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ADENOSINE PLAYS A ROLE IN POSTPRANDIAL INTESTINAL HYPEREMIA

By

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ABSTRACT

ADENOSINE PLAYS A ROLE IN POSTPRANDIAL INTESTINAL HYPEREMIA

By

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Blood flow to the small intestine increases following a meal, and the stimulus for this hyperemia is the products of nutrient digestion. The purpose of this study was to determine the role of adenosine in this hyperemia. The intestine was tested against criteria which were originally proposed by Berne for determining the role of adenosine in metabolic hyperemia in the heart. The criteria tested and results are summarized as follows.

1) Adenosine should be a potent vasodilator in the intestinal mucosa, i.e. the primary region of the food-induced hyperemia. Utilizing the microsphere technique, intra-arterial infusion of adenosine was found to increase mucosal and muscularis blood flows in the jejunum and ileum of anesthetized dogs and cats. In addition, selective application of adenosine and non-metabolizable analogues of adenosine (5'-N-ethylcarboxamide adenosine and N^6-cyclohexyladenosine) to the mucosal surface increased total jejunal blood flow in a dose-dependent manner. The hyperemias induced by the mucosal applications were not mediated by mucosal nerves, since they were unaltered by a local anesthetic. Therefore, adenosine is a vasodilator in the intestinal mucosa.

2) Adenosine concentration in the interstitial fluid of the jejunum (ISF [ADO]) must increase during the food-induced hyperemia. Placement of predigested food into the canine jejunal lumen increased jejunal blood flow, oxygen consumption and jejunal venous and lymphatic adenosine concentration and release, i.e. indices of ISF [ADO]. The estimated increase in ISF [ADO] appears to be sufficient to play a role in the food-induced hyperemia. The increases in jejunal adenosine concentration and release were not mediated by a decrease in oxygen

supply-to-demand ratio, since this variable did not change during the food-induced hyperemia. Placement of normal saline into the lumen did not alter blood flow, oxygen consumption nor adenosine concentration and release, indicating that the food-induced changes were due to the constituents of food.

3) Agents which potentiate or attenuate the action of administered adenosine should elicit a similar effect on the food-induced hyperemia. The food-induced hyperemia was attenuated by aminophylline and 8-phenyltheophylline, i.e. adenosine receptor antagonists, and by adenosine deaminase. The hyperemia was enhanced by dipyridamole, an inhibitor of cellular adenosine reuptake. The effect of aminophylline and dipyridamole on the hyperemia was complicated by counteracting effects of these compounds on motility and oxygen consumption. Aminophylline blocked the hyperemia when motility was low. However, when food increased motility, aminophylline enhanced the food-induced motility and had no effect on the hyperemia. Dipyridamole enhanced the hyperemia despite the fact that it attenuated the food-induced increase in oxygen consumption. Adenosine deaminase also attenuated the food-induced increase in oxygen consumption.

In conclusion, the present study indicates that adenosine indeed mediates the food-induced hyperemia. Adenosine also appears to regulate intestinal oxygen uptake or oxidative metabolism.

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INTRODUCTION

Several studies show that intestinal blood flow increases after a meal (39,80). Although the stimulus for this hyperemia has been shown to be the products of food digestion (39,40,80), the mechanisms of action has not yet been determined. Evidence to date indicate that the response is complex, with many factors such as nerves, gastrointestinal hormones and polypeptides, histamine, prostaglandins, villus hyperosmolarity, and oxidative metabolism playing a role (39,80).

Adenosine may be a mediator of metabolic hyperemia in a variety of tissues including heart, skeletal muscle, kidney, brain, liver and adipose tissue (11,12,147). In addition, recent studies suggest that adenosine may also mediate intestinal pressure-flow autoregulation (144), autoregulatory escape from norepinephrine (46) and reactive hyperemia (90,145). The present study determined the role of adenosine in the postprandial intestinal hyperemia by testing the intestine against criteria originally proposed by Berne (11) for determining the role of adenosine in coronary blood flow regulation. This study determined whether or not 1) adenosine is a vasodilator in the jejunal mucosa, i.e. the primary region of the food-induced hyperemia (38,82); 2) jejunal interstitial fluid adenosine concentration increases during the food-induced hyperemia; and 3) adenosine receptor antagonists, i.e. aminophylline and 8-phenyltheophylline, attenuate the hyperemia and dipyridamole, an inhibitor of cellular adenosine reuptake, enhances the hyperemia.

LITERATURE REVIEW

General Cardiovascular Response After a Meal

The circulatory events during a meal were first determined in the 1930s utilizing the thermostromuhr and ballistocardiogram methods and it was shown that feeding increases cardiac output (87,95) and blood flow through the femoral, carotid, coronary, renal and mesenteric arteries (60,113). Subsequent studies made before 1965 supported these results (1,48,95,188,203). Upon further refinement of the techniques for measuring blood flow, however, particularly after the development of electromagnetic and ultrasonic flowmetry, it was shown that the cardiovascular system responds to feeding in two distinctly different phases. During anticipation and ingestion of food, there is an increase in cardiac output, heart rate and aortic pressure (75,76,243-245), coronary vascular resistance decreases (244,245), renal resistance increases (75,245), limb resistance increases (75,76) or decreases (76,245) and mesenteric resistance increases (242-244) or does not change (75,76). These changes can be attenuated by adrenergic blocking agents (243) and appear therefore to result from activation of the sympathetic nervous system.

Within 5 - 30 min following a meal, cardiac output, heart rate, aortic pressure, and blood flows to the heart and kidney return to control levels, while blood flow through the superior mesenteric artery and pancreas start to rise and reach a maximum in 30-90 min (29,75,76, 82,243-245). At rest, limb blood flow is decreased, but the decrease is abolished or reversed if the animal stands or changes position (82,244, 245). These cardiovascular changes have been observed in man (21,183)

(dye dilution technique), conscious primates (242,245), dogs (20,29,74-76,82,113,114,128,233,243,244), cats (63,64), sheep (58,129) and rats (112,203).

The increase in blood flow to the digestive organs after feeding is not uniform and simultaneous, but appears to be localized to the area where tissue activity is greatest. In anesthetized dogs, intragastric placement of food promptly increases celiac blood flow which remains elevated for only 30-60 min (38). Superior mesenteric flow does not increase until 30 min following the intragastric food placement, and the increased flow is maintained for at least 3 hours. In conscious dogs, blood flows to the stomach, duodenum, jejunum and pancreas increase 30-90 min following a meal, flow to the terminal ileum does not increase until 45-90 min and colon blood flow does not change, as determined by the radioactive microsphere technique (20,82). Utilizing electromagnetic flowmetry in conscious dogs, however, Takagi et al. (233) and Kato et al. (128) recently show that there is only a quick and transient increase in celiac and left gastric blood flows after a meal of meat or milk. Superior mesenteric blood flow does not increase until 20 min after the meal and the increase is gradual and sustained.

Blood flow to the different layers of the gut wall are also not uniform during digestion. In anesthetized dogs, placement of glucose or alanine into the intestinal lumen increases mucosal blood flow without a significant change in blood flow through the muscular layer (38,82,190, 219,254). In conscious dogs, feeding increases total gastric and small intestinal blood flows, and these increases are confined only to the mucosal layer (82).

In summary, the above findings demonstrate that the circulatory response to digestion is confined to the digestive organs. Blood flow to the gastric mucosa increases during the first hour of feeding when gastric secretion is stimulated. Blood flow to the small intestine increases only in the segment containing chyme and the increase in blood

flow is primarily localized to the mucosal layer.

Constituents of Chyme Responsible for Postprandial Intestinal Hyperemia

Chou et al. (40) conducted the first systematic study to identify the constituents of chyme which are responsible for the postprandial intestinal hyperemia. They found that food digested in vitro with pancreatic enzymes increases jejunal blood flow, but undigested food or pancreatic enzymes per se does not. Furthermore, the supernatant of digested food produces hyperemia, while the precipitate is without effect. Gallbladder bile significantly enhances the hyperemic effect of the supernatant, even though bile alone has no effect on jejunal blood flow. It was therefore concluded that the water-soluble end products of food digestion are responsible for the food-induced increase in intestinal blood flow and that bile enhances their vasoactive effect by solubilizing the lipid nutrients.

In order to further identify the soluble constituents of food responsible for the postprandial hyperemia, Chou et al (40) and Kvietys et al (139) examined the vasoactive properties of glucose, amino acids, various dipeptides, fatty acids and various lipids. They found that jejunal blood flow is increased by a mixture of glucose, 16 amino acids and micellar lipids (oleic acid, monoolein, and taurocholate), all at physiological concentrations. When tested alone, both glucose and the micellar lipids increase blood flow, but the 16 amino acids, either in combination or individually, do not. However, when the concentration of these amino acids is increased 10 fold (252 mM), they produce a significant increase in flow. Further studies showed that the hyperemic effect of these amino acids is entirely due to that of glycine and aspartate.

Chou et al. (40) and Kvietys et al. (139) investigated the role of bile in postprandial intestinal hyperemia. They found that fatty acids,

lipids and amino acids require the presence of bile or taurocholate, a bile salt, to produce hyperemia. Glucose alone produces a slight increase in jejunal blood flow and bile enhances the hyperemia. Neither taurocholate nor bile alone alters jejunal blood flow. However, bile increases blood flow to the ileum, the site of active bile salt absorption (40,142).

The means by which bile exerts its effect on the vasoactivity of these nutrients are poorly understood. In the case of long-chain fatty acids, this action of bile is related to its role in micelle formation; addition of bile to a 20 mM oleic acid solution significantly increases lipid absorption and jejunal blood flow (42). The effects of bile on water-soluble nutrients, i.e. glucose, amino acids, and short-chain fatty acids, however, are unexplained. Sit et al (223,224) have shown that the enhancement by bile of the glucose-induced jejunal hyperemia is not accompanied by an increase in either the amount of glucose absorbed or the amount of oxygen consumed. Therefore, the enhancement is not mediated by enhancement of glucose transport or oxidative metabolism.

Mechanisms of Postprandial Intestinal Hyperemia

The effect of bile and various nutrients on intestinal blood flow is mediated by a variety of regulatory pathways; i.e., the postprandial hyperemia is multi-factorial. Recent studies have suggested that the constituents of chyme may act directly on the intestinal vasculature or indirectly by means of osmotic, neural, hormonal, paracrine or metabolic regulatory mechanisms.

The constituents of chyme which have a direct vasoactive effect on vascular smooth muscle include bile salts (42,140) and oleic and caproic acids (42). Intra-arterial infusion of taurocholate produces a dose-dependent decrease in jejunal (42) and ileal (140) vascular resistance. This vasodilation is not accompanied by an increase in $\dot{V}O_2$,

indicating that the response is independent of oxidative metabolism (140). Oleic and caproic acids increase jejunal blood flow, and the increase is equally shared by mucosal and muscularis layers (42).

Although ions such as Ca^{++}, K^{+} and Cl^{-} have no direct vasoactive effect in the intestine (39), changes in tissue electrolyte concentration might indirectly alter intestinal blood flow by changing tissue osmolality. Levine et al (151) showed that local i.a. infusions of hypertonic glucose and NaCl solutions decrease jejunal vascular resistance. They showed that the intestinal vasculature is more sensitive to changes in plasma osmolality than other organs such as heart, skeletal muscle, kidney and brain. In addition, active transport of electrolytes and nutrients is accompanied by an increase in interstitial fluid osmolarity (102,124). Direct measurements of Na^{+} concentrations in the rat villus and submucosa show that there is a close temporal relationship between the increases in Na^{+} concentrations and blood vessel diameter when the mucosa is suffused with isotonic glucose solutions (19).

Placement of hypertonic solutions of glucose or mannitol in the gut lumen produces a greater hyperemia than that induced by the corresponding isotonic solution (35,190). The hypertonic solution also increases venous osmolality. However, the vasoactive effect of the hypertonic solutions appear to be due to a higher concentration of the hexose rather than to hyperosmolality per se. Intra-luminal placement of solutions of non-absorbable polyethylene glycol with osmolalities up to 1000 mOsm also increase venous osmolality but have little or no effect on blood flow (35,141). Hypotonic food solutions produce the same hyperemia as isotonic solutions, when the concentration of food in these solutions is the same (141). Hypertonic solutions of glucose, prepared by adding glucose to isotonic Ringer-bicarbonate buffer, produces the same hyperemia as isotonic solutions with the same glucose concentration, prepared by diluting isotonic 5% glucose with Ringer-

bicarbonate buffer (198). Therefore, changes in luminal osmolality per se appear to have little effect on intestinal blood flow.

The role of nerves in food-induced intestinal hyperemia is controversial. Surgical denervation of the intestine as well as administration of methylsergide, hexamethonium, and tetrodotoxin do not alter the increase in jejunal blood flow and oxygen consumption produced by luminal placement of an isotonic glucose or oleic acid solution (184). Sympathoadrenergic blockade also has no effect on the postprandial hyperemia (39,64,233). Nyhof et al (185) and Kvietys et al (142) show that atropine enhances the intestinal vascular response to food and oleic acid. These studies indicate therefore that the food-induced jejunal hyperemia is not mediated by a direct vasodilator action of local intestinal nerves. However, other studies show that nerves may play a role in the food-induced hyperemia. Biber et al (15,16) show that the intestinal hyperemia induced by mechanical stimulation of the mucosa, as produced by the movement of chyme, is blocked by tetrodotoxin or a serotonin antagonist. The hyperemia induced by hypertonic glucose (35), isotonic glucose or isotonic oleic acid (184) are abolished after exposing the mucosal surface to a local anesthetic, dibucaine hydrochloride. In addition, Gallavan et al (78) found a close temporal relationship between the jejunal release of vasoactive intestinal peptide (VIP), a potent intestinal vasodilator, and bile-oleate-induced jejunal hyperemia. This increase in VIP release was not seen when either bile or oleic acid was placed in the lumen alone, treatments which also failed to produce a hyperemia. Rozsa and Jacobson (205) showed that VIP antiserum inhibit the bile-oleate-induced hyperemia in the rat jejunum. The hyperemia was also abolished by capsaicin, indicating that the hyperemia might involve afferent C-fibers. As VIP is found only in the neural tissue of the small intestine (143), a portion of the bile-oleate-induced jejunal hyperemia may be mediated by a neural pathway involving VIP-ergic neurons.

The possible role of gastrointestinal hormones in the postprandial intestinal hyperemia was first suggested in 1969 by Burns and Schenk (29). They found that intravenous infusions of secretin and gastrin increase superior mesenteric blood flow. Since then, systemic- and intra-arterial infusions of secretin, cholecystokinin (CCK), glucagon, VIP, enkephalins, neurotensin, substance P and gastric inhibitory peptide (GIP) have been shown to increase small intestinal blood flow in anesthetized cat, dog, and pig (41). The minimal plasma concentrations of CCK required to increase duodenal and jejunal blood flows and of neurotensin required to increase ileal blood flow are similar to those measured after a meal. Therefore, CCK and neurotensin may mediate the food-induced hyperemia. All of the other hormones only produce vasodilation at concentrations much higher than that measured postprandially. Recent more direct studies have demonstrated, however, that the food-induced changes in intestinal CCK, glucagon and neurotensin release are transient and on the order of 1 ng/min/100g. The concentration of these peptides in the venous blood were less than that required to dilate the intestinal vasculature (78,83,197). Furthermore, Rozsa and Jacobson (205) showed that CCK and substance P antisera have no effect on the bile-oleate-induced hyperemia in the rat jejunum.

Histamine is a locally produced substance which may play a role in the jejunal vascular and metabolic response to food. Chou and Siregar (43) have found that H1 - receptor blockade with tripelennamine abolishes the food-induced increase in jejunal oxygen consumption and significantly reduces the corresponding hyperemia. The H2 receptor antagonist metiamide has no effect on the food-induced increase in blood flow and oxygen consumption. Histamine is known to dilate the intestinal vasculature (65,101,171,191), increase capillary permeability (252) and has been implicated in the regulation of alkaline secretion (73) and motor activity (136). It is therefore possible that

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histamine mediates intestinal vasodilation by acting directly on the H1 receptors of the intestinal vasculature, or indirectly by stimulating intestinal metabolism.

Prostaglandins are other locally produced substances which may play a role in the food-induced intestinal hyperemia. Inhibition of prostaglandin synthesis by mefenamic acid or indomethacin enhances the food-induced hyperemia and increase in oxygen consumption (79). Conversely, arachidonic acid, a substrate for prostaglandin synthesis, inhibits the food-induced increases in jejunal blood flow and oxygen consumption (164). Thus, endogenous prostanoids might act to limit the food-induced hyperemia and oxygen consumption. The food-induced hyperemia is accompanied by increases in intestinal venous concentration and release of vasodilator, i.e. PGE_2 and PGI_2, and vasoconstrictor prostanoids, i.e. TXA_2 and PGF_2 (34). Intra-arterial infusions of the four prostanoids at doses which increase their blood concentrations by 0.5 - 20 times that which occurs during the food-induced hyperemia did not significantly alter jejunal vascular resistance. Therefore, the food-induced increases in prostanoid releases do not appear to act directly on the vasculature to alter blood flow. However, the inhibitory effect of prostaglandins on the food-induced hyperemia might be due to inhibition of food-induced oxidative metabolism. Prostaglandins inhibit intestinal absorption of glucose (6,45), and inhibition of prostaglandin synthesis increases the rate of glucose absorption and metabolism when food is in the jejunal lumen (81). In addition, inhibition of prostaglandin synthesis increases intestinal motility (79) and the enhancement of food-induced hyperemia could therefore be due to increased mixing of chyme or stimulation of intestinal oxidative metabolism.

Role of Oxidative Metabolism in Intestinal Blood Flow Regulation

In the metabolic model of local circulatory control, as originally proposed by Granger and Shepherd (91,92,216), tissue oxygenation is the regulated variable in autoregulating organs. This model is based on the premise that in response to inadequate oxygenation, parenchymal cells produce a metabolic feedback signal that, in turn, regulates both resistance vessels (i.e. arterioles) and precapillary sphincters. Therefore, two vascular mechanisms may be utilized to regulate tissue oxygenation: (1) vasodilation of arterioles to increase blood flow and convective flux of oxygen into capillary beds, and (2) relaxation of precapillary sphincters which facilitates oxygen diffusion by increasing capillary surface area and decreasing capillary-to-cell diffusion distance. These responses serve to maintain tissue PO_2 at a level which does not limit the rate of aerobic metabolism. This model of autoregulation has been supported by studies on the intestinal circulatory responses to arterial hypoxia, imposed changes in arterial perfusion pressure (i.e. pressure-flow autoregulation), brief occlusion of the intestinal artery (i.e. reactive hyperemia) and increases in intestinal metabolic activity (e.g. nutrient absorption). The results from these studies are summarized as follows.

In two studies (213,232), arterial hypoxia has been shown to increase intestinal blood flow and capillary density. Capillary density was estimated from measurements of capillary filtration coefficient (K_{fc}) and rubidium-86 extraction (PS product). When arterial PO_2 was reduced to 46 mm Hg, intestinal blood flow and capillary density increased to maintain intestinal oxygenation to within 26% of control (213). When intestinal loops were perfused at a constant blood flow, the same level of hypoxia increased capillary density, maintaining oxygenation to within 48% of control.

The ability of the intestine to maintain a relatively constant

blood flow despite imposed changes in perfusion pressure, i.e. pressure-flow autoregulation, is not the intense phenomenon seen in other organs such as the kidneys. However, oxygen delivery to the intestine is well regulated down to a perfusion pressure of 30 mm Hg (89,182). The degree of blood flow autoregulation is greater under hypermetabolic conditions, as during feeding, than under fasting conditions (89,182,215,217). Furthermore, blood flow regulation is more complete in the mucosa (where metabolism is highest) than in the muscularis (156). These results appear to support a metabolic mechanism in intestinal blood flow autoregulation.

The characteristic overshoot in blood flow that follows arterial occlusion, i.e. reactive hyperemia, is well characterized in the intestine (135,172). The metabolic model predicts that vasodilation and capillary recruitment in response to arterial occlusion should cause an overshoot in tissue PO_2 upon release of the occlusion. Microelectrode studies confirm the overshoot in PO_2 (135). The observation that both the magnitude and the duration of reactive hyperemia are related to the duration of the arterial occlusion further supports a metabolic mechanism in the hyperemia (172). In addition, reactive hyperemia is present in the mucosa, where metabolism is greatest, but is absent in the muscularis (218).

Intestinal metabolic activity may be increased by stimulation of mucosal or muscularis tissue activity. The primary activity of the muscularis tissue is motility. The effect of motility on intestinal blood flow has been extensively reviewed (33,66), and the increased motility produced after a meal may contribute to the observed hyperemia and increased oxygen consumption. Mucosal activity is increased during active absorption and intracellular metabolism of nutrients. It is well established that intestinal oxygen consumption increases when nutrients are in the lumen (21,22,23,43,79,89,139,141,164,190,214,224,237,241). When digested food and bile are placed in the canine jejunum, there is a

significant correlation between the increases in oxygen consumption and blood flow (79,164). However, the slope of this relationship may depend on the initial level of oxygen extraction (89). For the same increase in oxygen consumption, the corresponding increase in blood flow is greater when the initial arteriovenous oxygen difference is more than 6 ml/dl blood than when it is less than 5 ml/dl blood. This indicates that the contribution of oxidative metabolism to the development of food-induced hyperemia depends in part on the initial level of arteriovenous oxygen content difference (i.e. tissue oxygen extraction); the greater the initial level, the greater the hyperemia.

