RELATIONSHIP BETWEEN GASTROINTESTINAL PEPTIDES, INTESTINAL WALL COMPLIANCE, AND VASCULAR RESISTANCE

by

Andre Joseph Premen

Dissertation submitted to the Faculty of the Department of Physiology
Graduate Program of the Uniformed Services University of the
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Doctor of Philosophy 1983

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ABSTRACT

Title of Dissertation: Relationship between Gastrointestinal Peptides, Intestinal Wall Compliance, and Vascular Resistance

Andre Joseph Premen, Candidate for Doctor of Philosophy, 1983

Dissertation directed by: Dr. David Dobbins, Associate Professor, Department of Physiology, USUHS

Numerous peptides have been isolated from the wall of the gastrointestinal tract. Among the actions reported for these peptides are effects on vascular or intestinal smooth muscle. Yet, few reports cite the simultaneous actions of these peptides on both types of muscle. Thus, their vasoactivity in the intestine may be under or over-estimated. Therefore, the aim of this investigation was to ascertain the simultaneous effects of local intraarterial infusions of synthetic gastrointestinal peptides on both intestinal vascular and visceral smooth muscle. Studies were designed to indicate which peptides may be involved in the postprandial changes in ileal vascular and visceral smooth muscle. The experiments conducted allowed for the concurrent assessment of vascular resistance and ileal wall compliance. Previously fasted adult mongrel dogs were anesthetized, intubated and ventilated. A midline incision was produced through the abdomen and a segment of ileum exteriorized. The segmental artery was isolated and the ileum perfused at constant flow with arterial blood obtained from a cannulated femoral artery.

Perfusion pressure was measured from the ileal perfusion circuit through a needle-tipped catheter. Systemic pressure was measured from the contralateral femoral artery. Incisions were made at both ends of the exteriorized segment and a balloon-tipped catheter inserted into the ileum to allow changes in ileal volume and measurement of ileal intraluminal pressure. Adjacent segments were ligated and the mesentery cut to exclude collateral blood flow. Ileal wall compliance was determined by measuring the changes in intraluminal pressure produced by stepwise increases in luminal volume, $\Delta V/\Delta P$. An increase in compliance was considered to reflect a decrease in wall tension, while a decrease in compliance reflected an increase in tension. Changes in vascular resistance were inferred from changes in ileal perfusion pressure. When wall compliance achieved a steady state, infusion of a test peptide began. Wall compliance was again measured, as described above. Intraarterial infusion of saline, postprandial (76.1 pM) and 10x postprandial blood levels of gastrin did not affect ileal perfusion pressure or wall compliance. Postprandial blood levels of secretin (16 pM) or gastric inhibitory polypeptide (GIP) (191 pM) did not affect ileal perfusion pressure; however, wall compliance was significantly increased. One hundred or 10x postprandial blood levels of secretin and GIP, respectively, significantly lowered ileal perfusion pressure without increasing wall compliance further. Postprandial blood levels of cholecystokinin-octapeptide (CCK-8) (34.4 nM) did not affect ileal perfusion pressure; however, wall compliance was significantly decreased. Postprandial (36 pM) and 10x postprandial blood levels of vasoactive intestinal polypeptide (VIP) did not affect ileal perfusion pressure or wall compliance. Blood levels of substance P (SP) (47 pM, 470 pM and 4.7 nM) significantly lowered ileal

perfusion pressure in a dose-dependent fashion. Wall compliance was significantly decreased by the highest blood level of SP. Systemic pressure and heart rate were not altered by the infusion of any of these agents. These data suggest that postprandial blood levels of gastrointestinal hormones (gastrin, secretin, CCK-8, and GIP) do not contribute to the ileal hyperemia coincident with the digestive process since these hormones failed to lower ileal vascular resistance. However, secretin, CCK-8, and GIP do affect the postprandial activity of intestinal smooth muscle since all three hormones altered wall compliance. Postprandial blood levels of VIP do not affect the activity of either ileal vascular or visceral smooth muscle. Although postprandial blood levels of SP have not been reported, the potential for a physiological regulatory role on both vascular and intestinal smooth muscle may be suggested due to the marked sensitivity of both muscles to low doses of the peptide.

