the control data compared with the data from its respective adenosine receptor blocked pair, showed no difference. In the one deviant experiment, for unknown reasons resistance actually increased with increasing hypoxia in the control state hence the slope was significantly different from the adenosine receptor blocked slope.

The pooled slopes of the control hypoxia curves were also compared against the pooled slopes of the 8-PT hypoxia curves. The mean slopes of 0.29 ± 0.12 and 0.53 ± 0.15, before and after adenosine receptor blockade, were not significantly different from each other. In addition, if the data from the individual experiments were pooled to obtain a slope (Fig. 6), the control slope of 0.34 ± 0.11 was also not different from the slope of 0.59 ± 0.12 ($P<0.13$) during adenosine receptor blockade. Increasing the sample size of the control curve by including those experiments for which no corresponding adenosine blocked data was obtained, did not alter the slope (0.34 ± 0.09;

FIGURE 6 Hypoxia Curves

Plot of %^SMAR against $\%PO_2$ before (Figure 5a) and after (Figure 5b) adenosine receptor blockade with 8-phenyltheophylline. Control slope of 0.34 $\pm$ 0.11 is not different from the adenosine receptor blocked slope of 0.59 $\pm$ 0.12 as compared by t-test.


CONTROL
% Δ SMAR
10
0
-10
-20
-30
-40
-50
0
20
40
60
80
100
% PO2


ADENOSINE RECEPTOR BLOCKED
% Δ SMAR
10
0
-10
-20
-30
-40
-50
-60
-70
0
20
40
60
80
100
% PO2

$P<0.10$), or affect the significance of the difference between the two slopes.

The raw data from each experiment were also pooled, and PO_2 was plotted against SMAR. This resulted in a control slope of 0.018 ± 0.029 $min.ml^{-1}.Kg^{-1}$ which was not statistically different from the adenosine receptor blocked slope of 0.033 ± 0.013 $min.ml^{-1}.Kg^{-1}$ ($P<1$).

Examining the other parameters which were measured or calculated did not reveal any other relationship between hypoxia and intestinal vascular function. Neither the total oxygen content in arterial or venous blood, nor the partial pressure of oxygen in venous blood showed a better relationship to the change in resistance during hypoxia. The partial pressure of carbon dioxide (PCO_2) and the pH of arterial and venous blood bore no relationship to the resistance. In addition, the changes in PCO_2 also did not parallel the changes in pH.

Constant Flow vs Constant Pressure:

In two experiments, vasoconstrictor responses were obtained under constant flow and constant pressure conditions. To obtain the constant pressure response, the flow was varied during the course of the vasoconstriction so as to maintain the SMA pressure relatively constant. Nerve stimulation (5Hz) produced a %^SMAR of $116.3 \pm 5.1\%$ under constant flow conditions which was significantly

smaller than 133.6 ± 4.9% seen under the constant pressure conditions ($P<0.051$, n=4). However, the difference between the %^SMAR for norepinephrine infusions under constant flow or pressure conditions (64.6 ± 10.5% and 61.0 ± 13.2% respectively, n=3) was not significantly different.

Effect of Altered Initial Parameters:

In an attempt to further validate the constant flow preparation, the neuromodulation experiments were repeated, but with little success. In three experiments neither adenosine nor isoproterenol had any significant effect on the nerve stimulated or norepinephrine induced vasoconstrictions. Adenosine inhibited the nerve stimulated constrictions by -59.3±67.7% and the norepinephrine constrictions by -49.5±88.8%. Isoproterenol, on the other hand, inhibited the nerve and norepinephrine-induced constrictions by -123.0±31.6% and-68.0±86.4% respectively. By examining other parameters it appeared that the %^SMAR observed correlated modestly with the pressure from which the constriction started (initial pressure). If the initial pressure was high then the %^SMAR induced by nerve stimulation or norepinephrine infusion would be smaller than if the initial pressure had been lower. The vasoconstrictor responses from these two experiments were further examined by plotting the %^SMAR

against the initial pressure. This yielded slopes of-0.89$\pm$.3 and -0.44$\pm$.2 for nerve and norepinephrine constrictions (N=8 observations). Neither of these slopes had particularly high coefficients of determination (R^2= .66 and .56 for sympathetic nerves and norepinephrine respectively), however both slopes were significant.