While the increase in blood flow following ingestion of a mixed meal may be a complex phenomenon involving neural, humoral and metabolic factors, intraluminal placement of glucose produces an uncomplicated metabolically mediated hyperemia. This hyperemia has been documented in a variety of studies and is accompanied by a relatively large increase in oxygen consumption compared with the corresponding increase in blood flow, an increase in oxygen extraction and an increase in capillary permeability-surface area product (PS) (42,190,214,223,241). If the gut loop is perfused at a constant blood flow, the glucose placement increases oxygen extraction and PS product (^{86}Rb extraction technique) (214). Sit et al (224) demonstrated a close relationship between glucose metabolism and the accompanying hyperemia. Placement of glucose and bile in the jejunal lumen increases blood flow, oxygen consumption, and glucose absorption. When 3-O-methylglucose and bile are placed in the lumen, the rate of glucose absorption is the same as that produced by intraluminal glucose and bile placement. However, 3-O-methylglucose and bile produce a lesser increase in blood flow and there is no increase in oxygen consumption. As glucose is actively transported and metabolized by the epithelial cells of the mucosa while 3-O-methylglucose is transported but not metabolized, this study indicated that tissue metabolism is responsible for approximately

two-thirds of the glucose-induced hyperemia.

A number of vasodilator metabolites have been proposed to mediate the intestinal hyperemia in response to food. Hypercapnia is a potent vasodilator in the intestine (221), but PCO_2 must be changed beyond the physiological range to cause vasodilation sufficient to account for the food-induced hyperemia. Hypoxia also increases blood flow, and could serve as a feedback signal mediating the food-induced hyperemia. Bohlen (17,18) demonstrated that glucose absorption decreases PO_2 in the intestinal mucosa, and the decrease is sufficient to relax vascular smooth muscle in vivo. The time course of glucose absorption and the fall in PO_2 in intestinal villi are consistent with a causal relationship.

Role of Adenosine in Intestinal Blood Flow Regulation

Adenosine has been shown to play a role in the metabolic regulation of blood flow in many tissues including heart, skeletal muscle, brain, kidney, liver and adipose tissue (11,12,144). For these tissues, adenosine satisfies several of the eight criteria originally proposed by Berne for establishing adenosine as a mediator of metabolic vasodilation (11). In addition, preliminary evidence appear to suggest that adenosine might mediate metabolic regulation of blood flow in the intestine. The eight criteria proposed by Berne, and the studies which tested these criteria for the intestine, are discussed as follows.

1. Adenosine must be a potent vasodilator (Criterion #1).

Intestinal vasodilatory effects of adenosine have been known for some time. In 1932, Marcou (166) suggested that systemic hypotension after i.v. injection of adenosine was due to intestinal vasodilation. Subsequent studies show that i.a. injections (37 nM) or infusions (37

nmol/min) of adenosine decreases vascular resistance in the pump-perfused small intestine of the dog (36,105,161). In the naturally perfused small intestine, the minimal increase in arterial adenosine concentration required to produce vasodilation was shown to be 370 nM in the feline ileum (93), 0.1 μg/min/kg total body weight (or 70 nM) in the canine ileum (247), and 10 nM in the canine jejuno-ileum (90). The minimal arterial adenosine concentration producing a "maximal increase" in blood flow was 37 μM in the feline ileum (93), 10 μg/min/kg total body weight (or 3 μM) in the canine ileum (247), and 1 μM in the canine jejuno-ileum (90). These latter doses of adenosine increase total intestinal blood flow by 56-150%. In the pump-perfused feline ileum, 37 μM arterial adenosine decreased vascular resistance by -47% (93). Despite the variability in these results, it appears that adenosine produces threshold vasodilation at nanomolar concentrations and maximal vasodilation at micromolar concentrations. When suffused over the serosal surface of rat jejunal flaps, adenosine dilates submucosal arterioles at a threshold concentration of 10^{-5} M and produces maximal vasodilation at 10^{-2} M (199). However, 10^{-5} to 10^{-2} adenosine has no vasoactive effect on submucosal arterioles when suffused over the mucosal surface. This suggests that there is a mucosal barrier which limits the passage of adenosine from the luminal to submucosal spaces.

In addition to increasing total intestinal blood flow, adenosine has been shown to have different vasoactive effects in the major tissue layers of the gut wall. Utilizing the radioactive microsphere technique, Walus et al. (247) showed that i.a. adenosine (1 μg/min/kg total body weight or 460 nM arterial concentration) increases mucosal-submucosal blood flow by 145% of control in the canine ileum. The non-metabolizable adenosine analogue, 2-chloroadenosine (172 nM arterial concentration), increased mucosal-submucosal blood flow by 250% of control. Since this non-metabolizable adenosine analogue was a more potent mucosal vasodilator than adenosine, it appears that metabolism of

adenosine by the intestinal mucosal limits adenosine's vasoactivity. When mucosal blood flow was measured by laser Doppler flowmetry, i.a. adenosine (47 µg/ml or 174 µM arterial concentration) was shown to decrease mucosal blood flow by -33% and increase muscularis blood flow by +49% in the canine ileum (220). The difference in results between these two studies appears to be due to the difference in technique for measuring mucosal blood flow. Despite their finding that adenosine decreases ileal mucosal blood flow, the authors of the latter study (220) suggested that adenosine produces mucosal vasodilation but decreases the mucosal blood flow by a "vascular steal" phenomenon. Using the radioactive microsphere technique in the feline ileum, however, Granger et al. (93) showed that an arterial adenosine concentration of 37 µM decreases mucosal-submucosal blood flow by -45% of control and increases muscularis blood flow by +203% of control. Therefore, the effect of adenosine on mucosal blood flow may differ among species.

A. Mechanism of Adenosine Induced Vasodilation

Adenosine-induced intestinal vasodilation is attenuated by adenosine receptor antagonists such as theophylline and aminophylline (90,93,199,247). This indicates that the vasodilation is receptor mediated. There are 2 major classes of extracellular adenosine receptors which may mediate adenosine-induced biological effects, i.e. A1 and A2 (49,154,239). Both of these receptor types are linked to adenylate cyclase. The A1 receptor has a relatively high affinity for adenosine (i.e. nanomolar range) and inhibits adenylate cyclase activity. A2 receptors have a generally lower affinity (i.e. micromolar range) and stimulates adenylate cyclase. Both receptor subtypes are also defined by their relative affinities for adenosine (ADO) and the agonists N^6cyclohexyladenosine (CHA), R-N^6phenylisopropyl adenosine (R-

PIA), S-N^6phenylisopropyl adenosine (S-PIA), 2-chloroadenosine (2CA) and 5'-N-ethylcarboxamide adenosine (NECA). A1 receptors are stimulated in various biological tissues with an affinity order of R-PIA = CHA > ADO = 2CA > NECA > S-PIA. The affinity order for A2 receptors is NECA > ADO = 2CA > R-PIA = CHA > S-PIA. A so-called purine site (P-site) for adenosine is also localized in the internal site of cell membranes mediating inhibition of adenylate cyclase.

The vasoactivity of some adenosine analogues has been tested in the small intestine. Intra-arterial infusion of 2-chloroadenosine, a non-metabolizable adenosine analogue, was found to be six-fold more potent than adenosine for increasing total blood flow in the canine ileum (247). As indicated above, this suggests that adenosine metabolism by the intestinal tissues limits adenosine's vasoactivity. 9-beta-D-arabinofuranosyl adenine, an adenosine analogue with substitutions on the ribose ring, has no effect on intestinal blood flow. Recently, Proctor (200) showed that 10^{-6}M 5'-N-ethylcarboxamide adenosine (NECA) and 2CA increases blood flow when suffused over the serosal surface of rat jejunal flaps. In this study, blood flow was calculated from submucosal arteriolar diameter and red blood cell velocity. The NECA-induced hyperemia was quantitatively greater than that induced by 2CA. Serosal N^6-cyclohexyladenosine (CHA) induced no change in intestinal blood flow at 10^{-6} M, but increased the blood flow at 10^{-4}M. These results indicate that serosal A2 receptors mediate adenosine-induced intestinal vasodilation. Mucosal suffusion with 10^{-6}M CHA decreased jejunal blood flow, while mucosal 10^{-6} or 10^{-4}M NECA, 10^{-4}M CHA or 10^{-6}M 2CA had no effect on blood flow. The lack of vasodilatory potency of mucosal adenosine or analogue is consistent with Proctor's earlier hypothesis for the existence of a mucosal diffusion barrier (199).

B. Miscellaneous Hemodynamic and Metabolic Effects of Adenosine in the Intestine

Granger et al. (93) showed that the maximal vasodilatory dose of adenosine (37 μM arterial concentration) does not alter lymph flow in the feline ileum, suggesting that adenosine has no effect on capillary fluid exchange. However, they showed that this dose of adenosine decreases capillary filtration coefficient, an index of effective capillary surface area and permeability, while increasing capillary hydrostatic pressure. These latter changes appear therefore to offset each other and maintain capillary fluid exchange. Adenosine was also shown to depress oxygen consumption. The investigators postulated that the decrease in capillary filtration coefficient was due to: (1) a precapillary myogenic vasoconstriction resulting from the increased capillary hydrostatic pressure, (2) a redistribution of blood flow toward the more impermeable and less numerous capillaries of the muscularis (This is supported by their finding that adenosine redistributes total intestinal blood flow from the mucosa to the muscularis tissue layers. See above.) or 3) a metabolic reduction in capillary surface area secondary to the depression of tissue oxidative metabolism. Shepherd et al. (220) also show that adenosine decreases capillary permeability-surface area (PS) product (determined by the rubidium extraction technique) in the canine ileum. The decrease in PS product was accompanied by decreases in mucosal blood flow and oxygen consumption, as shown by Granger et al (93).

Several studies show that adenosine and adenosine analogues alter intestinal function. Forrest et al. (67-70,132,187) investigated the effects of adenosine on chloride secretion in the elasmobranch rectal gland. This gland actively secretes Cl^- against an electrochemical gradient by mechanisms involving hormone-sensitive NaCl transport. At concentrations of 5 μM and above, adenosine produces a dose-dependent increase in Cl^- secretion, which is accompanied by parallel increases in

tissue cyclic AMP content and membrane-bound adenylate cyclase activity. Adenosine analogues stimulate Cl^- secretion with a rank order of potency of NECA > adenosine > phenylisopropyl adenosine (PIA), indicating that the secretory effect is mediated by A2 receptors. The stimulation of Cl^- secretion requires the integrity of the ribose moiety, since substitutions on the ribose ring (9beta-D-arabinofuranosyl adenine or 2'-deoxyadenosine) abolishes the stimulation of Cl^- secretion or tissue cAMP. The increased Cl^- secretion is inhibited by theophylline, consistent with mediation by external adenosine receptors. Single isolated perfused tubules of the gland consistently generate a lumen-negative transepithelial voltage which is enhanced by vasoactive intestinal peptide (VIP), cyclic AMP and adenosine (70). These effects are inhibited by furosemide, an inhibitor of coupled sodium-potassium-chloride co-transport, and theophylline.

The first evidence of adenosine-induced stimulation of Cl^- secretion in the mammalian epithelium was provided by Dobbins et al (55). They show that adenosine and some of its analogues stimulate electrogenic Cl^- secretion in rabbit ileum with a potency order of NECA > 2CA > PIA > adenosine. The potency of adenosine is enhanced by deoxycoformycin, an inhibitor of adenosine deaminase (49), and dipyridamole, an inhibitor of adenosine reuptake into cells (43a,189, 238). Thus, the secretory effect of adenosine is limited by its reuptake and metabolism. The 2CA-induced increase in Cl^- secretion is inhibited in a dose-dependent manner by 8-phenyltheophylline, a highly potent adenosine antagonist (225). The 2CA-induced increase in Cl^- secretion is paralleled by increases in tissue cAMP content. These results suggest that the stimulation of Cl^- secretion is mediated by external A2 receptors. Grasl and Turnheim (94) show that adenosine stimulates electrogenic Cl^- secretion in isolated epithelium of rabbit colon. The adenosine-induced Cl^- secretion is inhibited by furosemide and dependent on the presence of Na^+ on the serosal side of this tissue.

The site of adenosine's action appears to be the extracellular surface of the basolateral membrane, since (a) luminal adenosine is ineffective, (b) intracellular metabolites of adenosine (i.e. 5'-AMP, inosine, adenine) are ineffective and (c) the effect of serosal adenosine is enhanced by nitrobenzylmercaptopurineribose (NBMPR), an inhibitor of cellular adenosine reuptake (189,238). The Cl^- secretion is stimulated by adenosine and its analogues with a rank order of potency of NECA > adenosine > CHA, and the stimulation is accompanied by parallel increases in cAMP and cGMP. Thus, external A2 receptor appears to mediate the Cl^- secretion.

Functional inhibitory A1 adenosine receptors are also present in secretory epithelia (131,132,195,196). In the elasmobranch rectal gland, 2CA and PIA at <1 μM inhibit forskolin- and VIP-induced Cl^- secretion. In addition, theophylline stimulates basal Cl^- secretion, suggesting that endogenous adenosine inhibits basal Cl^- secretion. Adenosine deaminase enhances forskolin-induced Cl^- secretion, and this stimulation is blocked by 1 μM 2CA. In addition, 2CA at < 1 μM inhibits forskolin-induced cAMP accumulation in tissue slices of the rectal gland (195). The presence of both A1 and A2 receptors in this gland is supported by ligand-binding studies with ^{3}H-NECA in enriched basolateral membrane preparations (196). These studies show the presence of two binding sites with Kd's of 43 nM (A1) and 6.6 μM (A2). In addition, NECA inhibits adenylate cyclase activity at nM and activates the activity at μM concentrations (IC50 = 30 nM; EC50 = 6 μM).

Several studies show that adenosine modulates intestinal motility. In longitudinal muscle of guinea pig ileum, adenosine inhibits acetylcholine release and contractions induced by transmural nerve stimulation (99,106,246). This inhibitory effect of adenosine is attenuated by theophylline and other methylxanthines (97,98,106,170,208, 246) and enhanced by dipyridamole or dilazep (97,106,169,206). In addition, theophylline and adenosine deaminase enhance, and dipyridamole

and dilazep attenuate, contractile responses and acetylcholine release induced by transmural nerve stimulation (96,97). Adenosine concentrations in the bath media of these muscle preparations are also enhanced during the nerve stimulation. Thus, endogenous adenosine might limit acetylcholine release and contractions induced by nerve stimulation.

The inhibition of nerve-induced contractions by adenosine appears to be mediated by pre- and post-junctional mechanisms (96). As indicated above, adenosine limits and theophylline enhances the release of acetylcholine induced by transmural electrical stimulation. Theophylline does not enhance contractions induced by transmural stimulation in the presence of tetrodotoxin, which selectively blocks Na^+ channels in nerves. These results support a pre-junctional mechanism for the action of adenosine. NECA, R-PIA and 2CA equally inhibit contractions and acetylcholine release induced by transmural nerve stimulation (100). Thus the A1 receptor appears to mediate the adenosine-induced inhibition of cholinergic neurons. In the presence of tetrodotoxin and atropine, contractions can be induced by direct smooth muscle stimulation. These contractions can be inhibited by 1 μM NECA, but are unaltered by 1 μM L-PIA or by 1 - 100 μM adenosine. The selective cAMP phosphodiesterase inhibitor ZK 62.711, which inhibits cAMP degradation (211), enhances the potency of NECA and renders adenosine potent. Therefore, post-junctional A2 receptors coupled to adenylate cyclase activation might be a supplementary site for the inhibition of nerve-induced contractions.

2. <u>There must be an endogenous source of adenosine (Criterion #2).</u>

Adenosine production by the intestine has not yet been previously determined. However, several other biological tissues, such as cardiac, skeletal, brain, renal and adipose tissues, release adenosine and this

adenosine appears to originate from two major chemical substrates, adenosine monophosphate (AMP) and S-adenosylhomocysteine (SAH).

A. Adenosine Monophosphate

AMP may be formed by degradation of ATP, when this compound is utilized as an energy source. Indeed, adenosine is released from many cells and tissues during ATP breakdown (10,153,167,180,181,253). In addition, AMP can be formed by degradation of cAMP, an intracellular second messenger. The major enzyme responsible for production of adenosine from AMP is 5'-nucleotidase. Several different 5'-nucleotidase enzymes have been characterized. The first enzyme characterized was a membrane-bound ecto-5'-nucleotidase, which has been shown to be present in heart (4,72,174,175,231), skeletal muscle (4,32,72), brain (4,28), liver (4,72,104) and small intestine (27,28). A recent study also shows that ecto-5'-nucleotidase is highly concentrated in capillary endothelial cells of the heart (52). The rat heart enzyme is a glycoprotein homodimer (MW = 74 kdaltons) and the preferred substrate is AMP (174). A similar enzyme has been isolated from beef liver (104) and guinea pig skeletal muscle (32). ATP, ADP and AOPCP (α,β-methylene adenosine 5'-diphosphate) inhibit whereas Mg^{++} and other divalent cations activate the enzyme (27,28,32,174,175,231). The pH optimum for this enzyme ranges between 7.4 and 9 and its Km for AMP ranges between 4.0 and 19 μM (27,32,104,174,175). Recent studies show that this enzyme might not be involved in cellular adenosine release. AOPCP or antiserum specific to ecto-5'-nucleotidase has little effect on adenosine formation by neonatal rat heart cells (167), rat hepatocytes (10), rat polymorphonuclear leukocytes (180) and guinea pig hearts (210) made hypoxic (10,210) or treated with the glycolytic false substrate 2-deoxyglucose (167,180). In addition, ischemia induces rapid formation of adenosine in pigeon heart, which lacks ecto-5'-nucleotidase (168).

However, the following evidence appears to suggest that adenosine originates from the cytoplasm. Inhibitors of adenosine transport, such as dipyridamole, hexobendine and dilazep, trap adenosine in the cytoplasm and reduce adenosine release from neonatal rat heart cells (167), rat hepatocytes (10) and guinea pig heart (210). In addition, adenosine accumulates rapidly in the cytoplasm, but slowly in the medium, of 2-deoxyglucose treated rat leukocytes (180).

Several cytosolic 5'-nucleotidases have been characterized. The most fully characterized cytosolic enzyme is an allosteric protein with four subunits (MW = 200 - 260 kdaltons) (117,118,176). This enzyme has been studied in hearts of chicken, pigeon and rat (86,118,119,155) and in livers of chicken and rat (115-118,176,240). ATP, ADP and Mg^{++} are activators, and Pi is an inhibitor. It is specific for IMP (Km = 0.2 - 1.2 mM), but also hydrolyzes AMP (Km = 8 - 25 mM) and its pH optimum ranges between 6.3 and 7. In rat leukocytes, cardiomyocytes and liver, the activity of cytosolic 5'-nucleotidase has been related to cytosolic adenylate energy charge {([ATP] + 1/2[ADP])/ ([ATP]+[ADP]+[AMP])}, an index of the supply of high energy adenine nucleotides (116,119,253). Within the range of adenylate energy charge values normally observed in living tissues (0.7-0.9), the cytosolic 5'-nucleotidase activity increases sharply with decreasing energy charge. In isolated guinea pig hearts, norepinephrine induces a phasic increase in adenosine release, which is accompanied by a phasic decrease in ATP phosphorylation potential ([ATP]/[ADP][Pi]) (107). These results suggest that the nucleotidase activity is enhanced during ATP degradation. A decrease in adenylate energy charge within the physiological range is accompanied by a marked increase in AMP concentration and a much smaller decrease in the sum of the concentrations of ATP and ADP, i.e. the activators of this enzyme. Therefore, the increase in AMP concentration may be the primary determinant of the cytosolic nucleotidase activity. Indeed, nucleoside release from hearts is positively correlated with free

cytosolic [AMP] during norepinephrine or isoproterenol infusion or hypoxia (25,26,108).

An AMP specific cytosolic 5'-nucleotidase has been isolated from rat, rabbit and pigeon hearts (44,179,235). Similar to the IMP specific cytosolic 5'-nucleotidase, this enzyme is activated by ATP and ADP and inhibited by Pi. Its pH optimum is about 7, and the Km for AMP is 3.3 - 5.2 mM. The rabbit heart enzyme has been dissociated and separated into catalytic and regulatory subunits (44). In addition, a cytosolic 5'-nucleotidase which is inhibited by ATP and ADP has been isolated from rat kidney (150) and human placenta (13,159). Its pH optimum is 7 - 9, and its Km for AMP is 9.5 - 18 μM. Since intracellular AMP levels are in the low micromolar range (25,26,108), it appears that this latter enzyme may play an important role in intracellular adenosine formation.

B. S-Adenosylhomocysteine (SAH)

An alternative pathway for adenosine formation involves degradation of SAH as part of the transmethylation pathway. S-adenosylhomocysteine, formed from S-adenosylmethionine (SAM) after the transfer of the methyl group of SAM to a variety of methyl acceptors, is hydrolyzed to adenosine and homocysteine by SAH hydrolase (51,152,236). Although the equilibrium of the reaction catalyzed by SAH-hydrolase favors synthesis of SAH, physiologically the reaction proceeds in the direction of hydrolysis because adenosine and homocysteine are further metabolized (51,236).