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DEDICATION

The author takes great pride and joy in dedicating the following dissertation to his parents, Joseph and Margarita Premen. This most important endeavor would not have been possible without the loving support given by them throughout the years. For that support, I am forever grateful.

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CHAPTER I

INTRODUCTION

Intestinal blood flow is primarily regulated by three mechanisms: intrinsic control (myogenic and local metabolic), extrinsic neural control (sympathetic nervous system), and circulating vasoactive agents (humoral). Each mechanism appears to contribute importantly to the overall regulation of intestinal blood flow (88).

Blood flow through the intestine is regulated through active and passive changes in vessel caliber. Active changes in caliber have been widely investigated and recently reviewed (88,215). Studies concerning the effects of intestinal motility on local blood flow have been conducted, but according to a recent review (1981) by Walus and Jacobson (237), more experimentation is needed to clarify the complex relationship between these two parameters. Because most of the blood vessels of the intestinal wall are imbedded within visceral smooth muscle which can impart both a continuous and rhythmic compressing action on the intramural vessels (204), passive changes in vessel caliber may be physiologically important in the regulation of local blood flow.

Along with their actions on vascular smooth muscle, some vasoactive agents are known to affect intestinal smooth muscle. Thus, these agents may affect vessel caliber passively through alterations in extravascular pressure induced by changes in intestinal smooth muscle tone. Generally speaking, agents which relax vascular smooth muscle, stimulate intestinal smooth muscle. This implies that the dilatory action of an agent may be attenuated or reversed in the intestine through its action on intestinal smooth muscle. Thus, the total effect of an agent in the

intestine may be over or underestimated if its action on intestinal smooth muscle is not considered. Therefore, to accurately determine the effects of an agent on intestinal vascular resistance, and hence blood flow, effects on both vascular and visceral smooth muscle need to be simultaneously assessed.

The effects of various vasoactive agents on both intestinal smooth muscle and vascular smooth muscle have been conducted and are fairly well delineated (42,43,54). However, a study of intestinal wall compliance and vascular resistance involving postprandial blood levels of endogenous gastrointestinal peptides is lacking. This question needs to be explored since there is evidence to support the contention that a "humoral substance" released from the gastrointestinal tract may, in part, be involved in the postprandial changes in intestinal blood flow and motility coincident with the digestive process (74,110).

Thus, a study on the effects of gastrointestinal peptides (mimicking their postprandial blood levels) on intestinal wall compliance and vascular resistance was conducted in the exteriorized canine ileum. Six peptides (gastrin, secretin, cholecystokinin-octapeptide, gastric inhibitory polypeptide, vasoactive intestinal polypeptide, and substance P), were infused into a local segmental artery and their actions on vascular and intestinal smooth muscle simultaneously assessed.

The objectives of the study were to: 1) ascertain the simultaneous effects of these gastrointestinal peptides on ileal wall compliance and vascular resistance, and 2) determine the potential roles, if any, these endogenous gastrointestinal peptides may have in the alterations

in intestinal blood flow and motility coincident with the digestive process.

CHAPTER II

SURVEY OF THE LITERATURE

Historical Perspectives on Intestinal Wall Compliance

Studies on the contractile state of intestinal smooth muscle are difficult to conduct *in vivo*. Changes in intestinal luminal pressure have been taken as being indicative of changes in intestinal wall tension or tonus. However, the measurement of luminal pressure alone is not sufficient to determine wall tension. This determination requires data on luminal pressure and luminal dimensions (length and radius). Wall tension (T) is a mathematical function of transmural pressure (p) and radius (r): $T = p \times r$ for circumferential tension, and $T = p \times r'/2$ for longitudinal tension where r' is the average of the two principle radii of the curvature of the intestinal segment (178). Thus, although a rise in luminal pressure may be observed, a rise in intestinal wall tension may not be indicated if the radius decreases.