From visual inspection of the experimental tracings in which neuromodulation was not attempted, it also appeared that the initial pressure had an effect on the net response to a constrictor or dilator stimulus. Here the response (%^SMAR) to each agent was plotted against the initial pressure or resistance for each experiment. Only the responses obtained under normoxic conditions were examined. Neither adenosine, nor isoproterenol nor norepinephrine showed any consistent relationship between the initial pressure or resistance and the %^SMAR. For adenosine in particular, a narrow range of pressure or resistance could show a large variation in response. The nerve stimulations, however, did show a general trend of decreasing %^SMAR with increasing pressure or resistance.

DISCUSSION

NEUROMODULATION

Adenosine is known to be a vasodilator of the intestinal vasculature (Granger et al., 1978). In particular it has been shown to exert more potent effects on the resistance vessels of the intestinal vasculature than on the larger conducting vessels (Walus et al., 1981). Adenosine also functions as a neuromodulator in the intestine as well as in many other tissues, including the brain, kidney, liver and heart. However, in many feline tissues adenosine does not inhibit norepinephrine release (Fredholm & Hedqvist, 1980), leaving doubt as to whether presynaptic adenosine receptors exist in the cat. The first study in this series examined adenosine neuromodulation in the feline superior mesenteric artery (SMA). The goals were to establish whether exogenous adenosine functions as a modulator and if so, whether it exerts its effects pre- or postsynaptically.

Peak Vasoconstriction

In the neuromodulation experiments, the control vasoconstrictions induced by sympathetic nerve stimulation showed a significant progressive decrease. Rather than a deterioration of the preparation, this may represent another phenomenon, in particular $alpha_2$-adrenoceptor-mediated feedback inhibition of neurotransmitter release.

This means that norepinephrine released into the synaptic cleft by nerve stimulation interacts with presynaptically located alpha$_2$ adrenoceptors. As a result of their stimulation, these receptors inhibit the amount of norepinephrine released by subsequent nerve stimulations. Researchers have found that rat mesenteric artery stimulated at 8Hz for 1 minute at 16 minute intervals showed a progressive decrease in norepinephrine efflux. This trend was reversible by administration of the alpha$_2$ antagonist, yohimbine (Su & Kubo, 1984). In rabbit mesenteric artery preparations, yohimbine was also observed to inhibit the decrease in excitatory junctional potentials (EJP) elicited by perivascular nerve stimulation. Trains of fifteen stimulation pulses at 1Hz produced ejp's whose amplitudes decreased after the first three pulses. Yohimbine had no effect on these first three pulses, but it reversed the decreased amplitude in subsequent EJP's (Illes & Norenberg, 1987).

The decrease in control vasoconstrictions seen with nerve stimulation was not as pronounced with the norepinephrine infusions. The response to the low dose of norepinephrine showed a tendency to decrease with time, but the high dose of norepinephrine produced vasoconstrictions which were all the same. Consequently, although this study did not examine any facet of feedback inhibition, it is a plausible hypothesis especially since

the nerve-induced constrictions showed the most pronounced effects. At the very least, this demonstrates that explanations other than a deterioration of the preparation could be evoked.

The most important finding of this study was that adenosine significantly reduced the vasoconstrictions in a dose-dependent manner when compared with their respective controls. The lowest dose of adenosine only had a clearly significant effect on the weakest vasoconstrictor stimuli. Both the mid and high dose of adenosine significantly attenuated all vasoconstrictor stimuli. The mid dose of adenosine attenuated the constrictions more than the low dose, while the high dose was more pronounced than both the low and mid doses.