In a recent study, Lloyd and Schrader (152) estimated the contribution of SAH hydrolysis to adenosine formation under normoxic and hypoxic conditions in the isolated perfused guinea pig heart. Rates of transmethylation and total adenosine production were determined and compared as follows. Under the normoxic condition, [^{3}H]adenosine and L-homocysteine were infused i.a. for 2 min in the presence of erythro-9(2-

hydroxy-3-nonyl)adenine (EHNA), which inhibits adenosine deaminase (111). This reverses the direction of the SAH hydrolase reaction toward synthesis, increasing tissue SAH concentration. In addition, this procedure selectively radiolabels the tissue SAH pool. During the postlabelling period, the SAH hydrolase reaction is in the direction of hydrolysis because in the absence of exogenous adenosine and homocysteine both of these compounds are rapidly metabolized and consequently SAH levels decrease. Simultaneously, endogenous synthesis of SAH from SAM via transmethylation reduces the specific radioactivity of SAH at a rate which is proportional to synthesis. From the $t_{1/2}$ of the specific radioactivity of SAH a transmethylation rate of approximately 750 $pmol \cdot min^{-1} \cdot g^{-1}$ was calculated. These hearts released adenosine with a mean value of 35 $pmol \cdot min^{-1} \cdot g^{-1}$. Therefore, the transmethylation rate under normoxic conditions is some 15 times greater than the adenosine release rate. In addition, it appears that over 90% of the adenosine formed from SAH is further metabolized intracellularly, i.e. phosphorylated and reincorporated into the cardiac nucleotide pool (see below). When adenosine kinase was inhibited, adenosine was produced at an overall rate of approximately 800 $pmol \cdot min^{-1} \cdot g^{-1}$, which is close to the calculated rate of transmethylation (750 $pmol \cdot min^{-1} \cdot g^{-1}$). Therefore SAH-derived adenosine can account for essentially all of the intracellularly formed adenosine during normoxia.

The transmethylation rate of guinea pig hearts was also determined under hypoxic conditions (152). ^{35}S-homocysteine was infused i.a. for 10 min, which enhanced the formation of ^{35}S-labelled SAH. However, the specific radioactivity of the ^{35}S-labelled SAH was less than that of the infused ^{35}S-homocysteine, due to the fact that SAH is also formed from endogenous SAM during transmethylation. From the specific radioactivity of the total tissue SAH relative to the infusion rate of ^{35}S-homocysteine, a transmethylation rate of 1200 $pmol \cdot min^{-1} \cdot g^{-1}$ was calculated. This is approximately 1.5 times more than the value

calculated under normoxia, yet adenosine release during hypoxia was 30-60 times greater. Therefore, the primary source of adenosine release during hypoxia cannot not be SAH.

C. Tissue Source of Adenosine

Although parenchymal cells are generally considered to be the major source of adenosine (11,12), several studies show that endothelial cells also release considerable amounts of adenosine. This has been shown in studies on cultured micro- and macrovascular endothelial cells, as well as in isolated organ and vessel preparations (9,53,56,85,110, 177,229). Microvascular coronary endothelial cells possess a cytosolic 5'-nucleotidase which is highly activated with falling pH (pH maximum of 5.0) (84). Endothelial cells are also equipped with a highly active cascade of ectonucleotidases at their luminal surface, catalyzing the extracellular dephosphorylation of ATP, ADP and AMP to adenosine (47,192-194)

Bardenheuer et al. (8) determined adenosine release from vascular endothelial cells of isolated perfused guinea pig hearts in response to catecholamines, acetylcholine and acidosis. Initially, ^{3}H-adenosine was infused intra-arterially in order to selectively radiolabel the adenine nucleotides of endothelial cells. Selective labelling of the endothelial adenine nucleotides was confirmed by measuring the relative specific activities of the nucleotides of endothelial cells, which were removed from the prelabeled hearts by intra-arterial infusion of collagenase and trypsin. Under resting conditions, the relative specific activity of released adenosine was similar to that of the endothelial adenine nucleotides. This indicates that under normoxic resting conditions, the released adenosine primarily originates from endothelial cells. Total adenosine release increased in response to isoproterenol and acetylcholine, and decreased in response to acidosis.

However, in response to all of these stimuli, the relative specific activity of the released adenosine decreased and approached that of the total heart. Therefore, under stimulated conditions, adenosine appears to be primarily released from the unlabelled adenine nucleotide pool of the cardiomyocytes. Utilizing the same technique, Deussen et al. (53) also showed that acidosis induces adenosine release from cardiomyocytes.

D. Adenosine Uptake and Metabolism

After adenosine is formed and released, it can be taken up and metabolized by many cell types. Adenosine uptake and metabolism might limit the increase in interstitial adenosine concentration during adenosine production. The adenosine uptake process common to most mammalian cells is mediated by a facilitated diffusion nucleoside transporter (122). This process is inhibited by a variety of compounds, such as dilazep, dipyridamole, diazepam, hexobendine, mioflazin and nitrobenzylthioinosine (NBMPR) (43a,122,189,238). Different tissues have different rates of adenosine transport. Coronary endothelial cells take up adenosine more rapidly than myocytes (204). Renal and intestinal epithelial cells possess an energy dependent, concentrative, Na^+-cotransport system (14,122,148,149,189,212). In the kidney, these active nucleoside transporters are localized on the brush border membrane (251). Compounds which inhibit the facilitated diffusion process do not inhibit the active transport process (122,189).

After adenosine is taken up by cells, it can be phosphorylated to AMP by adenosine kinase or deaminated to inosine by adenosine deaminase (4). Both of these enzymes are ubiquitous (4,52,84,177,210). The intestine contains a relatively greater level of adenosine deaminase than other tissues such as heart, skeletal muscle, liver, kidney and brain (4). The intestinal level of adenosine kinase, however, is similar to that found in these other tissues. Adenosine kinase has a

lower Km than adenosine deaminase, and therefore adenosine taken up into cells is preferentially phosphorylated. However, the higher Vmax of adenosine deaminase makes this enzyme a potentially important sink for adenosine, particularly when the intracellular adenosine concentration is high (162). The rate of adenosine metabolism appears to play a role in limiting adenosine uptake by myocytes. At low adenosine concentrations (below 32 μM), the adenosine transported into myocytes is rapidly phosphorylated to adenine nucleotides (204). This maintains intracellular adenosine concentration low and causes membrane transport to be rate-limiting for adenosine uptake. Little adenosine and deaminase products accumulate in the cells. At higher adenosine concentrations, however, adenosine kinase becomes saturated and adenosine phosphorylation becomes rate limiting for adenosine uptake. Additionally, more adenosine is deaminated to inosine and hypoxanthine.

Vascular endothelial cells possess a particularly high capacity to metabolize adenosine. Adenosine deaminase and xanthine dehydrogenase/ oxidase, which converts inosine to hypoxanthine, is highly localized in vascular endothelium (9,52,120,121). The dynamics of adenosine metabolism by vascular endothelial cells is similar to that by coronary myocytes. At low concentrations, adenosine is preferentially phosphorylated into adenine nucleotides (204). Only at high concentrations does intracellular deamination of adenosine become significant. The uptake and metabolism of adenosine is markedly different between endothelial cells of micro- and macrovascular origin. Cultured microvascular coronary endothelial cells possess a more rapid rate of adenosine uptake and metabolism than do aortic endothelial cells (84).

Adenosine release by the intestine has not been determined in vivo, and therefore the following four criteria have not been tested.

3. Adenosine should have access to the intestinal arterioles and be present under basal physiological conditions (Criterion #3).

4. The adenosine concentration reached in the interstitial fluid must be capable of eliciting vasodilation, and there must be a close relationship between the interstitial fluid adenosine concentration and intestinal blood flow (Criterion #4).

5. The time course of oxygen deficit (either decreased oxygen supply or increased oxygen demand) should parallel the increase in intestinal blood flow and interstitial fluid adenosine concentration (Criterion #5).

6. The physiological effect at different concentrations of endogenous adenosine should be mimicked by exogenous administration of adenosine (Criterion #6).

7. Agents that potentiate or attenuate the action of administered adenosine should elicit a similar effect on endogenously liberated adenosine (Criterion #7).

The effect of adenosine antagonists on the food-induced hyperemia has been studied twice (90,199), and the results were controversial. In one study by Granger and Norris (90), the adenosine antagonist theophylline did not alter resting blood flow or oxygen consumption in a jejunal-ileal preparation of anesthetized dogs, nor did it have a significant effect on the hyperemia produced by instillation of a digested dog food mixture without bile through the jejunal lumen. In

this study, intestinal blood flow was measured by a flow transducer placed on the superior mesenteric artery perfusing the jejunal-ileal preparation. In the other study by Proctor (199), theophylline and adenosine deaminase attenuated the hyperemia produced by mucosal suffusion of rat intestinal flaps with a solution containing oleic acid, glucose and bile. Blood flow was calculated from arteriolar diameter and red blood cell velocity measured from videomicroscopy of submucosal arterioles. The conflicting effects of theophylline in these two studies may be related to differences in species, intestinal preparation, methods of measuring blood flow, food solutions and routes of drug administration. Furthermore, these studies did not report measurements of other parameters, such as motility, which can influence blood flow (33,66).

8. A direct cause-and-effect relationship should be established under all physiological and pathophysiological conditions between changes in intestinal blood flow and adenosine release (Criterion #8).

Adenosine appears to play a role in intestinal reactive hyperemia (90,145), pressure-flow autoregulation (144) and autoregulatory escape from norepinephrine (46). These metabolically related hyperemias are attenuated by adenosine antagonists, i.e. 8-phenyltheophylline, aminophylline and theophylline, as well as adenosine deaminase.

METHODS

Mongrel dogs (15-25 kg) or cats (3.5-4.0 kg) of either sex were deprived of food for 24 hours and anesthetized with pentobarbital sodium (30 mg/kg; dogs, i.v.; cats, i.m.). All animals were ventilated with a positive-pressure respirator (Harvard Apparatus, Millis, MA) that was adjusted to achieve normal arterial blood pH, O_2 tension and CO_2 tension. Systemic arterial pressure was continuously monitored through a cannula in the femoral artery.

After making a midline abdominal incision, a segment of jejunum or ileum perfused by a single artery and vein was exteriorized from dogs (Figure 1), or an ileal segment perfused by several intestinal arteries and veins was exteriorized from cats. A rubber tube was placed into the lumen of the segment for placement and withdrawal of solutions, and both ends of the segment were tied and cut away from the adjacent intestine to exclude collateral flow. Luminal pressure was continuously recorded by connecting the rubber tube to a pressure transducer (Statham P23Gb) during the presence of a solution in the lumen. Motility index was calculated by dividing the sum of the heights of the pressure wave peaks by the number of pressure waves over a period of time usually as long as the time of a blood flow measurement.

Before cannulation of intestinal blood vessels, heparin sodium (500 U/kg) was administered intravenously. In dogs, the single vein draining the segment was cannulated for measurement of venous outflow by timed collection with a stopwatch and graduated cylinder. In some experiments, blood flow was also continuously monitored by an electromagnetic flow transducer (BL 2048-E04, Biotronex Laboratory, Silver Spring, MD) placed in the venous outflow line and connected to an

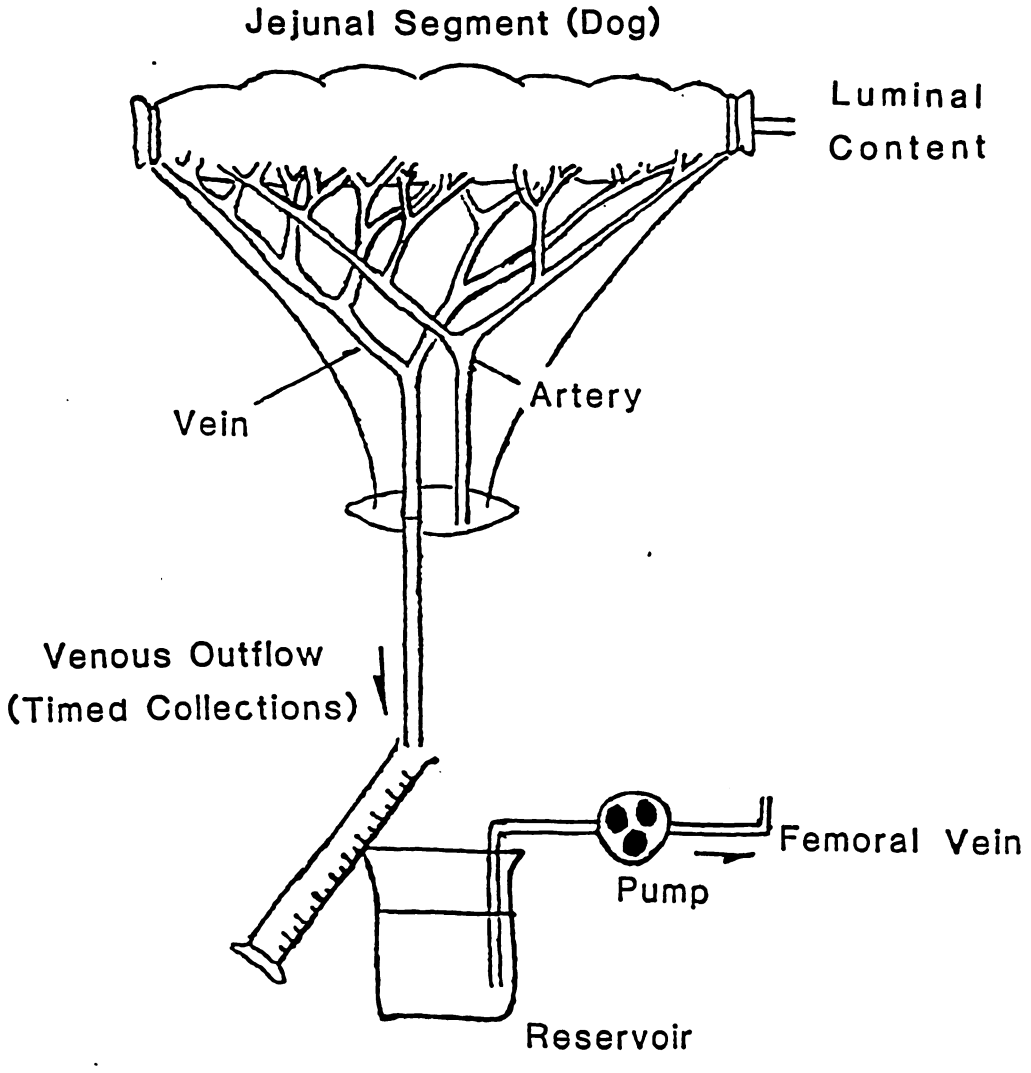

Figure 1

Intestinal preparation

electromagnetic flow meter (BL 610, Biotronex Laboratory). In cats, the duodenum, jejunum, pancreas and proximal large intestine were removed in order to be sure that the superior mesenteric artery and vein only perfused the ileal segment. The superior mesenteric vein was then cannulated for measurement of the ileal venous outflow. Arteriovenous oxygen content difference [$(A-V)O_2$] was determined continuously in some experiments by perfusing femoral arterial blood and a portion of the venous outflow at 6 $ml \cdot min^{-1}$ through separate cuvettes of an arteriovenous oxygen content difference analyzer (A-VOX Systems, San Antonio, TX) with a Gilson pump (Minipuls 2; Gilson Medical Electronics, Middleton, WI). The venous outflow and outflows from the cuvettes were allowed to drain into a reservoir, which initially contained 200 ml of 6% dextran in normal saline. The blood in the reservoir was pumped back to the animal via a femoral vein at a rate equal to the total outflows. The jejunal or ileal segment was covered with a plastic sheet and kept at 37°C with a heat lamp and thermoregulator (Yellow Springs Instruments, Model 63RC, Yellow Springs, OH). Oxygen uptake was calculated as the product of blood flow and $(A-V)O_2$. Blood flow and oxygen uptake were expressed as $ml \cdot min^{-1} \cdot 100g^{-1}$.

The following experiments were designed to determine 1) if adenosine is a vasodilator in the intestinal mucosa, i.e. the primary region of postprandial intestinal hyperemia (38,82) (Series I - IV); 2) the effect of intraluminal placement of food on jejunal venous and lymphatic adenosine concentration and release, i.e. indices of interstitial fluid adenosine concentration (163,227,234) (Series V - VII); and 3) the effect of adenosine antagonists, i.e. aminophylline and 8-phenyltheophylline, adenosine deaminase and an inhibitor of cellular adenosine reuptake, i.e. dipyridamole, on the food-induced hyperemia (Series VIII - XI).

Effect of Adenosine on Intestinal Mucosal Blood Flow

Series I: The effect of intra-arterial infusion of adenosine on mucosal-submucosal and muscularis-serosal blood flow was determined in segments of dog jejunum (22 ± 1g; n=6), dog ileum (19 ± 1g; n=4), and cat ileum (15 ± 3 g; n=5). An arterial circuit containing a mixing chamber was placed between a femoral artery and the artery perfusing the intestinal segment, and the segment was perfused with aortic blood at aortic blood pressure. After the venous outflow of the segment reached a steady state, approximately 2 x 10^5 microspheres (14 ± 0.7 μm diameter; 3 M Co., St. Paul, MN) labeled with either ^{85}Sr or ^{141}Ce were injected into the arterial circuit, between the femoral artery and the mixing chamber. Four to five minutes later, adenosine (Sigma Chemical, St. Louis, MO) was infused i.a. at 1 μmol·min^{-1}, and the second labeled microspheres were injected when the venous outflow reached a steady state. One μmol/min i.a. adenosine was shown by our preliminary observations to produce a near maximal increase in venous outflow. The microsphere stock solution contained 10^7 spheres with 0.2 mCi per ml; a drop of Tween 80 was added to prevent aggregation. Prior to the injection, the stock solution was shaken with a vortex mixer, and an aliquot of 0.1 ml was added to 0.9 ml of 6% dextran (MW 75,000; 309 mOsm·l^{-1} NaCl). This mixture was vortexed and treated with an ultrasonic cell disruptor to achieve a uniform dispersion of the microspheres, and 0.2 ml of this solution was then withdrawn into a syringe for immediate injection. The order of injection of the two labeled microspheres was randomized. The total venous outflow was collected for 3 min in a graduated cylinder for blood flow measurement during the injection of microspheres. The blood collected was not returned to the animal to avoid contamination of the animal with radioactivity.

Similar experiments were conducted in single canine jejunal segments perfused at a constant blood flow rate. An arterial circuit

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with an interposed Masterflex pump (Cole Parmer Instruments, Chicago, IL) was placed between a femoral artery and the single artery perfusing the jejunal segment. Perfusion pressure was monitored (Statham P23Gb) via a catheter placed in the arterial circuit close to the jejunal segment. Pump flow rate was adjusted initially until perfusion pressure was approximately 10 mmHg below systemic arterial pressure. When the perfusion pressure reached a steady state, microspheres were injected into the arterial circuit, between the femoral artery and the pump, before and during i.a. adenosine infusion at 0.1 $\mu mol \cdot min^{-1}$. Preliminary observations showed that this dose of adenosine is sufficient to produce near maximal vasodilation under constant flow conditions (also see Figure 17). The adenosine-induced vasodilations were observed as a decrease in perfusion pressure.

Four to five minutes after the second microsphere injection, the intestinal segment was excised, and carefully separated into mucosal-submucosal and muscularis tissue layers (82). Each entire tissue layer was divided into 1 g samples, and all samples from each tissue layer were counted in a gamma spectrometer (Packard Instruments Co., Downers Grove, IL). Corrections were made for the overlap in the energy spectra of the two nuclides. The radioactivities in all samples of each tissue layer were added to represent the total mucosal-submucosal or muscularis radioactivity. Blood flow to each tissue layer was calculated as the product of venous outflow and the fraction of total gut wall radioactivity distributed to each tissue layer, expressed as $ml \cdot min^{-1} \cdot 100g^{-1}$ tissue weight.

All of the following experiments were performed in jejunal segments of anesthetized dogs.

Series II: The effect of intraluminal placement of adenosine and analogue, N^6-cyclohexyladenosine (CHA) and 5'-N-ethylcarboxamide adenosine (NECA) (both from Warner Lambert Co, Milford, CN), on the venous outflow of jejunal segments was determined (n=8). The rationale

behind this approach is that these compounds when placed into the gut lumen would be primarily exposed to the mucosal vasculature, and therefore changes in total venous outflow would primarily reflect the action of adenosine or analogue on the mucosal vasculature. The non-metabolizable analogues, CHA and NECA, were used to avoid degradation of adenosine by the epithelial cells (4). Ten ml of normal saline (NS) were placed into the jejunal lumen for 15 min. This procedure was repeated several times until blood flow reached a steady state. At this time, the luminal content was changed to adenosine (10^{-3} and 10^{-2}M; n=5), CHA (10^{-7}, 10^{-5}, 10^{-4} and 10^{-3}M; n=6) or NECA (10^{-7}, 10^{-5}, 10^{-4} and 10^{-3}M; n=8). Blood flow was measured during the following four periods: 0-3, 4-7, 8-11 and 12-15 min after the placement. All concentrations of any given compound was sequentially tested in ascending order, starting from the lowest concentration. After the effect of one compound was tested, the luminal content was changed to NS, and an appropriate interval was allowed for venous outflow to reach a new steady-state before testing a different compound. The order of administration of the three compounds was randomized. The effect of each compound on venous outflow was expressed as percent change from the blood flow measured immediately before the compound placement, i.e. when the lumen contained NS.

Series III: In order to determine whether or not adenosine enters the intestinal mucosal tissue during its intraluminal placement, ^{3}H-radioactivity in the mucosal and muscularis tissue layers of jejunal segments was measured after intraluminal placement of ^{3}H-labeled adenosine (n=7). When the venous outflow of the jejunal segment reached a steady state after repeated intraluminal placements of 10 ml of NS, [2,8-^{3}H]-adenosine (10^{-2}M; 10 mCi·mole^{-1} or 1 mCi·mole^{-1}) (ICN, Chemical and Radioactivity Division, Irvine, CA) was placed into the jejunal lumen. After observing an increase in blood flow, which occurred 2 to 5 min after the placement, the jejunal segment was rapidly excised and separated into mucosal-submucosal and muscularis tissue layers. These

tissues were then rinsed with NS and blotted dry with cotton gauze. The ^{3}H-radioactivity in each tissue sample was determined as previously described by Chou et al. (42). 100-250 milligram samples from each tissue layer were placed into tared scintillation vials, and the vials were reweighed to determine wet tissue weight. The samples were digested by adding 100 µl of 70% perchloric acid to each vial and then incubating them at 60-70°C for 4 h in a Dubnoff metabolic shaking incubator (Precision Scientific, Chicago, IL). The digested tissue was then decolorized with 200 µl of 30% hydrogen peroxide, and 10 ml of liquid scintillation cocktail (Safety-Solv; Research Products International Co., Mount Prospect, IL) were added to each sample for counting in a liquid scintillation counter (Packard Instruments Co., Downers Grove, IL).