The precise definition of intestinal wall tonus has been difficult to assess in the past. In 1946, Kreuger (128) reported that tonus was elevated while intestinal tension increased, decreased, or remained unaltered. Four years later, Quigley and Brody (178) defined tonus as the total amount of tension exerted by a given number of muscle fibers in the intestinal wall. According to Evans (67), tonus was described as the relationship between a stretching force (f), and the final muscle length (1): $T = f/1$. Finally, Youmans (245) defined tonus in terms of the relationship between intestinal luminal pressure and luminal volume such that a decrease in luminal pressure at a constant volume indicated a decrease in intestinal wall tonus.

The dimensions of the intestinal lumen are impossible to measure accurately in vivo. However, changes in luminal volume can be accurately produced. The compliance of a hollow organ can be defined as the volume change per unit pressure change: $C = \Delta V/\Delta P$. This investigation employed the technique developed by Chou and Dabney (42) which allows for the simultaneous assessment of intestinal wall compliance and vascular resistance. The measure of wall compliance yields information about the contractile state of intestinal smooth muscle. This method takes into account the geometrical factor of tension and is a more sensitive index of intestinal wall tension, than is measurement of luminal pressure alone. An increase in wall compliance is considered to indicate a decrease in wall tension, while a decrease in wall compliance indicates an increase in wall tension.

Prior to the development of this technique, intestinal smooth muscle tension was largely examined in vitro using isolated muscle strips. However, a few studies were attempted in vivo starting with the work of White, Verlot, and Ehrentheil in 1940 (240). A preliminary study of the measurement of colonic compliance in man was undertaken to explore the neurogenic disturbances of the colon. Their method involved the infusion of water through the anus at a certain rate, while intraluminal pressure was simultaneously measured. Changes in the slope of the pressure-volume curve reflected changes in the compliance of the colon. Although the same protocol was used by Scott and Cantrell in 1949 (201), no further study of the measurement of compliance was undertaken.

Subsequent investigations using various vasoactive agents such as acetylcholine (15,202), serotonin (202), and plasma kinins (45), revealed that the dilating action of these substances could be attenuated

or reversed by a simultaneous action of the substance on intestinal smooth muscle. However, in these investigations, intestinal wall tension was inferred from changes in luminal pressure alone, an assessment subsequently thought not to be sensitive enough to detect small changes in intestinal wall tension. Consequently, Chou and Dabney (42) developed a method for the measurement of intestinal wall compliance with evidence to indicate that compliance was a better index of wall tension. Their method allows for the simultaneous assessment of intestinal vascular resistance through the establishment of an extracorporal circuit. Thus, it is possible to assess the simultaneous effects of various substances on intestinal vascular and visceral smooth muscle.

Using this method in the exteriorized canine ileum, Chou and Dabney (42) studied the effects of hemorrhage, epinephrine infusion, and alterations in local blood flow on wall compliance and vascular resistance. Subsequently, they studied the effects of epinephrine, acetylcholine, bradykinin, serotonin, adenosine, and ATP on these two parameters (43). The authors concluded that the direct vascular effects of these agents could be modified by their effects on intestinal smooth muscle tension, since the responses of vascular and visceral smooth muscle were often in opposite direction. A similar assessment was made for the effects of various cations on intestinal blood flow (54). The data of Chou and Dabney concerning the differential vasoactivity of acetylcholine (43) agreed with the findings previously reported by Bean and Sidkey (5). They were later corroborated by Brobmann, Jacobson, and Brecher in 1970 (21,22).

Effects of Digestion on Blood Flow and Motility

It is well established that following ingestion of a meal, a characteristic increase in blood flow is manifested throughout the mesenteric circulation, aptly named postprandial mesenteric hyperemia (18,41,47,49,50,74,81,130,132,224,246). Numerous studies conducted in both conscious and anesthetized animals have corroborated this physiological phenomenom, and include studies in man (20), dog (31,78,79,106, 226,227), rat (187), cat (74), and primate (228).

Although clearly identified, the mechanism(s) by which chyme in the lumen of the small bowel mediates the mesenteric hyperemia has yet to be fully elucidated. According to the recent review on intestinal blood flow by Granger et al. (88), five possible regulatory mechanisms may be involved. They include: 1) a local nervous reflex of the myenteric plexus, 2) metabolic alterations in mucosal cells, 3) myogenic activation of vascular smooth muscle, 4) changes in visceral smooth muscle tone, and 5) release of endogenous gastrointestinal hormones and/or peptides.