When the data were expressed as % inhibition the results were the same regardless of the constrictor stimuli, but adenosine dose had a significant effect. Pooling the constrictor responses by adenosine levels and comparing by Tukey's HSD test showed that the high level of adenosine was significantly different from both the low and mid levels of adenosine. However, the low and mid level were not different from each other in their ability to inhibit vasoconstriction. Separating the nerve from the norepinephrine-induced constrictions, but maintaining the pooling by adenosine dose, demonstrated that adenosine exerted an equal or greater inhibitory effect on

norepinephrine-induced responses. From table 2 the % inhibition for the 1Hz nerve stimulation and low dose of adenosine is clearly anomalous when compared to the rest of the values for the low dose of adenosine. This could speculatively represent presynaptic inhibition of neurotransmitter release, which is masked by postsynaptic effects of adenosine at higher concentrations. Thus, exogenous adenosine is clearly modulating vasoconstriction through a postsynaptic mechanism, since presynaptic modulation would involve inhibition of transmitter release and consequently affect the responses to sympathetic nerve stimulation more than the responses to infused norepinephrine.

In a companion study (Lautt et al., 1988a) it has been demonstrated that endogenous adenosine does not modulate vasoconstriction of the SMA during sympathetic nerve stimulation. When adenosine receptors were blocked with 8-phenlytheophylline the constrictions elicited by both nerve stimulation (1,3 and 9 Hz) and norepinephrine infusion (0.181 and 0.406 ug/(kg.min)) were not significantly altered.

Similar examples of neuromodulation by exogenous adenosine, but a lack of influence by endogenous adenosine have been found in the intestinal vascular bed of a number of other species. In isolated rabbit mesenteric arteries, adenosine suppressed excitatory junctional potentials

(EJP) in a dose-dependent manner. Stimulation of the perivascular nerves at 1 Hz in trains of 15 pulses produced EJP's whose amplitudes were significantly decreased by various adenosine analogues. The rank order of potency suggests this was via an A_1 adenosine receptor. The inhibition of the first EJP amplitude was less than for the next four pulses, after which the inhibition returned to the level of the first EJP. When the preparation was precontracted with norepinephrine, the addition of the relatively selective A_1 receptor agonist (-)-N^6-(R-phenylisopropyl)-adenosine (R-PIA) failed to have any effect on the norepinephrine-induced change in membrane potential. Addition of 8-phenyltheophylline or 8-cyclopentyltheophylline during the nerve stimulations also significantly enhanced the EJP's (Illes et al., 1988), indicating that endogenous adenosine must also be generated during nerve stimulation. Thus, in this preparation adenosine neuromodulates presynaptically via the A_1 adenosine receptor.

In both normotensive and hypertensive rats adenosine inhibited vasoconstriction of the SMA by nerve stimulation and norepinephrine infusion. In both cases the nerve-induced contraction was attenuated significantly more than the norepinephrine-induced constriction (Jackson, 1987). Therefore, both normotensive and hypertensive rats also show presynaptic neuromodulation by adenosine.

Elegant experiments by Kuan and Jackson (1988) studied the effects of adenosine on nerve stimulation and norepinephrine-induced vasoconstriction in two groups of rats; one receiving the adenosine receptor antagonist 1,3-dipropyl-8-sulfophenylxanthine (DPSPX) and the other saline. This protocol allowed examination of the effect of adenosine on vasoconstriction and the effect of adenosine receptor blockade on the constrictions, as well as the effectiveness of the adenosine receptor antagonist. An adenosine analogue was able to inhibit both the nerve and norepinephrine-induced constrictions, but had significantly less effect on the norepinephrine response. DPSPX was capable of reversing the modulatory effects of the adenosine analogue, however it had no significant effect on the vasoconstrictions. There was also no effect of DPSPX on norepinephrine spillover measured during sustained nerve stimulation. Again, this demonstrates presynaptic neuromodulation by exogenous adenosine, but apparently no participation by endogenous adenosine.

In isolated dog pancreatico-duodenal arteries adenosine also inhibited vasoconstriction by nerve stimulation, but not in response to norepinephrine infusion (Varga et al., 1984).

In the experiments conducted in cats and rats (Lautt et al., 1988a; Kuan & Jackson, 1988) the response to norepinephrine application was inhibited by adenosine.

Those experiments in which adenosine did not affect the norepinephrine induced-responses (Illes et al., 1987; Varga et al., 1984) were conducted on isolated arterial preparations. The type of preparation is likely the source of the difference in norepinephrine response sensitivity, rather than a species difference.