Series IV: In order to determine whether or not the hyperemia induced by intraluminal placement of adenosine, CHA or NECA is mediated by stimulation of intramural nerves, the adenosine-, CHA- and NECA-induced hyperemias were determined before and after treating the mucosal surface of jejunal segments with dibucaine, a local anesthetic. The procedure was similar to that used by Chou et al. (35). Two adjacent jejunal segments per dog (n=9) were exposed, and the venous outflow from both segments were measured as described above. When the venous outflows reached a steady state after repeated intraluminal placements of NS, dibucaine hydrochloride (0.4%; Sigma Chemical, St. Louis, MO) was placed into the lumen of one segment, while NS was placed into the other segment. This dose of dibucaine was previously shown to be the optimal dose for anesthetizing the mucosal nerves (35,184). This dose is also commonly used to produce local anesthesia of the skin and rectum (207). After 20 min, both segments were emptied, and the hyperemia induced by intraluminal placement of 10 ml adenosine (10^{-2}M), CHA (10^{-3}M) or NECA (10^{-4}M) was determined in both segments as follows. While NS was placed into the lumen of one segment, either adenosine, CHA or NECA was placed

into the lumen of the other segment. The venous outflow from both jejunal segments were then measured for consecutive 1 min periods until they reached a steady state. Both segments were then emptied, gently rinsed with NS, and the luminal contents of the two segments were then reversed. This procedure was repeated for each compound, and the order of placement of the three compounds was randomized. In six additional experiments, adenosine-, CHA- and NECA-induced hyperemias were determined in the same segment before and after dibucaine treatment. Normal saline was placed into the lumen between placement of each of the above compounds as the control.

Effect of Food on Jejunal Adenosine Concentration and Release

Series V: The effect of intraluminal placement of food and normal saline on jejunal venous adenosine concentration and release was determined (n=4). The food solution used contained equal parts by weight of fat, carbohydrate, and protein. It was prepared by adding 30 g high-fat test diet, 15 g high-protein test diet, and 5 g high-carbohydrate test diet (US Biochemical, Cleveland, OH) to 400 ml of 0.1 N $NaHCO_3$ containing 750 mg of a pancreatic enzyme preparation (Viokase, Viobin, Monticello, IL; Refs. 43,79,164). The mixture was then gently mixed with a magnetic stirrer at room temperature for 5 h to permit digestion. Before the experiment nine parts of digested food were mixed with one part of gallbladder bile, and the pH and osmolality of the mixture was adjusted to about 7.4 and 300 mosmol/kg, respectively. Both the digested food plus bile and the normal saline (NS) were kept at 37°C during the experiment. For simplicity, the term "food", instead of "digested food plus bile", is used throughout the following text.

When the jejunal venous outflow reached a steady-state after repeated intraluminal placements of NS, the lumen was emptied, and arterial and jejunal venous blood samples (3 ml) were collected

simultaneously, while jejunal blood flow was measured. After processing the blood samples for adenosine analysis (as described below), either NS or food was placed into the lumen for 15 min. Blood flow was measured during the following four periods: 0-3, 4-7, 8-11 and 12-15 min after the placement. Blood samples were collected at 3 and 11 min following the placement for adenosine analysis. The lumen was then emptied again and blood samples collected. After this, either food or NS was placed into the lumen for 15 min, and blood samples collected at 3 and 11 min after the placement. The order of placement of food or NS was randomized in these experiments.

Series VI: The purpose of this series of experiments was to determine in more detail the time course of adenosine release during food placement. When blood flow and $(A-V)O_2$ reached a steady state after repeated placements of normal saline into the jejunal lumen, the lumen content was changed to food. Arterial and venous blood samples were collected before (normal saline in the lumen) and at either 3 and 11 min (n=14) or 7 and 15 min (n=6) after the food placement. The reason for allowing only three sample periods for each experiment was that immediate processing of the samples was essential for accurate determination of adenosine concentration, and the processing required 5-6 min per sample period.

In some experiments, venous adenosine concentration and release were determined before and during reactive hyperemia, which followed a 1 min arterial occlusion produced by a snare placed around the jejunal artery. Blood flow was measured before and after the occlusion by 1 min collection with a stopwatch and graduated cylinder, and also continuously monitored by an electromagnetic flow transducer. Arterial and venous blood were sampled before and after the arterial occlusion, at the time when blood flow reached a peak.

Procedures for blood sample processing and adenosine analysis were the same as those of Thompson et al. (234). All blood samples were

collected into iced test tubes containing 250 µl "collecting solution" to prevent cellular uptake and degradation of adenosine. The "collecting solution" contained 26 µM dipyridamole (Sigma Chemicals, St. Louis, MO), 3 µM erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) (Burroughs Wellcome, Research Triangle Park, NC), and 5% methanol in isotonic saline. Dipyridamole inhibits cellular adenosine reuptake (43a,189,238); alcohol is needed to dissolve dipyridamole in normal saline; EHNA is an inhibitor of adenosine deaminase (111). After the samples were thoroughly mixed by inversion, they were immediately centrifuged at 4°C for 3.5 min. One ml plasma was then removed from each tube and placed in a separate tube containing 250 µl 35% perchloric acid. The tubes were then vortexed and stored in ice. At the end of the experiment, all samples were centrifuged at 4°C for 15 min. Aliquots of the supernatant were transferred to another set of tubes and neutralized (pH 6-8) with K_2CO_3 (1 g/ml). All samples were stored at -70°C until adenosine analysis.

Series VII: Lymph flow and lymphatic adenosine concentration and release were determined after placement of food into the jejunal lumen (n=4). A cannula was placed into a lymphatic vessel within the mesentery of the intestinal segment, adjacent to the major segmental artery and vein. To facilitate and maintain the lymph flow, 40 ml/kg Krebs buffer was infused i.v. during the first hour of surgery, and thereafter infused at a rate of 3 ml/min. Intestinal lymphatic effluent was collected for 2-5 min into tared collecting tubes containing 10 µl 70% perchloric acid. The collecting tubes were then vortexed and reweighed for determination of lymph flow. After centrifugation, aliquots of the supernatant were neutralized with K_2CO_3.

Adenosine concentration in plasma and lymph was assayed by reverse-phase high pressure liquid chromatography (HPLC; Waters Associates, Milford, MA) (163,234). Samples of neutralized plasma or lymph extract were injected onto a 5 µm Radial Pac cartridge (Nova Pac

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C18; Waters Associates) using an automatic injector. Adenosine was separated using an isocratic (1.4 ml/min) elution mixture containing 90% 4 mM potassium phosphate buffer (pH 7.0) and 10% 70/30 methanol/water (vol/vol). Absorbance was continuously monitored at 254 nm on a strip-chart recorder. Because of the frequent presence of substances coeluting with adenosine, effluent corresponding to the adenosine peak but containing no inosine was collected and evaporated to dryness. After resuspension in 500 µl water, 0.5 U of adenosine deaminase was added to convert the adenosine to inosine. After 20 min, the adenosine deaminase was inactivated by addition of 500 µl methanol. The samples were evaporated again, resuspended in water, and reinjected onto the 5 µm Radial Pac cartridge for quantification of inosine. The inosine peak was identified by comparison with retention times of inosine standards. With the use of this peak shift method, peak areas of inosine and appropriate dilutional factors of the plasma samples were used to calculate plasma adenosine concentration. Adenosine release into the venous effluent ($\dot{R}ado$, in $nmol \cdot min^{-1} \cdot 100g^{-1}$) was calculated as follows (163):

$$\dot{R}ado = ([ADO]_v - [ADO]_a) \times BF \times (1-Hct) \times 10^{-3}$$

where $[ADO]_v$ and $[ADO]_a$ equal adenosine concentration (in nM) in venous and arterial plasma samples, respectively, BF equals blood flow (in $ml \cdot min^{-1} \cdot 100g^{-1}$) and Hct equals hematocrit. Adenosine release into the lymphatic effluent was calculated as the lymphatic flow multiplied by the lymphatic adenosine concentration.

Effect of Aminophylline, 8-Phenyltheophylline, Adenosine Deaminase and Dipyridamole on the Food-Induced Hyperemia

Series VIII: The effect of the adenosine receptor blocker, aminophylline, on the postprandial jejunal hyperemia was determined (n=13). When venous outflow reached a steady-state, the luminal content was changed to digested food. After determining the response to food,

aminophylline (Sigma Chemical, St. Louis, MO) was infused into a side branch of the single artery of the segment at a rate of 1 $mg \cdot kg^{-1} \cdot min^{-1}$ (approximately 45 μmol/min) for 10 min. This resulted in a blood concentration of 10^{-4} M, a concentration used extensively to attenuate adenosine-induced hyperemia in skeletal muscle (234), and intestine (90,93,247). Our preliminary study (n=7) also showed that this concentration of aminophylline produces 100% and 70% reduction of adenosine-induced hyperemia during and 20 min after stopping aminophylline infusion, respectively. Adenosine (Sigma Chemical) was infused into the arterial side branch (at a rate of 1 μmol/min) before and after aminophylline in every experiment to determine the degree of adenosine blockade. After demonstrating attenuation of adenosine-induced hyperemia, food was placed into the intestinal lumen and the resulting hyperemia was determined again.

Series IX: The effects of 8-phenyltheophylline (8-PT; Sigma Chemical, St. Louis, MO) on food-induced hyperemia and adenosine-induced vasodilation were determined (n=6). Because the effect of 8-PT on adenosine-induced vasodilations has never been evaluated before except in the liver (146), the minimal dose required to attenuate adenosine-induced vasodilations in the intestine was determined. This is best done under constant flow conditions in order to precisely control the plasma concentrations of adenosine and 8-PT. An arterial circuit with an interposed Masterflex pump was established between a femoral artery and the jejunal artery. The perfusion circuit was designed so that the intestinal segment could be perfused by the pump at a constant flow rate with the aortic blood, or by aortic blood pressure at a natural flow condition via a bypass around the pump. A three-way stopcock was used to alternate the perfusion between constant and natural flow. Under constant flow conditions, perfusion pressure was monitored via a catheter in the perfusion line attached to a pressure transducer (Statham P23Gb).

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After measuring the food-induced hyperemia under natural flow conditions, the perfusion circuit was switched from natural to constant flow to determine the efficacy of 8-PT in blocking adenosine-induced vasodilations. Pump flow rate was adjusted until perfusion pressure was 10 mmHg below the systemic arterial pressure. Once a steady state had been reached, adenosine was infused intra-arterially at rates of 0.1, 0.2, 0.4 and 1.0 μmol/min. Under these conditions, adenosine-induced decreases in perfusion pressure represented decreases in vascular resistance. 8-PT was then infused intra-arterially at 0.1, 0.2 or 0.4 μmol/min, and adenosine infusions were repeated after every dose of 8-PT. All infusions were made into the arterial circuit upstream from the pump. Our experiments showed that 10-15 min of 0.4 μmol/min 8-PT infusion was sufficient to attenuate adenosine-induced vasodilations by more than 50%. After determining the attenuation of adenosine-induced vasodilation, the perfusion circuit was switched back to the natural flow condition and the hyperemic response to food was determined during 8-PT infusion.

Series X: The effects of dipyridamole on the food-induced hyperemia and adenosine-induced vasodilation were determined (n=6). After determining the response to food, as described above, normal saline was placed into the lumen and dipyridamole (Sigma Chemical, St. Louis, MO) was infused at 10-20 nmol/min i.a. This infusion produced an arterial concentration of 1.49 $\pm$ 0.24 μM, which is sufficient to enhance adenosine-induced hyperemia in the heart (127) and skeletal muscle (133) and block adenosine reuptake by red blood cells (134) and rat and guinea pig hearts (43a). In addition, preliminary observations showed that this dose of dipyridamole produces a 43 - 344% enhancement of the vasodilations induced by 1 - 100 nmol/min i.a. adenosine. During the dipyridamole infusion, food was placed into the intestinal lumen and the resulting hyperemia was determined again.

Series XI: This series determined the effect of adenosine

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deaminase on the food-induced increases in blood flow and oxygen consumption. The protocol was similar to that used in Series X, i.e. the effect of intraluminal placement of food on blood flow and oxygen consumption was determined, adenosine deaminase (Type VIII; Sigma Chemical) was then infused at 100-200 Units/min, i.a. and the effects of food were determined again. The adenosine deaminase infusion produced a local arterial concentration of 8.71 $\pm$ 1.31 U/ml, which is sufficient to attenuate various metabolic hyperemias in the heart and other tissues (185a). The adenosine deaminase was dissolved in Kreb's solution to yield a final infusate concentration of 400 U/ml. In some experiments, the adenosine deaminase was purified as described by Kroll and Feigl (137a). However, the results using native and purified adenosine deaminase were similar and therefore pooled in this study.

All values were expressed as mean $\pm$ S.E. The response of blood flow, luminal pressure, motility index and oxygen uptake to food were expressed as change from control (NS in the lumen) before food placement. The data were analyzed using paired Student's t test modified for comparison of two paired sample means and analysis of variance. Significance was assessed at the 95% confidence level.

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Effect of Adenosine on Intestinal Mucosal Blood Flow

Series I: The effect of i.a. adenosine on mucosal-submucosal and muscularis-serosal blood flow was determined in segments of dog jejunum, and dog and cat ileum, utilizing the microsphere technique. In these studies, the intramural distribution of wet tissue weight was 73 ± 2% mucosa-submucosa and 28 ± 2% muscularis-serosa in the dog jejunum (n=10), 66 ± 3% mucosa-submucosa and 34 ± 3% muscularis-serosa in the dog ileum (n=4), and 57 ± 2% mucosa-submucosa and 42 ± 2% muscularis-serosa in the cat ileum (n=5). These values are not significantly different from those observed previously (37a).

As shown in Figure 2A, intra-arterial infusion of adenosine at 1 $\mu mol \cdot min^{-1}$ significantly increased venous outflow as well as the mucosa-submucosa and muscularis-serosa blood flows of naturally perfused jejunal segments. The adenosine-induced increase in blood flow in the mucosal layer was not significantly different from that in the muscularis layer. In additional experiments, the jejunal segments were perfused at a constant blood flow rate of 55 ± 12 $ml \cdot min^{-1} \cdot 100g^{-1}$. Intra-arterial infusion of adenosine at 0.1 $\mu mol \cdot min^{-1}$ significantly ($P < 0.05$) decreased the perfusion pressure from 118 ± 8 mm Hg to 79 ± 4 mm Hg (-32 ± 6%) and the vascular resistance from 3.0 ± 0.8 to 1.9 ± 0.4 mm $Hg \cdot min \cdot 100g \cdot ml^{-1}$ (-31 ± 7%) (n=4).

Figure 2B shows the percent distribution of total gut wall blood flow (i.e. total gut wall microsphere radioactivity) to the mucosal and muscularis layers of the segments perfused under the natural or constant flow conditions. The flow distribution to the two compartments was not significantly altered by i.a. adenosine, despite the fact that it

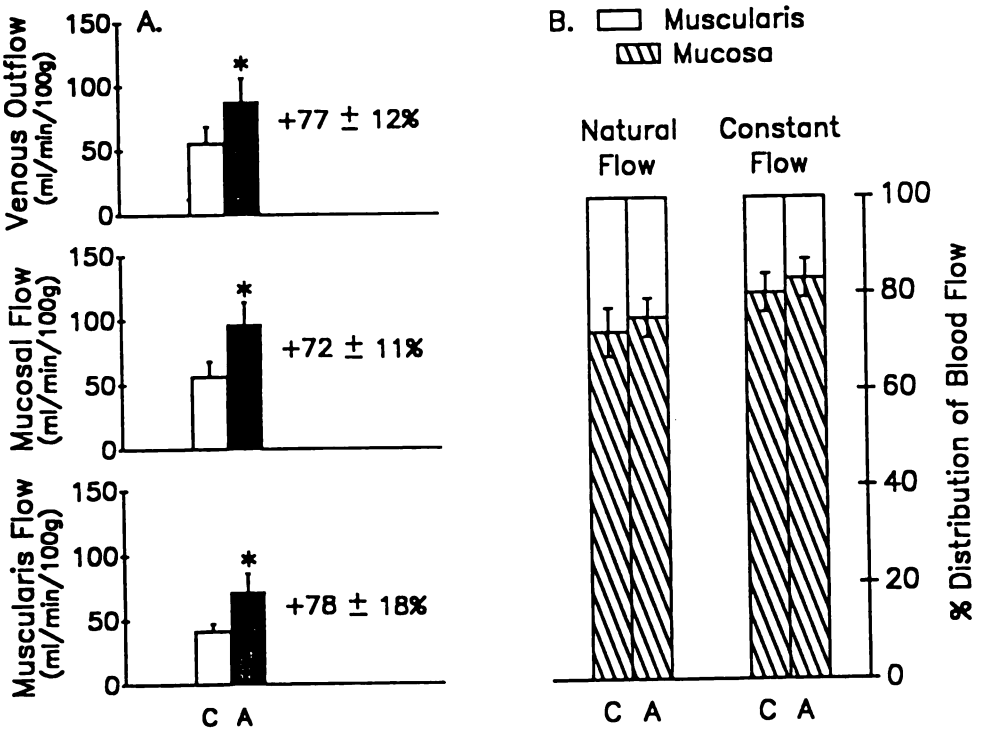

Figure 2

Effect of i.a. adenosine on jejunal compartmental blood flow.
A. Jejunal venous outflow, and mucosal and muscularis blood flows before (C, open bars) and during (A, closed bars) i.a. infusion of adenosine at 1 μmol/min. Also shown are the percent increases in blood flow in response to adenosine, and all these increases are statistically significant ($P < 0.05$). *$P < 0.05$ relative to blood flow before adenosine infusion. N=6.
B. Percent distribution of total gut wall blood flow to the mucosal and muscularis layers of jejunal segments perfused under natural (n=6) or constant (n=4) flow conditions, before (C) and during (A) i.a. adenosine infusion.

significantly increased the venous outflow under the natural flow condition (Figure 2A) and decreased jejunal vascular resistance under the constant flow condition. These results therefore demonstrate that adenosine is a a vasodilator in the mucosa and muscularis of the canine jejunum.

In two additional jejunal segments, the two types of microspheres were injected within 5 sec of each other, and their distribution was determined. In one experiment, the fraction of the total radioactivity distributed to the mucosa was 64% for ^{141}Ce and 58% for ^{85}Sr, and that to the muscularis was 35% for ^{141}Ce and 42% for ^{85}Sr. In the other experiment, the distribution to the mucosa was 85% for ^{141}Ce and 82% for ^{85}Sr, and that to the muscularis was 15% for ^{141}Ce and 18% for ^{85}Sr. Therefore, the blood flow distribution is not altered by microsphere injection per se.

Figure 3 shows the effect of i.a. adenosine in the ileum of the dog and cat. Adenosine significantly increased ileal venous outflow as well as the mucosa-submucosal and muscularis-serosal blood flows in both dog and cat. The hyperemia in the mucosa-submucosa was not significantly different from that in the muscularis-serosa. Furthermore, the increases in venous outflow and mucosal and muscularis flows did not significantly differ between intestinal region (jejunum vs. ileum) or species (dog vs. cat) (analysis of variance).

Series II: In order to confirm that adenosine is a vasodilator in the jejunal mucosa, the total venous outflow of segments of canine jejunum was measured during mucosal application, by luminal placement of adenosine, CHA and NECA. The rationale of taking this approach is that the mucosal vasculature will be primarily exposed to adenosine and, therefore, changes in total blood flow will represent changes in mucosal blood flow. As shown in Figure 4, mucosal application of adenosine, CHA and NECA significantly increased the venous outflow, in a dose-dependent manner, with a rank order of potency of NECA > CHA > adenosine. The minimal concentration required to produce significant increases in

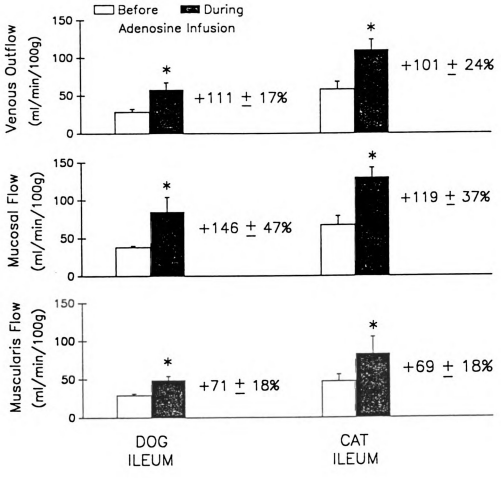

Figure 3

Venous outflow and mucosal and muscularis blood flows before (open bars) and during (closed bars) i.a. infusion of adenosine at 1 μmol/min in the ileum of dogs (n=4) and cats (n=5). Also shown are the percent increases in blood flow in response to adenosine, and all these increases are statistically significant ($P < 0.05$). *$P < 0.05$ relative to blood flow before adenosine infusion.