Historically, it was suggested that a redistribution of the cardiac output, in favor of the splanchnic viscera, occured during the digestive state since it was apparent that the gastrointestinal organs were metabolically more active at that time and thus, required a greater blood flow. In 1934, Herrick, Essex, Mann, and Balders (106), using a modification of the thermostruhr method of Rein (186), reported on the effects of digestion on blood flow distribution in various vascular beds in the conscious, fasted dog. They were the first ones to provide direct experimental evidence that: "...the increased blood flow to the intestinal tract during digestion is not obtained at the expense of the blood flow

to the somatic tissues..." (106).

The ingestion of foodstuffs results in a dual response in the splanchnic vascular bed. The initial response is observed during the anticipatory and ingestion phases while the subsequent response, observed during the absorption of chyme from the intestinal lumen. The initial response is mediated by the generalized arousal of the sympathetic nervous system, resulting in transient increases in cardiac output, heart rate, aortic pressure, and renal vascular resistance. Along with these responses, a simultaneous transient decrease in coronary vascular resistance is observed (78,79,226,227,228). Within 5-30 minutes after meal ingestion, cardiac output, heart rate, aortic pressure, and flows to both renal and coronary vascular beds return to preprandial levels. Concurrently, intestinal blood flow through the superior mesenteric artery markedly increases, and reaches a peak flow between 28-132% above preprandial control levels within 30-90 minutes. Inconsistent decreases in limb blood flow have been reported during this time. Flow through the intestine returns to preprandial levels 4-5 hours after meal ingestion (31,78,79,226,227,228). Coincident with the intestinal hyperemia are reports of elevated blood levels of several gastrointestinal hormones (gastrin, secretin, cholecystokinin, and gastric inhibitory polypeptide) (14,16,34,99,100,123,129,137,145,156,195,198,199,218,235). Thus, while the anticipatory/ingestion phase of digestion is mediated by a more generalized, sympathetically-mediated cardiovascular response, cardiovascular responses to digestion appear to be confined to the gastrointestinal organs. The precise localization of the hyperemia remains controversial but generally can be said to occur along the entire length of the small bowel.

Little data exists concerning the effects of gastrointestinal peptide hormones on both vascular and visceral smooth muscle during the digestive state. A possible role for a humoral component in mesenteric postprandial hyperemia was suggested by Burns and Schenk (30) when they reported that intravenous infusions of gastrin and secretin increased blood flow through the superior mesenteric artery. Hsieh and Chou (110) reported that intraduodenal infusion of food or acid increased local blood flow and decreased vascular resistance in an isolated jejunal segment perfused by the duodenal venous outflow. They suggested that humoral substances, i.e., endogenous gastrointestinal hormones, may be involved in the postprandial hyperemia and increases in intestinal motility coincident with the digestive process. Similar findings were reported by Fara, Rubinstein, and Sonnenschen (74). They reported that instillation of corn oil, acid, or phenylalanine into the duodenum produced a selective increase in mesenteric blood flow associated with increases in gall bladder and duodenal motility, and elevations in pancreatic secretions which were both enzyme and bicarbonate rich. These responses could be reproduced by intravenous infusions of low doses of secretin and cholecystokinin. Cross-perfusion studies further indicated that intraduodenal instillation of corn oil not only increased local blood flow in that animal but also in an unfed animal in which the superior mesenteric artery was being supplied with arterial blood obtained from the aorta of the animal receiving corn oil (74).

Therefore, it appears that contact of chyme with the mucosa of the intestinal wall can cause the release of some humoral, vasoactive substance into the intestinal circulation. The humoral candidates have included three well known gastrointestinal hormones: gastrin, secretin,

and cholecystokinin (CCK). These three hormones are released into the intestinal circulation following a meal and are known to mediate changes in intestinal secretory and motility patterns coincident with the digestive process. Gastric inhibitory polypeptide (GIP), a putative gastrointestinal hormone, may also be involved in the hyperemia since a vasodilatory action has been demonstrated in the feline intestine (75).