Thus, in the intestinal vascular bed of dogs, rabbits, and rats adenosine inhibits neurotransmission through a presynaptic mechanism. In the cat the mechanism of neuromodulation is postsynaptic. This same mechanism of adenosine neuromodulation has been established in the hepatic artery of the cat (Lautt & Legare, 1985). Thus, postsynaptic modulation in the cat may be a generalized phenomenon, representing a species difference. Regardless of species there is no evidence for neuromodulation by endogenous adenosine in any of these vascular beds.

Competitive vs Non-competitive Inhibition

The kinetic analysis of the effect of adenosine dose on the nerve-induced vasoconstriction revealed features characteristic of non-competitive inhibition. The frequency producing half maximal vasoconstriction (K_r), was constant regardless of the concentration of adenosine modulating the vasoconstriction (K_r- 2.1 to 2.3 Hz). However, the maximal vasoconstriction (R_{max}) decreased with increasing doses of adenosine. This suggests that

adenosine modulates the net response of norepiniphrine after it binds to its postsynaptic receptor as opposed to inhibiting binding to the receptor itself. These experiments do not indicate any possible mechanisms of inhibition, however adenosine has been shown to affect second messenger systems.

The A_1 receptor is believed to be functionally coupled to an inhibitory guanine nucleotide regulatory protein (G-protein) (Stiles, 1986), thus adenosine could be exerting its modulatory effects by decreasing intracellular cAMP levels. Presynaptically, adenosine decreases transmitter release through inhibition of Ca^{+2} entry into the cell (Fredholm & Dunwiddie, 1988; Fredholm et al., 1988). Postsynaptically, adenosine suppressed calcium influxes in CA1 hippocampal neurons (Schubert & Kreutzberg, 1987). Norepinephrine-induced vasoconstriction of rabbit mesenteric arteries has also been demonstrated to be elicited through activation of voltage-dependent Ca^{+2} channels (Nelson et al., 1988). In view of adenosine's established involvement with calcium it is more likely that this is its mechanism of action rather than alterations in cAMP levels.

HYPOXIA SERIES

This series of experiments characterized the responses to hypoxia in the feline intestine. In addition, it presented an opportunity to develop an experimental technique in which a single variable in one organ at a time was manipulated. In these experiments the only organ receiving a reduced oxygen supply was the intestines. It is possible that the oxygen supply to the liver was compromised, but since hepatic arterial blood flow is independent of hepatic arterial metabolism (Lautt, 1980), hypoxia is unlikely to have produced direct vascular effects at sites other than the intestines. This experimental design has an advantage over other experimental protocols, in that it is an in vivo experiment in which the intestine is not an isolated organ, but it is the only organ which is experimentally manipulated. Most other experiments studying the effects of hypoxia on an organ are either isolated organs, or else hypoxia is induced in the whole animal and one or more organs is studied. Generalized hypoxia will have ramifications on the brain, heart, kidneys, lungs, liver and other organs. Depending on the method of generating the hypoxic condition, hypo- or hypercarbia and/or hypotension may also be present. In this type of situation it is impossible to differentiate the effects of hypoxia on other organs which may indirectly affect the

organ of interest, either through hormonal, metabolic or neural influences.

It has been found that the use of hemorrhage to study hypoxia in the intestine elicits neurogenic reflexes which probably conceal the effect on the tissue itself. The net effect of hemorrhagic hypotension is an increase in resistance, accompanied by a decrease in responsiveness to sympathetic vasoconstrictor nerve stimulation (Lundgren, 1983). Since unloading the baroreceptors by carotid occlusion produces the same results this is not an effect of reduced oxygen delivery to the intestine.

In concious dogs, the hypercapnia has been shown to significantly enhance hypoxic vasodilation. This effect is neurally mediated since aortic sinus denervation causes the preparation to behave in the same manner as an eucapnic preparation during hypoxia (Koehler et al., 1980).