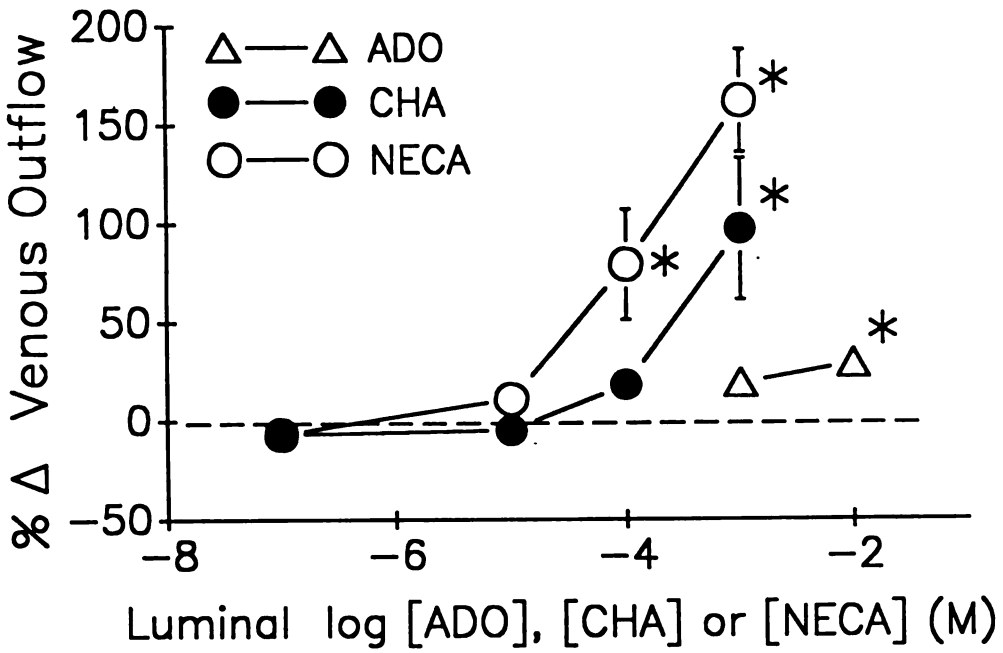

Figure 4

Percent changes in canine jejunal venous outflow in response to mucosal application, by luminal placement, of adenosine (ADO; n=5), N^6-cyclohexyladenosine (CHA; n=6) and 5'-N-ethylcarboxamide adenosine (NECA; n=8). *P<0.05. Resting venous outflow was 56 ± 5 ml·min^{-1}·100g^{-1}.

venous outflow were NECA 10^{-4}M, CHA 10^{-3}M and adenosine 10^{-2}M. Figure 5 shows the time course of increases in venous outflow in response to luminal placement of 10^{-2}M adenosine, 10^{-3}M CHA and 10^{-3}M NECA. Venous outflow significantly increased within 3 min after placement of adenosine, CHA and NECA. While the hyperemia induced by CHA and NECA lasted as long as the 15 min placement period, the hyperemia induced by adenosine waned during the 15 min placement period.

Series III: In 7 experiments, the total amount of ^{3}H-radioactivity in the mucosa and muscularis layers was determined after luminal placement of 10^{-2}M [2,8-^{3}H] adenosine. This placement significantly increased venous outflow from 56 $\pm$ 15 to 84 $\pm$ 15 ml$\cdot$min$^{-1}\cdot$ 100g^{-1}, and 1.7 $\pm$ 0.4% of the placed adenosine was found in the mucosal tissue with an insignificant (less than 0.03%) amount of the radioactivity found in the muscularis-serosal tissue. Therefore, only the mucosal vasculature should be exposed to luminally placed adenosine and its analogues.

Series IV: In order to determine whether or not the hyperemia produced by luminal placement of adenosine and its analogues was mediated by stimulation of the mucosal nerves, the hyperemia produced by these compounds was compared before and after 20 min luminal placement of dibucaine, a local anesthetic. Figure 6 shows the increase in venous outflow induced by luminal placement of adenosine, CHA or NECA in two adjacent jejunal segments, one of which was treated with dibucaine and the other left untreated. Adenosine, CHA and NECA significantly increased venous outflow in both segments. The magnitude of the hyperemia produced by each compound in the dibucaine-treated segment was not significantly different from that in the adjacent untreated segment. In six experiments, the effect of these compounds was determined in the same segment before and after dibucaine treatment. Adenosine, CHA and NECA significantly increased venous outflow +51 $\pm$ 11%, +60 $\pm$ 13% and +146 $\pm$ 35%, respectively, before dibucaine treatment, and +51 $\pm$ 9%, +75 $\pm$ 14% and +136 $\pm$ 60%, respectively, after the treatment. The hyperemia

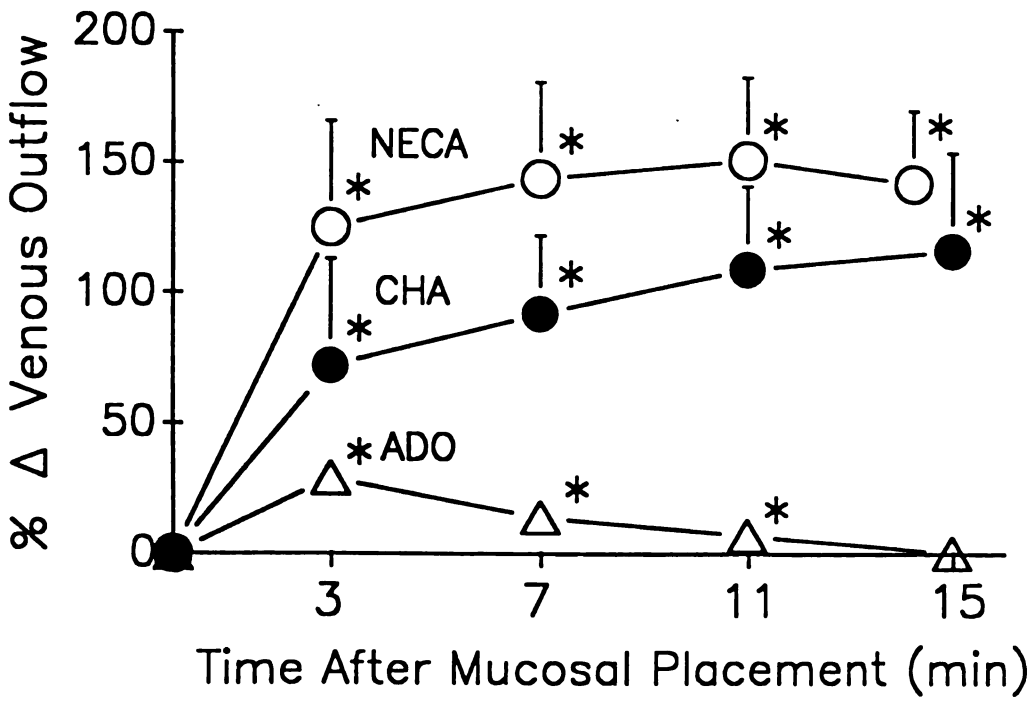

Figure 5

Time course of increase in canine jejunal venous outflow in response to mucosal application, by luminal placement, of adenosine (ADO; n=5), N^6-cyclohexyladenosine (CHA; n=5) and 5'-N-ethylcarboxamide adenosine (NECA; n=7). *P<0.05.

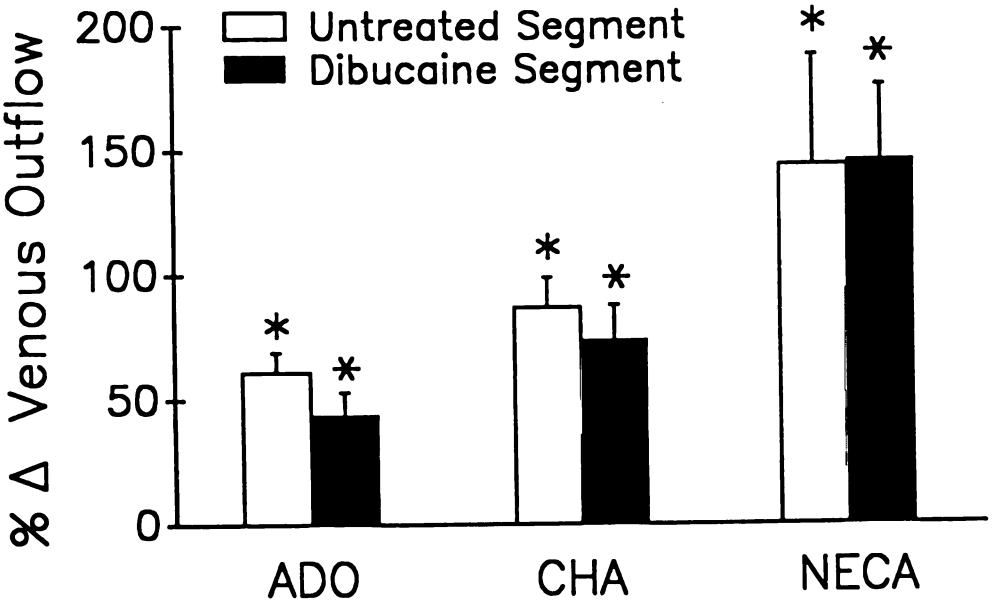

Figure 6

Percent increase in canine jejunal venous outflow in response to mucosal application of adenosine (ADO), N^6-cyclohexyladenosine (CHA) and 5'-N-ethylcarboxamide adenosine (NECA) before and after treating the jejunal mucosa with dibucaine (0.4%). N=9. *P<0.05. The resting venous outflow was 74 ± 11 $ml \cdot min^{-1} \cdot 100g^{-1}$.

produced by each of these compounds after dibucaine was not significantly different from that produced before the treatment. Therefore, dibucaine had no significant effect on the hyperemia induced by intraluminal placement of adenosine, CHA or NECA, whether its effect was tested between two adjacent jejunal segments or within the same segment. Dibucaine per se also did not alter resting blood flow (blood flow was 80 ± 15 before and 76 ± 14 $ml \cdot min^{-1} \cdot 100g^{-1}$ during the dibucaine placement), but did increase motility (luminal pressure increased from 4 ± 1 before to 10 ± 1 mm Hg during dibucaine placement), as observed previously (35). This increase in motility, however, waned and disappeared shortly after the removal of dibucaine from the lumen and its replacement with NS.

Effect of Food on Jejunal Adenosine Concentration and Release

Series V: Figure 7 shows that placement of normal saline into the jejunal lumen did not significantly alter jejunal blood flow, $(A-V)O_2$, oxygen consumption, venous adenosine release nor arterial and venous adenosine concentrations. Placement of food, however, significantly increased jejunal blood flow and oxygen consumption for the entire 15 min placement period. Venous adenosine concentration significantly increased in all four dogs at 3 min after the food placement, while arterial adenosine concentration remained unaltered. The venous adenosine concentration was not significantly different from the arterial adenosine concentration, producing zero adenosine release, before and 11 min after food placement. However, at 3 min following the food placement the venous adenosine concentration was significantly higher than the arterial concentration, producing a significant increase in adenosine release.

Series VI: Figure 8 shows the results from experiments in which blood samples were obtained either at 3 and 11 min (n=14) or 7 and 15 min (n=6) following luminal placement of food. The control values, when

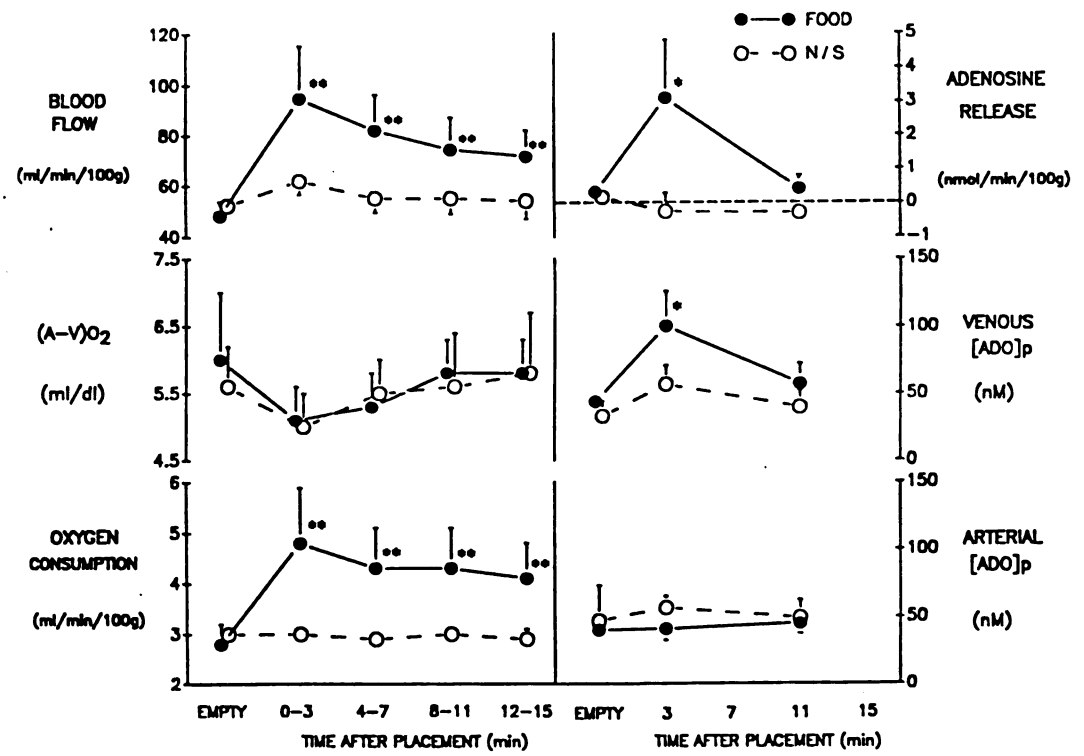

Figure 7

Jejunal blood flow, arteriovenous O_2 content difference [(A-V)O_2], oxygen consumption, adenosine release and adenosine concentration ([ADO]p) in jejunal venous and systemic arterial plasma before (lumen EMPTY) and after placement of normal saline (N/S) or food into the jejunal lumen. *P<0.07; **P<0.05 relative to EMPTY. N=4.

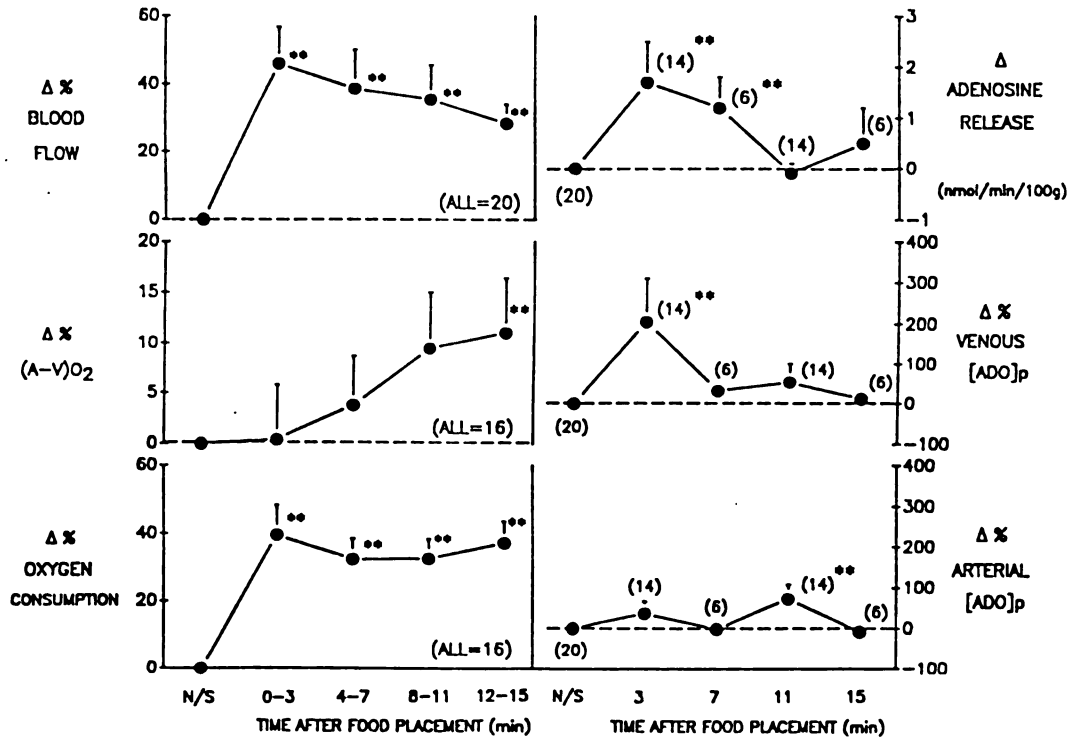

Figure 8

Percent change from control (N/S in lumen) of jejunal blood flow, arteriovenous O_2 content difference [$(A-V)O_2$], oxygen consumption, adenosine release, and adenosine concentration ([ADO]p) in jejunal venous and systemic arterial plasma following placement of food into the jejunal lumen. **P<0.05, paired Student's t test. Number in parenthesis indicates number of experiments.

the lumen contained NS, were blood flow: 48 ± 3 $ml \cdot min^{-1} \cdot 100g^{-1}$, $(A-V)O_2$: 5.3 ± 0.3 $ml \cdot dl^{-1}$, oxygen consumption: 2.5 ± 0.1 $ml \cdot min^{-1} \cdot 100g^{-1}$, adenosine release: 0.3 ± 0.3 $nmol \cdot min^{-1} \cdot 100g^{-1}$, jejunal venous adenosine concentration: 62 ± 11 nM, arterial adenosine concentration: 56 ± 9 nM. Placement of food into the jejunal lumen significantly increased blood flow and oxygen consumption for the entire 15 min placement period. The increase in adenosine release, however, occurred only during the first 7 min of food placement. Venous adenosine concentration significantly ($P<0.05$) increased at 3 min and tended to increase ($P<0.1$) at 7 min following food placement, and arterial adenosine concentration significantly ($P<0.05$) increased at 11 min following the placement. Venous adenosine concentration was not significantly different from the arterial adenosine concentration, producing zero adenosine release, before and 11 and 15 min after food placement. However, at 3 and 7 min following the food placement, the venous adenosine concentration was significantly ($P<0.05$) higher than the arterial concentration, producing a significant increase in adenosine release. Arteriovenous oxygen content difference was not altered until 12-15 min after the food placement, at which time this variable was significantly ($P<0.05$) increased.

Table 1 shows oxygen supply, oxygen demand and oxygen supply-to-demand ratio before and after placement of food into the jejunal lumen. Oxygen supply and demand increased to the same extent following the food placement, resulting in no change in the oxygen supply-to-demand ratio. Therefore, the food-induced increase in jejunal adenosine concentration and release is not mediated by a decrease in oxygen-to-supply ratio. However, a decrease in oxygen supply, as during arterial occlusion, can increase adenosine release. As shown in Figure 9, jejunal blood flow and venous adenosine concentration and release significantly increased ($P<0.05$), while arterial adenosine concentration remained unaltered ($P>0.05$), following a 1 min occlusion of the artery perfusing the intestinal segment.

Table 1

Jejunal O_2 supply, O_2 demand and O_2 supply-to-demand ratio before (0 min) and after placement of food into the jejunal lumen.

	Time After Food Placement				
	0 min	3 min	7 min	11 min	15 min
O_2 Supply	9.3 ± 0.6	13.5 ± 1.4*	12.5 ± 1.2*	11.5 ± 0.9*	11.6 ± 0.8*
O_2 Demand	2.4 ± 0.1	3.4 ± 0.3*	3.2 ± 0.3*	3.2 ± 0.3*	3.3 ± 0.2*
O_2 $\frac{\text{Supply}}{\text{Demand}}$	4.0 ± 0.2	4.0 ± 0.2	3.9 ± 0.2	3.8 ± 0.3	3.7 ± 0.2

O_2 supply and O_2 demand (mean ± SE) in $ml \cdot min^{-1} \cdot 100g^{-1}$. Oxygen supply equals blood flow multiplied by arterial oxygen content, which is assumed to be 20 ml/dl. Oxygen demand equals blood flow multiplied by $(A\text{-}V)O_2$. N=16. *$P<0.05$ relative to the corresponding value at 0 min.

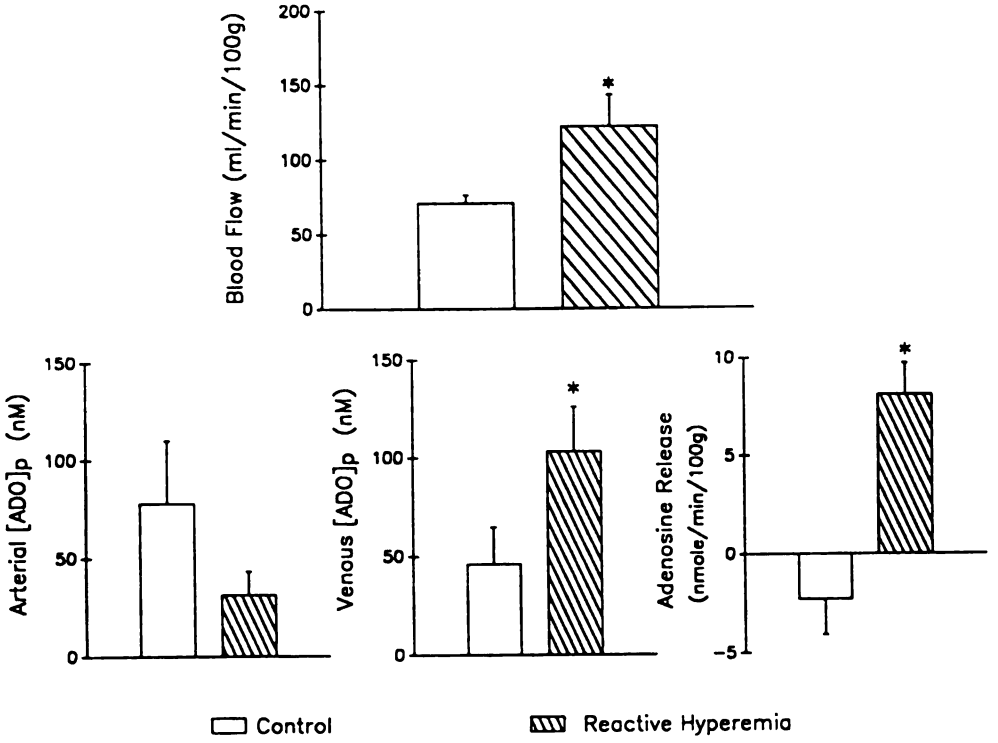

Figure 9

Jejunal blood flow, adenosine release and adenosine concentration ([ADO]p) in jejunal venous and systemic arterial plasma before and after a 1 min occlusion of the jejunal artery. *P<0.05 relative to before occlusion.