Other endogenous vasoactive peptides of the intestine should be considered as possible physiological regulators of the hyperemia since many of them have been shown to possess vasodilatory activity. In particular, neural concentrations of both vasoactive intestinal polypeptide (VIP) and substance P (SP) are found in the small bowel forming dense plexuses around the ganglionic cell bodies of the myenteric plexus (111). Thus, a possible regulatory role on intestinal blood flow for these two peptides is plausible since these substances are known to dilate in other vascular beds.

Most of the previous investigations supporting the role for endogenous gastrointestinal hormones as possible humoral mediators of the mesenteric hyperemia have utilized partially purified, extracted forms of the hormones. Therefore, the possibility of contamination by some unknown, potentially vasoactive substance in the extract obtained from the mucosal wall cannot be ruled out. On this point, it has been recently disclosed that some commercial preparations of secretin used in clinical studies were contaminated with cholecystokinin and VIP (88). Thus, the vasodilatory activity of secretin in the intestine may have been largely due to the action of these vasoactive contaminants. Furthermore, GIP was first isolated from a side fraction during the purification process of CCK (23,24,25) and subsequently shown by Fara and Salazer (75) to

dose-dependently increase superior mesenteric blood flow in the cat. Thus, the mesenteric vasodilatory actions ascribed to CCK in previous studies (9,10,44,48,72,73,74,76,136,191) may have included a significant contributing action of GIP.

Early radioimmunassays (RIA) developed for gastrointestinal peptides were relatively insensitive and unspecific. Therefore, the concentration of the substances infused into the intestine may not have accurately reflected the true arterial postprandial blood levels of the peptides. With the advent of specific, accurate RIAs for these peptides (84), recent literature has provided postprandial blood levels for many of the peptides found in the gastrointestinal tract.

Endogenous Gastrointestinal Peptides

Gastrin

Gastrin belongs to a family of endogenous gastrointestinal peptides which includes cholecystokinin-pancreozymin (CCK-PZ). These two hormones are structurally identical in their carboxy-terminal five amino acids (Gly-Trp-Met-Asp-Phe-NH_2). Thus, they share similar biological activities (119,147,233). Multiple forms of gastrin exist. Gastrin, in the heptadecapeptide form (G-17), is principally found in the antral mucosal G-cells of the stomach (92,146). G-17 and a larger 34 amino acid form (G-34), known as "big-gastrin" are found in the circulation (62,63). All of the biological activity of gastrin can be reproduced by the carboxy-terminal tetrapeptide (G-4) (90,91).

Gastrin (G-17) is released from the antral mucosal G-cells into the circulation principally by feeding (64). It is additionally released by calcium in the gastric lumen, activation of cholinergic-vagal reflexes, antral distension, and infusion of bombesin (205,235). Of the two major circulating forms, G-17 is some five times more potent on gastric acid stimulation than equimolar concentrations of G-34. Yet, G-17 comprises only one-third of the total immunoreactive gastrin released into the circulation by feeding.

In dogs, the half-life of G-17 is approximately 3 minutes, while that for G-34, between 9-15 minutes (208,229,234). The disappearance of these forms in the human circulation is some 50% slower as compared to the dog (236). The major forms of gastrin do not appear to be inactivated by the liver. However, smaller fragments of gastrin (G-4) have been shown to be inactivated by this route (209). It appears that the kidneys

may have an important role in the clearance of G-34. Many vascular beds, including both gastric and mesenteric, have been shown to remove G-17 from the circulation (210). Thus, a local metabolism of G-17 may be suggested.

The literature indicates that gastrin possesses many biological actions (119,147,233). However, many of the actions demonstrated were obtained using extremely high doses of gastrin. According to Johnson (118,119), the primary physiologic actions of gastrin are to stimulate gastric acid output from the oxyntic gastric mucosa, and to exert a trophic influence on gastrointestinal mucosal cells.

Previous studies on the cardiovascular actions of gastrin in the mesenteric circulation employed either crude extracts obtained from the antral mucosa, or the pentapeptide analogue, pentagastrin. Using extracted gastrin, Burns and Schenk (31) reported that subcutaneous injection of 2 U/kg (U = units) into conscious dogs increased superior mesenteric arterial blood flow for up to 3 hours. In anesthetized dogs, local intra-arterial infusion of extracted gastrin (0.2 mg/ml) decreased both superior mesenteric vascular resistance (44) and the vascular resistance in the gastric circulation (136). Vasoactive contaminants may have been implicated in these vascular responses.