In ketamine-sedated lambs reducing the inspired oxygen to generate hypoxia also produced a hypocapnic situation. A decrease in PaO_2 from 80 to 29 mmHg also caused a drop in $PaCO_2$ from 36 to 23 mmHg. After 15 minutes of hypocapnic hypoxia blood lactate levels increased significantly by over three fold (Moss et al., 1988). These types of changes, if not controlled for, can mask the true effect of hypoxia on the resistance vessels of the intestine leading to difficulties in interpeting

results and identifying their relevance to overall cardiovascular homeostasis.

Basal Parameters

Because of the invasive nature of the long-circuit many controls were instituted in the protocol to ensure the viability of the preparation. The SMA pressure did not change over the course of the experiment because the flow was adjusted to maintain this pressure. However, in some animals it was not possible to maintain this pressure without the aorta collapsing due to a suction effect by the circuit. The SMA resistance tended to decline over the course of the experiment, although the decrease was not significant until the end of the experiment, after the administration of 8-phenyltheophylline. The portal venous and central venous pressures did not change significantly, indicating a generalized hemodynamic stability. The mean arterial pressure had decreased significantly by the end of the experiment because the increased blood flow through the intestines was at the expense of the rest of the body.

Paradoxically, in this experimental protocol, the administration of 8-PT tended to cause vasodilation instead of vasoconstriction, as would be expected if it were causing blockade of endogenous adenosine. In similar experiments in the intestine which did not involve an

arterial long circuit, 8-PT had no effect on basal parameters, and overdoses caused massive vasoconstriction (Lautt, 1986 a).

It is difficult to determine the reason for this relaxation. From the one experiment in which the carrier solution was examined for its effects on the condition of the preparation and the dilatory responses, the possibility exists that it may be responsible for this gradual relaxation. Since the carrier solution is quite alkaline (approximate pH 12) it is conceivable that it could have damaged the endothelial cells causing release of endothelium-derived relaxing factor (EDRF). Since neither adenosine or isoproterenol (Furchgott, 1983) are dependent on EDRF for their effects this would also account for the maintance of their responses after carrier administration.

The administration of 8-PT itself may have caused hemodynamic changes by inhibiting adenosine-mediated effects in other organs such as the liver, kidney and brain, thereby causing vasodilation. As the experimental protocol took approximately 8 hours after surgery was completed, the preparation may have been fatigued by that time. However, there were experiments in which 8-PT was not administered, but the protocol was equally as long. These preparations did not show the same sort of deterioration indicating that 8-PT or its carrier solution

must in some way be responsible for precipitating the decline in stability.

To ensure these changes were not compromising the preparation the responses to isoproterenol infusion at the beginning and the end of the experiments were compared and found to be similar. A similar comparison of responses to adenosine infusion revealed that 8-PT administration was selectively blocking the vasodilator response to adenosine.

To summarize, this experimental design was capable of producing rapid changes in the partial pressure of oxygen in the blood supplying the intestines without affecting the oxygenation of other tissues. There were no concurrent changes in the partial pressure of carbon dioxide or pH. This was accomplished without compromising the integrity of the preparation over the course of the experiment.

The Effects of Altered Baselines

It would appear there are some differences between constant flow and constant pressure preparations which could complicate the interpretation of results. In the constant flow preparation it was not possible to duplicate the neuromodulation experiments. This could be a result of having only obtained results in three animals, but since there is not even a trend toward modulation, this

does not appear likely.

The fact that altering baselines also seems to affect responses is also a complicating factor. In the same experiments in which neuromodulation was attempted, varying the initial pressure (through varying the initial flow) demonstrated a relationship, albeit weak, between the initial pressure and the magnitude of the response (%^SMAR). This method has a fault in that it varies two parameters, flow and pressure, at the same time and it is not possible to separate their effects. When just the varying pressure was examined by collating the data from the remaining hypoxia experiments, the results were not significant, although the trend remained. In a blood-perfused canine hindpaw under constant flow conditions the response to nitroglycerine (a vasodilating agent) with respect to initial resistance was found to be constant only if the results were expressed as percent change in resistance. In the same preparation the constrictor responses to norepinephrine, nerve stimulation, tyramine and angiotensin amide were found to be constant only if expressed as change in resistance. If these results were expressed as percent change in resistance then an inverse relationship to initial resistance existed (Greenbery and Wilson, 1974). These results support the trend for vasoconstrictors seen in this study.