Series VII: Figure 10 shows jejunal lymph flow and lymphatic adenosine concentration and release before and after placement of food into the jejunal lumen in 4 dogs. Lymph flow increased by +70 ± 23 $\mu l \cdot min^{-1} \cdot 100g^{-1}$ ($P<0.05$), and an increase was observed in all 4 dogs. Lymphatic adenosine concentration and release tended to increase by +61 ± 38 nM ($P=0.1$) and by +10 ± 5.7 $pmol \cdot min^{-1} \cdot 100g^{-1}$ ($P=0.09$), and the increases were observed in 3 out of 4 dogs.

Effect of Aminophylline, 8-Phenyltheophylline, Adenosine Deaminase and Dipyridamole on the Food-Induced Hyperemia

The next 4 series of experiments examined the effects of aminophylline and 8-phenyltheophylline (adenosine receptor blockers), adenosine deaminase (enzyme which specifically degrades adenosine into inosine) and dipyridamole (inhibitor of cellular adenosine reuptake) on resting intestinal blood flow and food- and adenosine-induced vasodilations.

Series VIII: Intra-arterial infusion of aminophylline per se significantly increased blood flow during infusion (38 ± 9.4 before vs. 74 ± 11.2 $ml \cdot min^{-1} \cdot 100g^{-1}$ during aminophylline, $P<0.05$). This hyperemia, however, only lasted during the infusion, and upon termination of the infusion resting blood flow returned to a level not significantly different from that before aminophylline (49 ± 5.9 before vs. 41 ± 4.8 $ml \cdot min^{-1} \cdot 100g^{-1}$ after aminophylline, $P>0.05$). The effect of aminophylline on food-induced hyperemia was influenced by changes in motility which can be classified into two categories. As shown in Figure 11, placement of food into the lumen increased motor activities, lasting for 6-7 min, in Group I before (Figure 11A) and after (Figure 11C) aminophylline administration; the food-induced hypermotility was enhanced by aminophylline. In Group II, however, food placement did not alter motility at all (Figure 11, D and F). In both groups, aminophylline attenuated resting motor activities if the activities were present when

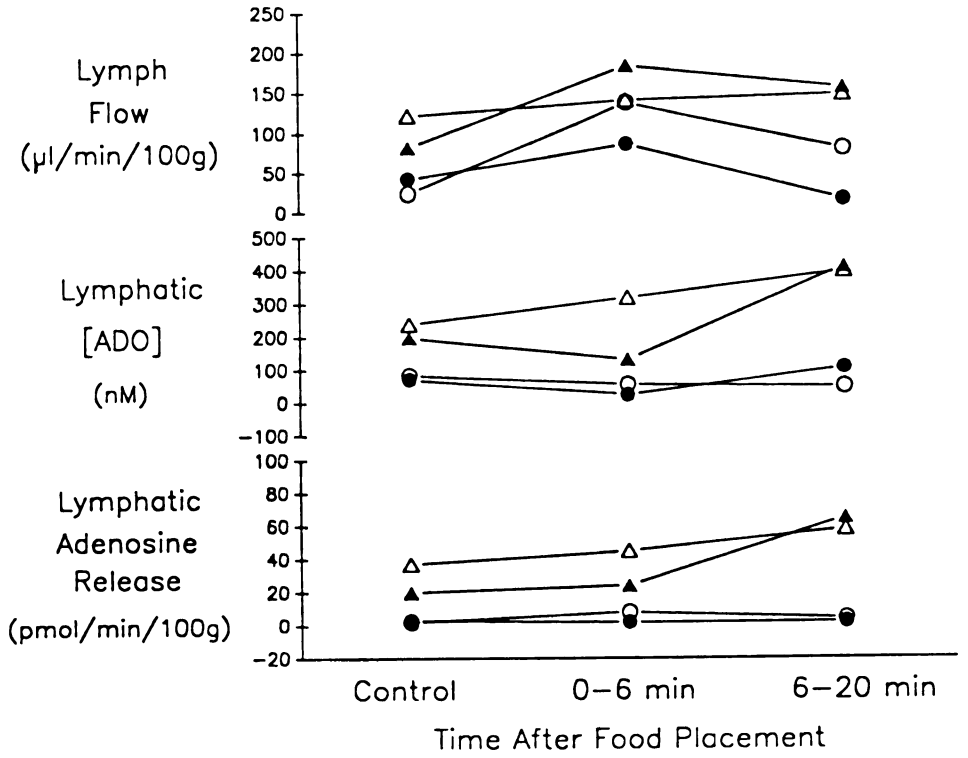

Figure 10

Jejunal lymph flow, and lymphatic adenosine concentration and release before and after placement of food into the jejunal lumen. Each symbol represents an individual experiment.

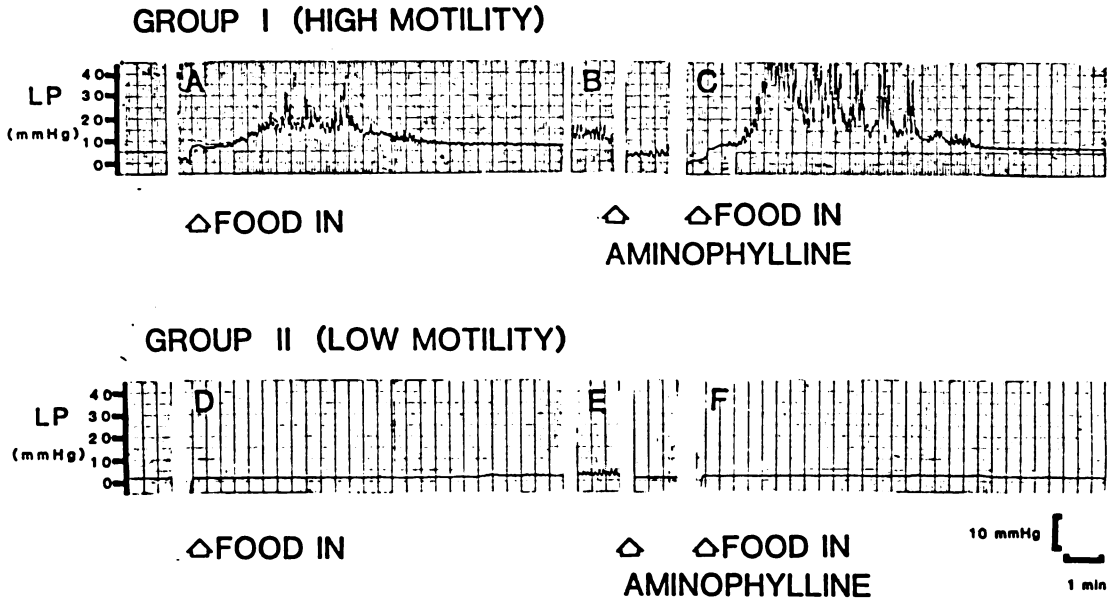

Figure 11

Effects of food on motility before and after aminophylline and effect of aminophylline per se on motility in representative experiments from *group I* (high motility) and *II* (low motility).

the lumen contained no food (Figure 11, B and E). Adenosine also attenuated motor activities, particularly in the presence of hypermotility.

Figure 12 shows the effect of luminal placement of food on blood flow, lumen pressure and motility index before and after administration of aminophylline in Group I. Luminal pressure and motility index was slightly but significantly increased before aminophylline, particularly between 4 and 11 min after food placement. After aminophylline, food placement also significantly increased lumen pressure and motility index, particularly during the first 7 min of food placement. The food-induced increase in lumen pressure and motility index measured 0-7 minutes after food placement was significantly larger after aminophylline than before aminophylline ($P<0.05$). These motility responses to food occurred in all dogs from this group. In this group of dogs, aminophylline had no significant influence on the food-induced hyperemia at any time of food placement ($P>0.05$). In Group II (Figure 13), food had no significant effect on luminal pressure or motility index at any time before or after aminophylline, but aminophylline significantly attenuated the food-induced hyperemia ($P<0.05$). As a matter of fact, food did not significantly alter blood flow at all after aminophylline. Control blood flow, luminal pressure and motility index were not significantly altered by aminophylline per se in Groups I or II ($P>0.05$). Figure 14 compares the effect of aminophylline on adenosine-induced jejunal hyperemia in Groups I and II. Exogenous adenosine significantly increased blood flow in both groups before and after aminophylline. The increase in blood flow induced by adenosine was significantly attenuated by aminophylline in both groups ($P<0.05$), and the degrees of attenuation were not significantly different between the two groups ($P>0.05$).

Series IX: Figure 15 shows the effect of food on blood flow, luminal pressure and motility index before and during i.a. infusions of 8-phenyltheophylline (8-PT). Food significantly increased blood flow

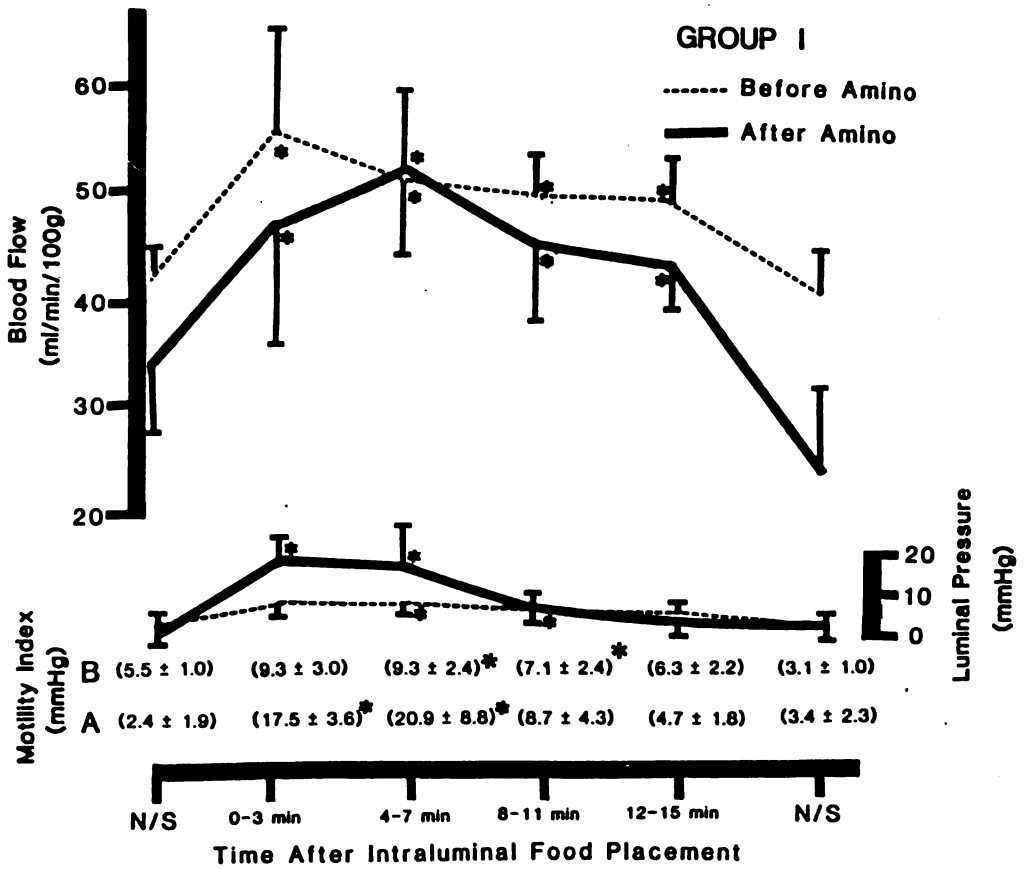

Figure 12

Effect of aminophylline on food-induced changes in blood flow, luminal pressure, and motility index in *group I* (high motility). *B*, before aminophylline: *A*, after aminophylline. *P<0.05 relative to normal saline (N/S) level before food placement; n=5.

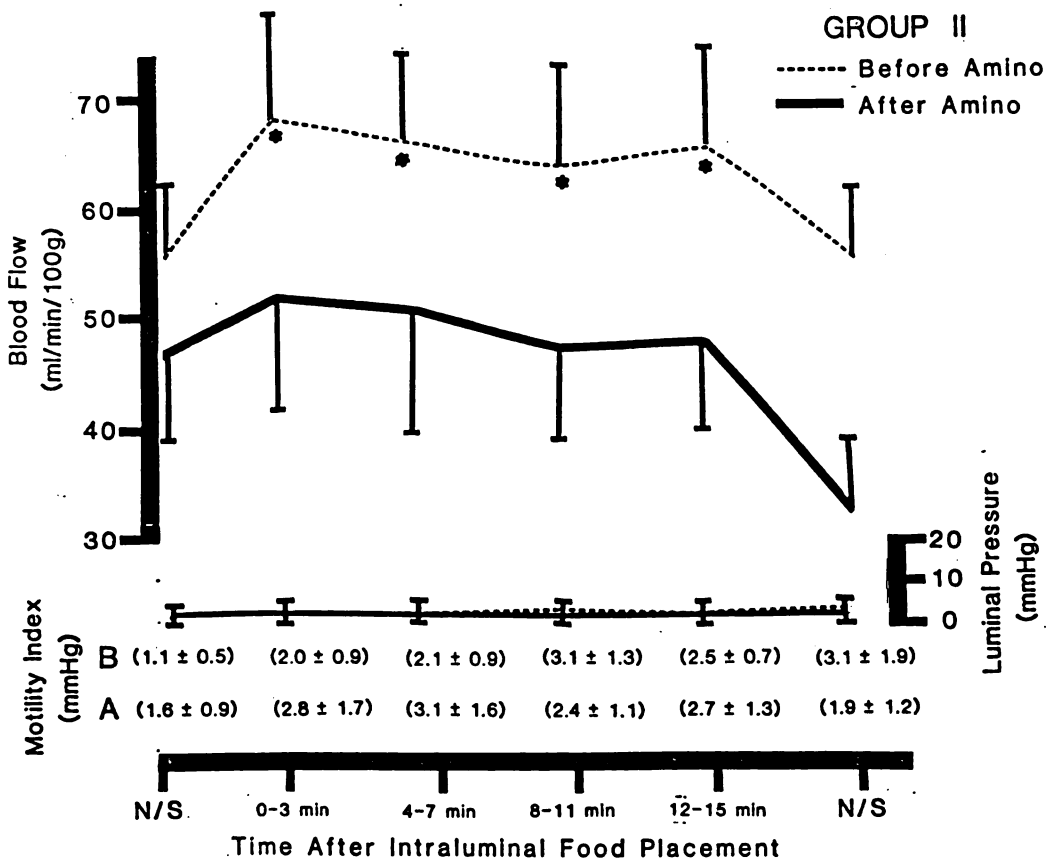

Figure 13

Effect of aminophylline on food-induced changes in blood flow, luminal pressure, and motility index in *group II* (low motility). *B*, before aminophylline; *A*, after aminophylline. *P<0.05 relative to normal saline (N/S) level before food placement; n=8.

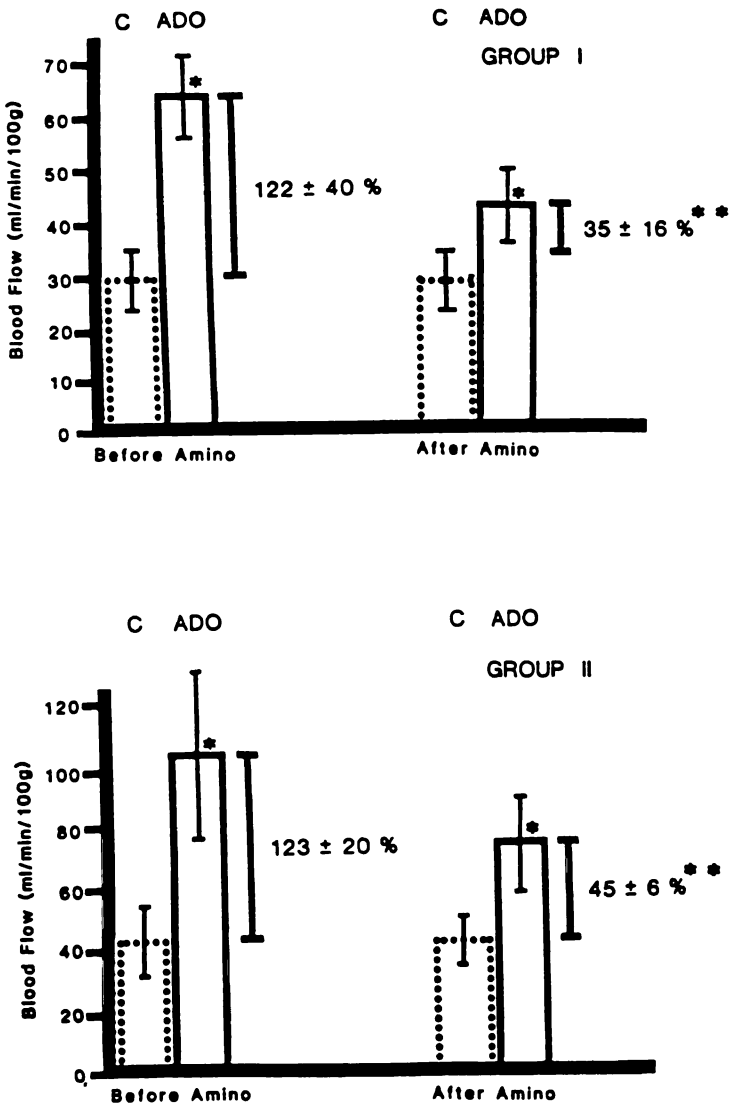

Figure 14

Effect of intra-arterial infusion of adenosine on intestinal blood flow before and after aminophylline in *groups I* and *II*. Adenosine (1 µmol/min) was given intra-arterially in each experiment to test efficacy of aminophylline. C, control blood flow; ADO, blood flow during adenosine infusion. *P<0.05 relative to C. **P<0.05 relative to before aminophylline. N=5 for *group I*; N=8 for *group II*.

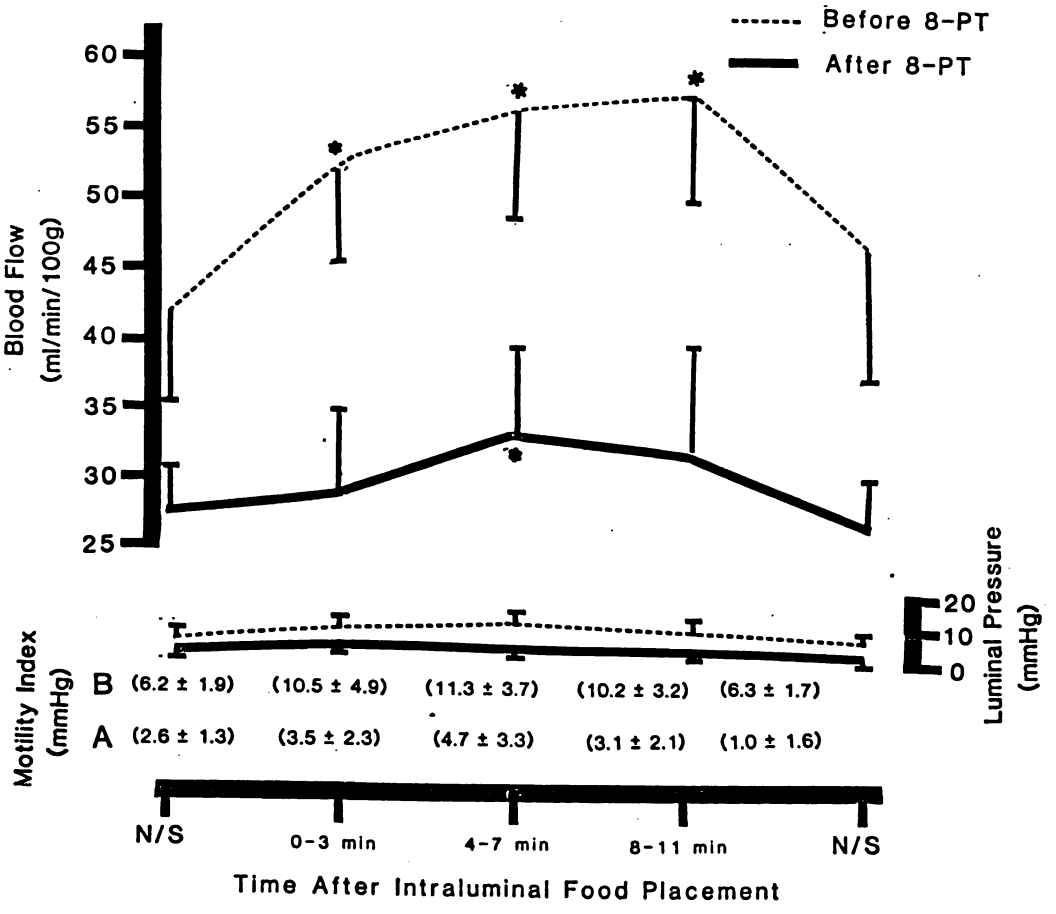

Figure 15

Effect of 8-phenyltheophylline (8-PT; 0.4 µmol/min) on food-induced changes in blood flow, luminal pressure and motility index. *B*, before 8-PT; *A*, during 8-PT. *P<0.05 relative to normal saline (N/S) level before food placement; n=6.

before 8-PT, and 8-PT significantly attenuated the hyperemia ($P<0.05$). The motility responses to food in this series of experiments also fell into two groups. In 3 of 6 animals, food increased luminal pressure (from 5 $\pm$ 2 to 15 $\pm$ 3 mmHg) before 8-PT, and the increases were smaller after 8-PT (from 3 $\pm$ 2 to 4 $\pm$ 4 mmHg) than before. In the remaining animals, food had no significant effect on luminal pressure before (from 5 $\pm$ 3 to 4 $\pm$ 2 mmHg) or after (1 $\pm$ 1 to 1 $\pm$ 1 mmHg) 8-PT. Because 8-PT attenuated the food-induced hyperemia in both groups to the same degree (+14 $\pm$ 10% and +19 $\pm$ 15% increases in flow after 8-PT in high and low motility groups, respectively), the data were analyzed together as one population. Control blood flow, luminal pressure and motility index was not significantly affected by 8-PT ($P>0.05$). As can be seen in Figure 16, food markedly increased oxygen consumption, and the increase was unaffected by 8-PT.