Employing the pentapeptide analogue, pentagastrin, Rudick et al. (192) and Swan and Jacobson (214) reported that infusions into the dog at 1-6 μg/kg/hr and 12.5-200 μg/hr, respectively, significantly elevated gastric mucosal blood flow. A similar increase in blood flow has been demonstrated in gastric submucosal arterioles (95). Local intra-arterial infusion of pentagastrin into either the feline (3 μg/kg/min) (76) or canine intestine (0.5 μg/kg/min) (19) significantly increased

superior mesenteric blood flow, which in the dog (19) could be attenuated by atropine. Chou, Hsieh, and Dabney (48) reported that local intra-arterial infusion of pentagastrin into several organs (2-1500 ng/ml) produced a selective vasodilation in the duodenum and jejunum (minimum concentration requirements of 50 and 25 ng/ml, respectively). Yet, in other studies where doses of pentagastrin known to stimulate gastric acid secretion were infused into the cat intestine, no significant changes in jejunal vascular resistance were observed (74,110,188).

Most of the investigations conducted on the effects of gastrin on visceral smooth muscle activity of the gut have employed in vitro preparations. Early studies conducted by Bennett (6) suggested that the contractile response of guinea pig ileal muscle strips to extracted gastrin (0.1-4 μg/ml) was mediated through a stimulation of postganglionic parasympathetic nerves and subsequent release of acetylcholine. Subsequent work in the guinea pig and other species (141,230,231,232), using the pentapeptide analogue, pentagastrin, corroborated the neurally-mediated contractile response to gastrin administration. Likewise, contractile responses to pentagastrin have been produced in the lower esophageal sphincter and gastric antrum (114,141).

In the isolated, vascularly perfused canine intestine, Stewart and Burks (207) corroborated the previous findings (6,141,230,231,232). Five or 25 μg pentagastrin, in a volume no greater than 0.1 ml, increased intestinal motility which was shown to be mediated through an interaction of the peptide with non-nicotinic receptors on intramural cholinergic ganglia and subsequent release of acetylcholine at the myoneural junction. Yet, isolated human duodenal, jejunal, or ileal muscle strips were insensitive to gastrin administration (150) and cat or opposum

circular and longitudinal muscle strips were unresponsive to 10^{-7}-10^{-5}M pentagastrin (1).

Fasth et al. (76) demonstrated that although local infusion of pentagastrin (3 μg/kg/min) into cat intestine increased superior mesenteric blood flow, the dilatory response was only transient due to a mechanical impedance of blood flow produced by pentagastrin-mediated increases in intestinal smooth muscle tone. After atropine blockade, the dilatory response was sustained.

The cardiovascular and visceral smooth muscle actions of pure gastrin have not been adequately assessed. It appears possible that the vascular actions described for the pentapeptide analogue, pentagastrin, may not be shared by the naturally occuring forms (G-17 and G-34). Also, many of the previous studies conducted on intestinal vascular and visceral smooth muscle used high doses of pentagastrin and thus, the actions ascribed might not have reflected a physiological response. The effects of postprandial blood levels of gastrin (G-17) on both of these muscle types has, as yet, not been critically evaluated in vivo.

It has been recently shown that after a meal arterial serum levels of gastrin in the dog increase and peak at a level of 160-200 pg/ml (expressed as heptadecapeptide gastrin equivalent and equal to 0.086 pmol/ml) (137,235).

Secretin

Secretin is a linear polypeptide containing 27 amino acids which belongs to a family of structurally related polypeptides including vasoactive intestinal polypeptide (VIP), glucagon, and gastric inhibitory polypeptide (GIP). Since these substances contain overlapping amino acid

sequences, they share similar biological activities (119,147,162,233). Although the entire 27 amino acid secretin structure is necessary for full biological potency, the 5-27 carboxy-terminal fragment retains a minimal capacity to stimulate pancreatic secretions (144).