The Effects of Hypoxia on Intestinal Blood Flow

In keeping with previous reports, (Granger et al., 1980; Nelson et al., 1987; Nowick et al., 1987; Shepherd, 1978; Shepherd, 1980) a significant decrease in the oxygen supply to the intestines also caused a significant decrease in the resistance.

In a constant flow preparation in areflexic dogs, decreasing the partial pressure of oxygen (PO_2) to 32 mmHg caused the resistance to decrease to 65% of control. When the experiment was repeated in a constant perfusion pressure preparation, reducing PO_2 to 46 mmHg resulted in a resistance of 70% of control (Shepherd, 1978). These results compare favourably with the values obtained in this study. With a constant flow preparation including an arterial long circuit, decreasing PO_2 from 109 to 38 mmHg caused resistance to drop to 74% of control.

In contrast, when hemorrhage was used as a model to decrease oxygen delivery to 16% of control, resistance did not change, remaining at 95% of control (Nelson et al., 1987). Since hemorrhage has been demonstrated to cause intestinal vasoconstriction through baroreceptor unloading, this reinforces the inappropriateness of hemorrhage as a model of hypoxia.

In the control state both adenosine and isoproterenol were capable of inducing significantly larger vasodilations than hypoxia, indicating that the

preparation was not maximally vasodilated. By comparing these vasodilations, it is also apparent that severe hypoxia is not a strong stimulus of vasodilation of the intestine. During adenosine receptor blockade, hypoxia may cause near maximal vasodilation since the resulting resistance was close to that seen with isoproterenol infusion. However, isoproterenol was still capable of causing greater vasodilation. This was evident when the resistances were compared by percent changes to examine the amount of vasodilation relative to the starting baseline.

By plotting all the points on the hypoxia curve it can be seen that the profile of the curve is also not changed by adenosine blockade. These graphs also revealed the nature of the relationship between hypoxia and resistance. In the individual experiments 3 of the control slopes and 5 of the adenosine-blocked slopes showed statistical significance in their slopes. In addition, these 8 slopes plus one more adenosine-blocked slope also showed a strong relationship between the two variables as demonstrated by their coefficients of determination ($R^2>.74$). In other words, the manipulation accounted for a high proportion of the variability. However, when the data were pooled, while the statistical significance remained, the strength of the relationship between the two variables was markedly reduced. Here the pooling increased the overall

variability to eradicate the relationship seen in the individual animals. Of interest however, is that the relationship remained slightly stronger in the 8-PT animals.

From this study it is evident that adenosine is not involved in hypoxic vasodilation of the feline intestine. If adenosine was mediating the dilation it would be expected that adenosine receptor blockade would decrease the amount of vasodilation seen with hypoxia. However, this was not observed, and on the contrary adenosine receptor blockade tended to increase, although not significantly, the amount of vasodilation in response to severe hypoxia. It does not appear that the involvement of adenosine in intestinal hypoxia has been studied in other species. None-the-less these results are somewhat surprising since adenosine mediates hypoxic vasodilation in the brain, kidney, skeletal muscle, and perhaps the heart (Winn et al., 1981; Osswald, 1988; Ballard et al., 1987; Gidday et al., 1988).

In summary, these studies have demonstrated that the effects of exogenous adenosine are not an indication of its endogenous functions. Exogenous adenosine is capable of modulating intestinal vasoconstriction but it is clear that endogenous adenosine does not (Lautt et al., 1988a). Endogenous does mediate both the postprandial (Proctor, 1986) and the postocclusive hyperemias (Lautt, 1986b) in

the intestine. However, from these studies, it is also evident that endogenous adenosine does not mediate hypoxia induced intestinal vasodilation. Perhaps this reflects the priorities of the intestine - the needs of digestion, absorption and iminent damage must first be are attended. Alternatively, other endogenous vasodilators may modulate intestinal vasoconstriction or mediate hypoxic vasodilation.

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