Figure 17 shows the effect of 8-PT on adenosine-induced vasodilations. Adenosine significantly decreased perfusion pressure in a dose-dependent fashion before 8-PT, and 8-PT dose-dependently attenuated the adenosine-induced vasodilations. During 0.4 μmol/min 8-PT infusion, adenosine did not significantly alter vascular perfusion pressure at infusion rates equal to or less than 0.4 μmol/min. The vasodilation induced by 1 μmol/min adenosine during 0.4 μmol/min 8-PT was significantly less than that produced before 8-PT ($P<0.05$). This dose of 8-PT was the dose used to test its effect on the food-induced hyperemia.

Series X: Figure 18 shows the effect of luminal placement of food on jejunal blood flow and oxygen consumption before and during dipyridamole. Intra-arterial infusion of dipyridamole significantly increased resting blood flow, i.e. normal saline in lumen, from 39.7 $\pm$ 3.4 to 43.9 $\pm$ 4.5 $ml \cdot min^{-1} \cdot 100g^{-1}$ ($P < 0.05$), but had no significant effect on resting oxygen consumption (2.44 $\pm$ 0.22 before vs. 2.55 $\pm$ 0.22 $ml \cdot min^{-1} \cdot 100g^{-1}$ during dipyridamole) ($P > 0.05$). Before dipyridamole, food significantly increased blood flow and oxygen consumption for the

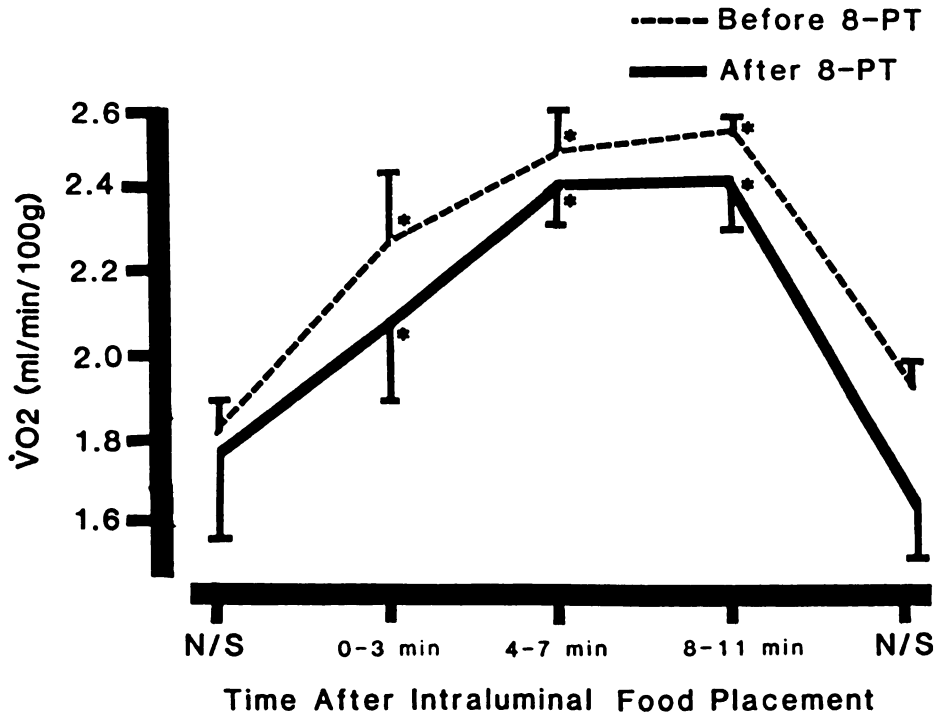

Figure 16

Effect of food on jejunal oxygen consumption before and during 8-phenyltheophylline (8-PT; 0.4 μmol/min). *P<0.05 relative to normal saline (N/S) level before food placement.

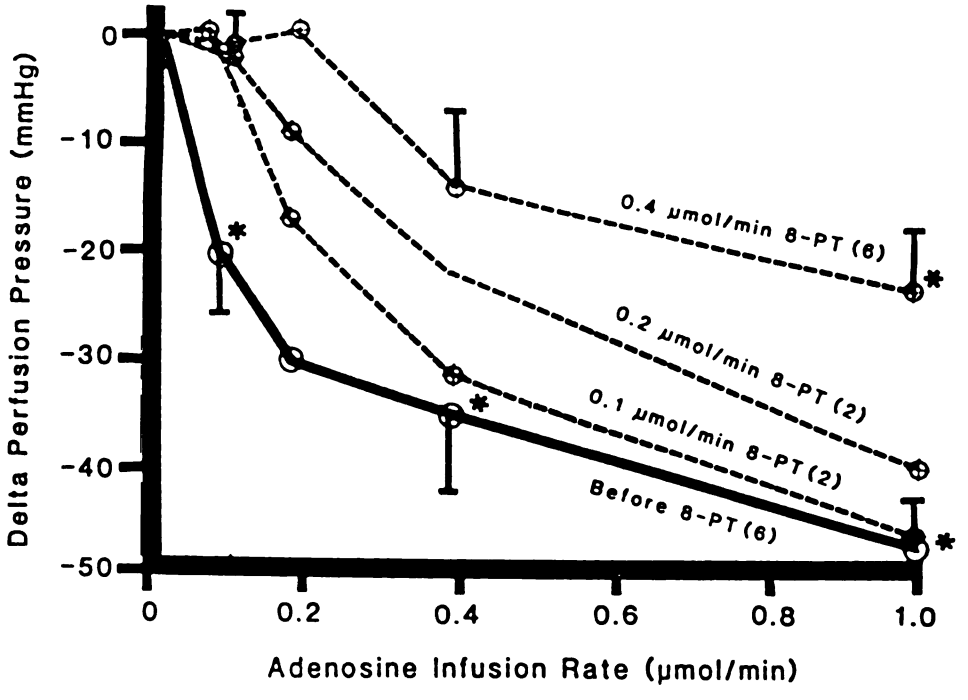

Figure 17

Effect of 8-phenyltheophylline (8-PT) at 3 infusion rates on adenosine-induced vasodilation. *P<0.05 relative to no adenosine infusion. Resting perfusion pressure were 110 ± 5 mm Hg before 8-PT and 113 ± 9 mm Hg during 8-PT (0.4 μmol/min). Number in parenthesis indicates number of experiments.

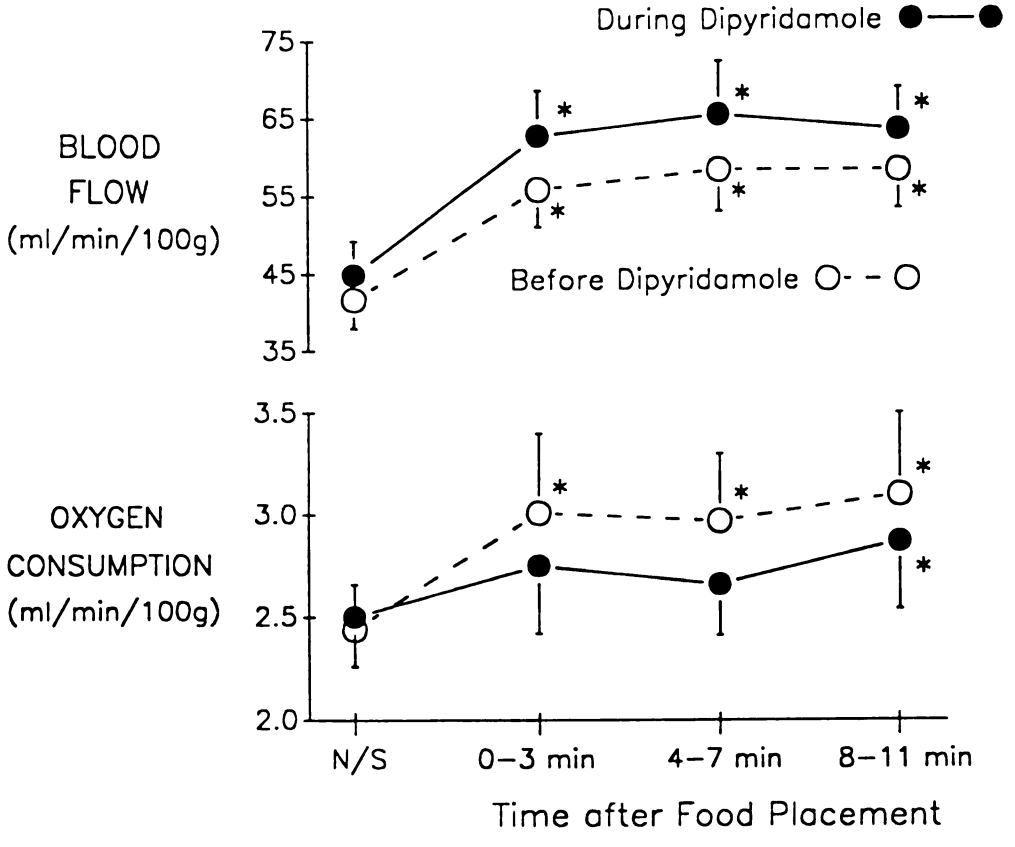

Figure 18

Effect of dipyridamole on the food-induced changes in blood flow and oxygen consumption. *P<0.05 relative to normal saline (N/S) level before food placement. N=9.

entire 15 min placement period. During dipyridamole, food increased blood flow for the entire 15 min period, but did not increase oxygen consumption until 8-11 min after the food placement. Table 2 shows the percent changes in blood flow and oxygen consumption which were induced by food before and during dipyridamole. Dipyridamole significantly ($P < 0.05$) enhanced the increase in blood flow which occurred during the 4-7 min period after food placement. During this period, blood flow increased by 42.6 $\pm$ 8.0% before and 49.4 $\pm$ 10.6% during dipyridamole. However, dipyridamole had no effect on the increase in blood flow which occurred during the 0-3 or 8-11 min period after the food placement. As was shown in Figure 18, food increased oxygen consumption before dipyridamole, but did not alter oxygen consumption during dipyridamole until the 8-11 min period after placement. The increase in oxygen consumption which occurred during this latter period of food placement was not significantly attenuated by dipyridamole ($P < 0.05$).

Series XI: The effect of adenosine deaminase on the food-induced increase in blood flow and oxygen consumption is shown in Figure 19. Food increased blood flow and oxygen consumption before adenosine deaminase. During adenosine deaminase, however, food did not alter blood flow at any time, and increased oxygen consumption only during the 8-11 min period after placement. During this latter placement period, oxygen consumption increased by +26.9 $\pm$ 9.9% before adenosine deaminase and by a similar magnitude ($P > 0.05$) during adenosine deaminase (+36.8 $\pm$ 12.3%). Adenosine deaminase did not significantly alter resting control blood flow or oxygen consumption.

Table 2

Effect of dipyridamole on food-induced changes in jejunal blood flow and oxygen consumption.

	Time After Food Placement		
	0-3 min	4-7 min	8-11 min
Before Dipyridamole			
%Δ Blood Flow	36.1 ± 5.3%*	42.6 ± 8.0%*	46.0 ± 12.0%*
%Δ Oxygen Consumption	21.9 ± 7.2%*	21.6 ± 7.6%*	25.5 ± 8.4%*
During Dipyridamole			
%Δ Blood Flow	40.0 ± 6.5%*	49.4 ± 10.6%*†	43.4 ± 10.2%*
%Δ Oxygen Consumption	8.2 ± 5.3%	6.5 ± 3.2%	13.7 ± 5.1%*

All values are percent change from resting level before food placement, i.e. normal saline in lumen, which were blood flow 39.7 ± 3.4 before and 43.9 ± 4.5 ml/min/100g during dipyridamole and oxygen consumption 2.44 ± 0.22 before and 2.55 ± 0.22 ml/min/100g during dipyridamole. * Denotes that the increase is significant at $P < 0.05$. † $P < 0.05$ relative to corresponding level before dipyridamole.

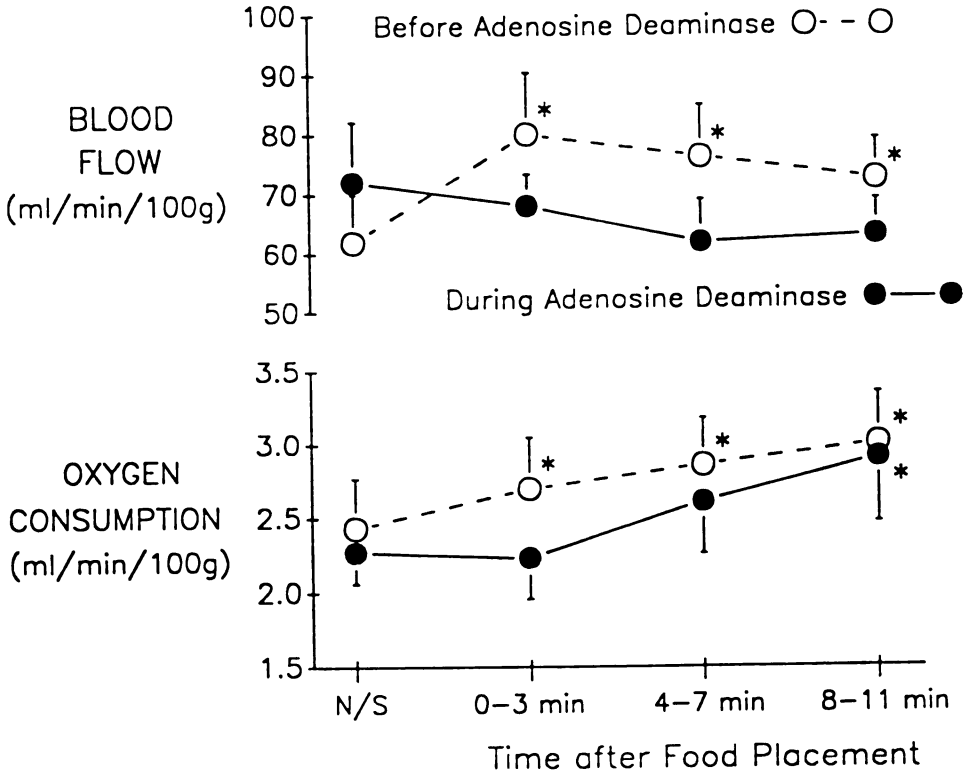

Figure 19

Effect of adenosine deaminase on the food-induced changes in blood flow and oxygen consumption. *P<0.05 relative to normal saline (N/S) level before food placement. N=7.

DISCUSSION

Adenosine is a Vasodilator in the Intestinal Mucosa

Postprandial intestinal hyperemia is primarily localized in the mucosa of the jejunum, where nutrients are absorbed (38,82). Therefore, before establishing adenosine as a mediator of this hyperemia, adenosine must be shown to be a vasodilator in this tissue. The results from the first series of experiments show that adenosine is indeed a vasodilator in the intestinal mucosa. Under the natural flow condition, intra-arterial adenosine increases mucosal as well as muscularis blood flows in the proximal jejunum, and the increases in blood flow in these two compartments are not significantly different (Figure 2A). As a result, i.a. adenosine has no significant effect on distribution of total gut wall blood flow to the mucosal and muscularis layers (Figure 2B). Under the constant flow condition, adenosine decreases jejunal vascular resistance and does not significantly alter the blood flow distribution between the mucosal and muscularis layers (Figure 2B). Therefore, adenosine produces equal vasodilation in both mucosal and muscularis layers of the jejunum. The doses of adenosine utilized in these studies were those which produce a near maximal increase in total blood flow, i.e. 0.1 and 1.0 μmol/min under constant and natural flow conditions, respectively. Less adenosine was required for maximal vasodilation under constant flow than under the natural flow condition because blood flow increased under the natural flow condition, thereby diluting the administered adenosine.

In order to assess further if adenosine is a vasodilator in the jejunal mucosa, venous outflow was measured while adenosine or its non-metabolizable analogues CHA and NECA were placed into the jejunal

lumen. NECA at concentrations above 10^{-4} M, CHA above 10^{-3} M, and adenosine at 10^{-2} M significantly increased venous outflow (Figure 4). The increased venous outflow is likely to result from exposure of the mucosal vasculature to these compounds because almost all ^{3}H-adenosine which entered the jejunal tissue are localized in the mucosa. At concentrations below that stated above, these compounds did not significantly alter blood flow. This finding agrees with that of Proctor (199,200), who also found that mucosal application of NECA at 10^{-6} and 10^{-4} M, CHA at 10^{-6} and 10^{-4} M, and adenosine at 10^{-5} to 10^{-2} M did not significantly alter steady-state jejunal blood flow in rats as calculated from the diameter of submucosal arterioles and red blood cell velocity. As shown in Figure 5, adenosine at 10^{-2} M did not produce a sustained hyperemia throughout the entire 15-min placement period. This finding is also in agreement with that of Proctor (199), who has found that adenosine at 10^{-2} M did not produce a sustained increase in blood flow. We did not test the effect of adenosine at higher concentrations, because adenosine saturated in aqueous solution at 10^{-2} M. The relative impotency and transiency of the adenosine-induced hyperemia found in our study (Figure 4 and 5) and by Proctor (199) might be due to a rapid reuptake and degradation of adenosine by the parenchymal cells of the intestinal mucosa (4,157,189,200). In contrast, NECA and CHA are non-metabolizable, and therefore are protected from degradation, producing a sustained and more potent hyperemia.

The increased blood flow induced by luminal placement of adenosine and its analogues might be due to stimulation of mucosal nerves. In order to rule out this possibility, the effect of luminal placement of these compounds was determined before and after anesthetizing the mucosal surface with a local anesthetic, dibucaine. As shown in Figure 6, dibucaine treatment did not alter the hyperemia induced by these compounds. In addition, the hyperemia produced by these compounds developed slowly, reaching peak levels a few minutes after their placement (Figure 5), while the hyperemia induced by stimulation of

mucosal nerves develops rapidly and reaches a peak within several seconds after the stimulation (15,16). Therefore, the vasodilatory action of luminal placement of these compounds is unlikely due to stimulation of the mucosal nerves.

Although the vasoactivity of adenosine has not been determined previously in the jejunal mucosa, its vasoactivity has been studied in the ileal mucosa (93,220,247). The results from these studies, however, are conflicting. Our finding that adenosine increases mucosal blood flow by 146 $\pm$ 47% in the canine ileum (Figure 3) is in agreement with that of Walus et al. (247). Utilizing the microsphere technique they found that adenosine increases mucosal blood flow by 147 $\pm$ 39% in the canine ileum. Utilizing laser Doppler flowmetry, however, Shepherd et al. (220) found that adenosine decreases mucosal blood flow in the canine ileum. The discrepancy between these results might be due to the difference in technique. For example, the microsphere technique measures total blood flow to the entire mucosal layer, whereas laser Doppler flowmetry might measure only the blood flow to the superficial portions of the mucosa. Laser Doppler flowmetry has a spatial resolution of 0.5-1.0 mm, which is less than the total thickness of the canine mucosa, i.e., 2.5 mm (88). Despite the finding that adenosine decreased the ileal mucosal flow, the authors of this study (220) have suggested that adenosine might be a vasodilator in the mucosa. They predicted from their model that the decreased mucosal flow results from a "vascular steal" mechanism. In other words, they predicted that adenosine produces greater vasodilation in the muscularis than in the mucosa and, as a result, redistributes total intestinal blood flow from the mucosa to the muscularis. Utilizing the microsphere technique, adenosine has been shown to decrease mucosal blood flow in the feline ileum (93), suggesting that the vasoactivity of adenosine in the ileal mucosa varies between cats and dogs. However, the present study shows that adenosine is a vasodilator in the feline ileum (Figure 3). Unknown technical differences might explain the discrepancy between the present

results and the results of these previous microsphere studies.

Interstitial Fluid Adenosine Concentration Increases During Postprandial Intestinal Hyperemia

The aim of the next series of experiments was to test another criterion of the adenosine hypothesis, i.e., interstitial fluid adenosine concentration (ISF [ADO]) must increase during the postprandial hyperemia (11). As shown in Figures 7 and 8, placement of food into the jejunal lumen significantly increased adenosine release into the local venous blood and its adenosine concentration, i.e. indices of ISF [ADO]. The food placement also tended to increase lymphatic adenosine concentration and release (Figure 10). Placement of normal saline into the jejunal lumen, on the other hand, did not alter blood flow, oxygen consumption, nor adenosine release and venous adenosine concentration (Figure 7). This indicates that the increase in adenosine release was not due to physical (e.g., distension) or time factors, but was due to the presence of food constituents in the jejunal lumen. Our study, therefore, provides further evidence to support the hypothesis that adenosine plays a role in postprandial intestinal hyperemia.

The increase in adenosine release into the venous blood occurred only during the first 7 min of food placement, while the increases in blood flow and oxygen consumption remained elevated for the entire 15 min placement period. A similar transient increase in adenosine release with sustained increases in blood flow and oxygen consumption have also been shown in metabolic hyperemia of the heart (8,54,71,107,209,248). This transient increase of adenosine release in the heart has been predicted from the hypothesis that adenosine is formed from ATP of parenchymal cells in response to a decrease in oxygen supply-to-demand ratio (227). When metabolism is initially increased, oxygen demand is higher than oxygen supply, and adenosine formation increases. The enhanced adenosine increases blood flow until a point is reached where

oxygen supply equals oxygen demand, and then adenosine formation falls. In the present study, both oxygen supply and oxygen demand increased to the same extent after placement of food into the jejunal lumen (Table 1). As a result, food placement did not significantly alter the oxygen supply-to-demand ratio throughout the entire 15 min placement period. The increased adenosine formation in the present study, therefore, cannot be explained by a decrease in oxygen supply-to-demand ratio. In the heart, adenosine formation also increases in response to a decrease in cytosolic ATP phosphorylation potential, as produced by i.a. administration of norepinephrine or isoproterenol (25, 107,108). This decrease in ATP phosphorylation potential appears to be a response to an increase in cardiac energy output and might occur independently of a decrease in oxygen supply-to-demand ratio (25). In isolated guinea pig hearts, norepinephrine produces a phasic increase in adenosine release, which is accompanied by a phasic decrease in ATP phosphorylation potential (107). Whether or not jejunal ATP phosphorylation potential decreases during the food-induced hyperemia is presently unknown. In addition to parenchymal ATP, other cellular and metabolic sources of adenosine exist. These sources include vascular endothelium, purinergic nerves, and degradation of cAMP and S-adenosylhomocysteine (227). The regulators of adenosine release from these sources are not fully known.

The Food-induced Increase in ISF [ADO] is Sufficient to Play a Role in Postprandial Intestinal Hyperemia

The above results indicate that adenosine is a vasodilator in the intestinal mucosa (Figure 2 - 5), i.e. the primary site of the postprandial intestinal hyperemia (38,82), and that the hyperemia is accompanied by increases in jejunal adenosine concentration and release (Figure 7 - 10), i.e. indices of interstitial fluid adenosine concentration (ISF [ADO]). However, one cannot definitely conclude that adenosine mediates this hyperemia without showing that mucosal ISF [ADO] increases sufficiently to produce mucosal vasodilation. Intestinal ISF

[ADO] has not yet been determined, but data from the heart and skeletal muscle can be utilized to speculate on the ISF [ADO] and whether or not adenosine could mediate the food-induced hyperemia. In these organs, adenosine is produced and released into the interstitial fluid, and then parenchymal cells and cells lining the vascular system, such as endothelial and smooth muscle cells, rapidly take adenosine up and metabolize it by adenosine deaminase and adenosine kinase (227,228). As much as 80-90% of intra-arterially infused adenosine is removed by re-uptake and subsequent metabolism, chiefly by endothelial cells, during a single passage through these organs. Interstitial fluid adenosine concentration is estimated to be 2-10 times greater than venous [ADO] (103,109,186,227,228,234,249). In the intestine, epithelial cells take up adenosine by equilibrative, facilitated diffusion and high affinity, concentrative, energy-dependent Na^+-cotransport (189). Degradation of adenosine by adenosine deaminase is another potentially important route of adenosine removal in the intestine mucosa, since this tissue has a high level of this enzyme (4,55,94,157). In addition, blood cells such as lymphocytes and erythrocytes also incorporate and metabolize adenosine (163,173). Therefore, the venous adenosine concentration during nutrient absorption might reflect only 10-20% of the adenosine in the interstitial space.

In the present study, jejunal venous [ADO] increased by 0.056 μM (range: 0.002-0.202 μM) during the food-induced hyperemia (Figure 8). Assuming that jejunal mucosal ISF [ADO] is 10 times that of its venous [ADO], the nutrient-induced increase in mucosal ISF [ADO] could be about 0.56 μM (range:0.02-2.02 μM). Near maximal vasodilation was produced in the intestinal mucosa by intra-arterial infusions of adenosine at 1 μmol/min (in natural flow condition) and 0.1 μmol/min (in constant flow condition) (Figure 2), which increased local arterial [ADO] by 44 ± 1 μM (range: 42-48 μM) and 11 ± 2 μM (range: 5-15 μM), respectively. Assuming that only 10% of the infused adenosine in the present study reaches the vascular smooth muscle of the mucosal vasculature, the

increases in vascular smooth muscle [ADO] could be only 0.5-4.8 μM. Walus et al (247) have shown that mucosal vasodilation can be produced by increasing arterial [ADO] by 0.4-3 μM, which might increase mucosal vascular [ADO] by 0.04-0.3 μM. The expected mucosal ISF [ADO] during luminal placement of adenosine (Figure 4) is even more difficult to estimate. Only 1.7% of the tritium placed into the jejunal lumen entered the mucosal tissue. Assuming that this is all adenosine and that water represents 60% of the mucosal tissue, the mucosal tissue [ADO] could be increased by 247 ± 44 μM (range: 94-367 μM). However, much of the tritium entering the mucosa might represent adenosine metabolites since adenosine had 100-300 less vasodilatory potency than the non-metabolizable analogues, CHA and NECA (Figure 4). If this lower vasodilatory potency is due to adenosine degradation, the increase in mucosal ISF [ADO] might be as little as 0.3-1.2 μM. The estimated mucosal vascular [ADO] during i.a. infusions of adenosine in the present study (0.5-4.8 μM) and Walus et al's study (0.04-0.3 μM), and during luminal placement of adenosine (0.3-1.2 μM) appears to be within the range of the estimated increase in mucosal ISF [ADO] during nutrient absorption as described above, i.e., 0.56 μM (range: 0.02 - 2.02 μM). Therefore, the increase in ISF [ADO] during nutrient absorption might be sufficient to be a mediator in the nutrient-induced hyperemia.

Postprandial Intestinal Hyperemia is Attenuated by Adenosine Receptor Antagonists and Enhanced by Dipyridamole

Another method to determine whether or not adenosine plays a role in the postprandial hyperemia is to test the effect of adenosine receptor antagonists, i.e theophylline, and adenosine deaminase on the hyperemia. The effect of adenosine antagonists and adenosine deaminase on the food-induced hyperemia has been previously investigated (90,199), but the results are conflicting. In one study performed by Granger and Norris (90), theophylline had no significant effect on resting blood

flow or oxygen consumption in a jejuno-ileal preparation of anesthetized dogs, nor did it have a significant effect on the food-induced increase in blood flow or oxygen consumption. In the other study performed by Proctor (199), serosal suffusion of rat jejunal flaps with theophylline or adenosine deaminase had no effect on resting control blood flow, but these two chemicals attenuated the hyperemia produced by mucosal suffusion with a solution containing oleic acid, glucose and bile.

The discrepancy between the above two studies may be related to differences in preparation, food solution, method of measuring blood flow, route of drug administration, and the occurrence of other parameters (e.g., motility) which can affect blood flow. First of all, while Proctor measured blood flow in the rat jejunum, Granger and Norris measured blood flow to the region of the dog intestine ranging from the proximal jejunum to the distal ileum. Because most of the absorption of carbohydrates, proteins and fats after a meal occurs in the jejunum, we decided to measure the postprandial hyperemia specifically in this region. Secondly, the techniques of blood flow measurement are different. Granger and Norris measured blood flow from electromagnetic flowprobes placed on the superior mesenteric artery perfusing their intestinal preparation, while Proctor calculated blood flow from arteriolar diameter and red blood cell velocity measured from video microscopy of submucosal arterioles. Finally, neither investigator reported measurements of other parameters, such as motility, which can also affect blood flow (33,37,138). This is important since adenosine decreases intestinal motility (96-100), Na^+ and Cl^- absorption (55,94) and oxygen consumption (93,220), and all of these intestinal functions play a role in regulating intestinal blood flow (33,37,80,138,160,224). In the presence of adenosine antagonists, one or more of these intestinal functions can be enhanced, thereby altering the effect of the adenosine antagonist on the food-induced hyperemia. With regards to motility, Granger and Norris did not measure motility, and Proctor suppressed motility by isoproterenol administration. Therefore, the

differences in their results may be due to presence and absence of motility.

Our results show that the effect of aminophylline on food-induced hyperemia was indeed influenced by intestinal motor activities. Aminophylline, at the dosage which inhibited exogenous adenosine-induced vasodilation (Figure 14), blocked the food-induced hyperemia when motility was low (Group II; Figure 13) but had no effect on the hyperemia when motility was high (Group I; Figure 12). The former observation is in agreement with the result of Proctor (199) whereas the latter observation agrees with that of Granger and Norris (90). It is possible that when aminophylline enhanced food-induced motility in Group I (Figures 11 and 12), the enhanced motility increased blood flow (33,37,138) which masked the attenuating effect of aminophylline on the food-induced hyperemia (Figure 13). Another possibility is that the enhanced motility produced a greater and sufficient amount of adenosine which competed with aminophylline for adenosine receptors, thereby reducing the aminophylline inhibitory effect. The competition between adenosine and theophylline for adenosine receptors has been observed in the intestine and the heart (11,247). The enhancement of motility by methylxanthines has been demonstrated earlier in hypermotile strips of guinea pig ileum (96,97,130) and may be related to inhibition of adenosine receptors responsible for modulating cholinergic nerve transmission. Adenosine inhibited motility in our study, particularly when baseline motility was high, and this has also been observed by others.

Aminophylline and theophylline have been shown not only to block adenosine receptors but also to inhibit cAMP phosphodiesterase, the enzyme responsible for degradation of cAMP (225). Phosphodiesterase inhibition increases intracellular cAMP concentration, which is known to decrease intestinal motility (96,97,100), Na^+, Cl^- (55,94,158) and water (59) absorption and vascular resistance (137) and increase Cl^- secretion (55,94,158). Although the concentration of aminophylline producing

these effects is not exactly known, the concentrations of theophylline required to produce these effects are much higher than those of aminophylline used in our study. Therefore, it is unlikely that aminophylline inhibited phosphodiesterase to a significant degree in this study. Indeed, aminophylline increased rather than decreased motility. However, in the interpretation of the aminophylline data one should consider its effect on phosphodiesterase, which might influence its effect on adenosine receptor blockade. For example, alteration in intestinal absorption could influence absorption-related hyperemia.

8-phenyltheophylline (8-PT) was used in this study because it has been shown to be a more potent and specific adenosine antagonist than aminophylline or theophylline (225). In this study, 8-PT was approximately 100-X more potent than aminophylline, on a molar basis. 8-PT has less phosphodiesterase inhibitor activity than theophylline or aminophylline (55,225), has minimal inhibitory effects on motility (96), and no effects on Na^+ and Cl^- absorption (55). The present results show that 8-PT had minimal and statistically insignificant motility effects. 8-PT also inhibited the food-induced hyperemia (Figure 15) whether the intestinal motility was high or low.

The purpose of the next two series of experiments was to further test the role of adenosine in the intestinal hyperemia by utilizing dipyridamole and adenosine deaminase. Dipyridamole is recognized as a potent inhibitor of cellular uptake of adenosine by numerous cell types (122,134,238). Inhibition of adenosine uptake should result in accumulation of adenosine in the interstitial space because the major fate of interstitial adenosine is uptake and metabolism (4,122,227,228). If the postprandial hyperemia is mediated by an increase in interstitial fluid adenosine concentration (ISF [ADO]), then dipyridamole should enhance the hyperemia by potentiating the increase in ISF [ADO]. The present study shows that dipyridamole indeed mildly enhanced the postprandial hyperemia (Figure 18 and Table 2). In contrast, adenosine deaminase degrades adenosine into inosine and therefore should attenuate

adenosine accumulation in the interstitial space. Proctor (199) has already shown that this enzyme attenuates the hyperemia induced by glucose-oleic acid in rats. As shown in Figure 19, adenosine deaminase similarly blocked the hyperemia which is induced by a mixture of carbohydrates, proteins and fats in dogs. These results further support adenosine's role in the postprandial intestinal hyperemia.

As shown in Figure 18, dipyridamole significantly enhanced resting blood flow when the lumen contained normal saline. This indicates that under the resting condition, inhibition of adenosine uptake can increase interstitial adenosine to vasoactive levels. Dipyridamole also increases resting blood flow in the heart (127,249) and skeletal muscle (133), and these increases are accompanied by increases in interstitial fluid adenosine concentration (249). However, it is unlikely that resting blood flow is normally maintained by adenosine, since it is not altered by adenosine receptor antagonists, i.e. aminophylline and 8-phenyltheophylline or adenosine deaminase (199) (Figure 12, 13, 15 and 19).

Dipyridamole blocked the increase in oxygen consumption which occurs during the first 0-7 min after intraluminal food placement (Figure 18 and Table 2). Endogenous adenosine may act to limit oxidative metabolism during this initial period of nutrient absorption. As described above, adenosine decreases intestinal motility (96-100), Na^+ and Cl^- absorption (55,94) and oxygen consumption (93,220). These effects of adenosine are consistent with the concept that adenosine is a "retaliatory metabolite", which decreases energy demand during enhanced energy utilization (178). The decrease in intestinal oxygen consumption might be expected to attenuate the food-induced hyperemia, since these two variables are closely related (79,80,164). However, dipyridamole did not alter or enhanced the food-induced hyperemia (Figure 18 and Table 2). The presence of the food-induced hyperemia despite an attenuated oxygen consumption might reflect an increase in interstitial adenosine concentration despite an attenuated oxidative metabolism and

adenosine production. This could only occur if dipyridamole indeed inhibited adenosine uptake and metabolism.

Adenosine deaminase also attenuated the food-induced increase in oxygen consumption (Figure 19). This is a paradoxical finding if endogenous adenosine indeed limits oxidative metabolism, since adenosine deaminase would be expected to enhance oxygen consumption. Perhaps adenosine deaminase reduced oxidative metabolism by a mechanism unrelated to adenosine degradation, and the inhibition of the food-induced hyperemia may have been mediated by this reduction in oxidative metabolism. However, such an effect of adenosine deaminase on oxidative metabolism has never been shown. Another possibility is that adenosine plays an essential role in mediating the food-induced increases in capillary surface area (80,165) as well as blood flow, i.e. two factors which regulate intestinal oxygen supply (80). Adenosine deaminase might block the food-induced increases in blood flow and capillary surface area sufficiently to decrease oxygen supply, thereby limiting the rate of oxidative metabolism during nutrient absorption. These and other mechanisms for the adenosine deaminase-induced decrease in oxygen consumption are important areas for future investigation.

Role of Adenosine in Postprandial Intestinal Hyperemia

The above studies show that jejunal venous adenosine concentration and release increase only during the first 3-7 min of intraluminal food placement (Figure 7 and 8). This is consistent with the results utilizing dipyridamole, since this drug enhanced the food-induced hyperemia and attenuated the increase in oxygen consumption only during this initial period (Figure 18). However, adenosine deaminase, aminophylline, theophylline and 8-phenyltheophylline attenuated the hyperemia which occurs during the entire period of intraluminal food placement (Figure 13, 15 and 19) (199). A similar discrepancy exists in the heart. Aminophylline inhibits both the initial and plateau

components of the pacing-induced hyperemia in the heart (202), but adenosine release is increased only during the initial phase (109,227). The authors have not made comments on this discrepancy, and the reason for the discrepancy in their and our studies is unclear. There are only two possible ways to reconcile this discrepancy. The first possibility is that adenosine mediates the entire food-induced hyperemia. If this is true, then venous adenosine concentration observed during the last 7 min of the hyperemia must not reflect interstitial fluid adenosine concentration. The second possibility is that adenosine only mediates the initial 7 min of the hyperemia. This could occur if aminophylline, 8-PT and adenosine deaminase block not only the effect of endogenous adenosine, but also the vasodilation produced by other factors which maintain food-induced hyperemia. At the present time, there are no direct experimental data for the intestinal circulation to support or refute either possibility. These possibilities can only be speculated upon based on indirect data and the data from other circulations.

The first possibility is that adenosine production and its interstitial concentration are increased throughout the entire food placement period and play a role in the hyperemia during this entire period. During the initial 7 min, adenosine production might overwhelm its removal, resulting in a significant increase in venous adenosine concentration and release. However, the rate of adenosine removal could gradually increase and eventually equal the rate of production during the last 7-8 min of food placement, resulting in no significant increase in venous adenosine concentration and release. For example, adenosine uptake by vascular endothelium increases when capillary surface area increases (234), as will happen during the postprandial hyperemia (165). As shown in Figure 8, as well as in our previous study (223), tissue oxygen extraction (arteriovenous oxygen content difference) progressively increases after placement of food into the jejunum. The progressive increase in tissue oxygen extraction would suggest a parallel progressive increase in capillary surface area. Other

mechanisms, such as adenosine deaminase activity, might increase with time as well. In order to assess this hypothesis, the rates of adenosine re-uptake and degradation by the intestine should be determined during resting and absorptive states.

The alternative to the above hypothesis is that adenosine only directly mediates the initial 7 min of the food-induced hyperemia. The factors which have been proposed to play a role in the maintenance of the food-induced hyperemia include histamine, gastrointestinal peptides, local nerves, and villus hypertonicity (39,80). These factors might act either directly or indirectly (mediated by their actions on intestinal functions and metabolism) on vascular smooth muscle to produce a sustained vasodilation. As described above, aminophylline and 8-PT inhibit cAMP phosphodiesterase, which will increase cAMP and decrease intestinal functions and metabolism. Therefore, aminophylline and 8-PT could attenuate the hyperemia via their action on cAMP levels which in turn decrease intestinal functions and metabolism. Secondly, methylxanthines decrease norepinephrine degradation, and increase intracellular Ca^{++} (201), which could produce vasoconstriction and directly attenuate the hyperemia. Thirdly, it is possible that the initial hyperemia, induced by adenosine, triggers an accommodation response which maintains the hyperemia, i.e. flow-mediated vasodilation (62,77). If this were the case, then blockade of the initial hyperemia by aminophylline and 8-PT might abolish the flow-mediated vasodilation triggered by the initial adenosine-induced hyperemia. This is supported by the results utilizing adenosine deaminase since this enzyme is expected to specifically act by decreasing interstitial fluid adenosine concentration.

SUMMARY AND CONCLUSIONS

Intestinal blood flow increases after a meal, and the stimulus for this hyperemia are the products of food digestion. Factors such as nerves, gastrointestinal hormones and polypeptides, villus hyperosmolarity, histamine, oxidative metabolism and prostaglandins have been proposed to play a role in this hyperemia. The purpose of the present study was to determine the role of adenosine in the hyperemia. The following results were obtained and conclusions drawn from these studies.

A. Adenosine is a vasodilator in the intestinal mucosa.

1. Intra-arterial infusion of adenosine increases mucosal as well as muscularis blood flows in the canine jejunum and canine and feline ileum, as determined by microspheres. The increases in blood flow in the mucosal and muscularis tissues are not significantly different.

2. Under the constant flow condition, i.a. adenosine decreases jejunal vascular resistance, but does not alter the blood flow distribution between the mucosal and muscularis layers. Thus, adenosine produces equal vasodilation in these two tissue layers.

3. Intraluminal placement of adenosine and non-metabolizable analogue [5'-N-ethylcarboxamide adenosine (NECA) and N^6-cyclohexyladenosine (CHA)] dose-dependently increases jejunal blood flow.

   a. The hyperemia resulted from exposure of the mucosal surface to these compounds, since almost all of the adenosine absorbed from the lumen was localized in the mucosal tissue.

   b. The hyperemia was not mediated by stimulation of mucosal nerves, as the hyperemia was unaltered after treating the mucosal surface with a local anesthetic.

B. Jejunal interstitial fluid adenosine concentration (ISF [ADO]) increases during the food-induced hyperemia.

1. The food-induced hyperemia is accompanied by increases in jejunal venous and lymphatic adenosine concentration and release, i.e. indices of ISF [ADO].

   a. The increase in ISF [ADO] is not due to a decrease in oxygen-to-supply ratio, since this variable does not change during the hyperemia.

   b. The estimated increase in ISF [ADO] appears to be sufficient for adenosine to play a role as a vasodilator in the food-induced hyperemia.

2. Intraluminal placement of normal saline does not increase venous adenosine concentration or release, indicating that the food-induced increases are due to the constituents of food and not related to physical (i.e. luminal distension) or time factors.

3. Jejunal venous adenosine concentration and release increase during reactive hyperemia following a 1 min arterial occlusion.

C. Adenosine antagonists, i.e. aminophylline, 8-phenyltheophylline and adenosine deaminase, attenuate and an inhibitor of cellular adenosine reuptake, dipyridamole, enhances the food-induced hyperemia.

1. The effect of aminophylline on the hyperemia is complicated by motility. When motility is low, aminophylline blocks the hyperemia. However, when food increases motility, aminophylline enhances the food-induced motility and has no effect on the hyperemia.

2. The more potent and specific adenosine antagonist, 8-phenyl-theophylline, blocks the food-induced hyperemia whether motility is high or low.

3. The effect of dipyridamole on the hyperemia is complicated by an inhibitory effect of this compound on the food-induced increase in oxygen consumption.

In conclusion, adenosine appears to play a role in the food-induced intestinal hyperemia. The effects of aminophylline and dipyridamole on the hyperemia are complicated by counteracting effects of these compounds on motility and oxygen consumption.

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