In the dog, the circulating half-life of either exogenously infused or endogenously released secretin is between 2.5 and 3 minutes (53,138). In man, a half-life of 4.1 minutes has been reported (125). Investigation has suggested that the kidneys play a major role as the site of removal of secretin from the circulation (71,138).

Secretin is distributed within mucosal endocrine cells (S cells) primarily concentrated in the duodenum and jejunum with little distribution in the ileum (33, 175). There is general agreement that acidification of the duodenum causes the release of secretin into the circulation which is not dependent upon cholinergic-vagal mechanisms (17,93,238). Many other biological actions have also been ascribed for secretin (147, 233), however, according to Johnson (119), most of these demonstrated effects were observed following high doses of administered peptide; doses which increase blood levels well above those attained under normal physiological conditions. Accordingly, it is suggested that the primary physiological actions of secretin include only: the stimulation of large volumes of pancreatic and biliary secretions rich in bicarbonate and a potentiating action on cholecystokinin-mediated stimulation of pancreatic enzyme secretion.

A possible vasodilatory action for secretin was first reported by Bayliss and Starling in 1902 (4). Since then, the vasoactive potency of secretin has been widely investigated throughout the gastrointestinal tract, predominately with the use of extracted forms of the peptide.

Intravenous injection (1-1.5 U/kg) (31,82) or infusion (5 U/kg or 0.4-10.7 U/kg/hr) (72,74,85) of natural secretin in either cats or dogs has been shown to produce a marked increase in superior mesenteric blood flow. Jejunal (74) and duodenal (85) blood flows were especially elevated following intravenous infusion. Furthermore, within the jejunum, blood flow was reported to be redistributed away from the mucosa to the submucosa (72). Additionally, local intraarterial infusion (100-500 mU/min) (76,189) or rapid intraarterial injection (1-10 U) (191) of natural secretin in cats, produced similar increases in mesenteric blood flow. Rapid intraarterial injection (1-2.5 U) increased venous outflow from the in situ feline jejunum (10). Furthermore, after administration of natural secretin by rapid intravenous injection (191) or infusion (189), or by intraarterial injection into the mesenteric vascular bed (191), the elevations in superior mesenteric blood flow were attended by decreases in systemic arterial blood pressure which manifested itself as a brief hypotension, followed by a long-lasting hypertension (191).

Only a few studies have been conducted in the mesenteric circulation using synthetic secretin (19,95). Employing an in vivo microscopic technique, Guth and Smith (95) reported that although close intraarterial infusion of natural secretin produced a dilation of gastric submucosal arterioles, similar infusions of synthetic secretin were unable to mimic the dilatory response. Thus, it was suggested that a vasoactive contaminating agent was present in the natural form of secretin. Further investigation by Bowen et al. (19) corroborated the "contaminating agent" suggested by Guth and Smith (95) since local infusion of synthetic secretin (0.03 or 0.3 μg/kg/min) into the canine intestine produced no significant effect on either mesenteric blood flow or oxygen consumption.

Most of the work on the effects of secretin (natural or synthetic) on gastrointestinal smooth muscle activity has been conducted using the in vivo experimental approach. Chey et al. (39) reported that natural or synthetic secretin (1 U/kg) produced an inhibition of spontaneous motor activity of the stomach and duodenum in both man and dog. In dogs, it was further shown that intravenous injection of 0.5-4 units of natural or synthetic secretin inhibited the motor activity of the canine gastric antrum, an effect also observed in man (56).

It has been suggested that a possible regulatory role for secretin on intestinal motor function exists since cholecystokinin-stimulated upper small intestinal motility could be inhibited by natural secretin and conversely, secretin-induced inhibition reversed by cholecystokinin (96). Yet, in vitro studies by Anuras and Cooke (1) demonstrated that administration of secretin ($4x10^{-5}M$) had no effect on the contractile activity of either circular or longitudinal smooth muscle strips obtained from either the cat or opossum.

Caution should be observed when attempting to interpret the data accumulated on vascular and visceral smooth muscle activity during secretin administration, especially in studies using natural forms. Until recently, pure, synthetically-derived secretin was difficult to obtain and in some commercial preparations previously used in experimentation, secretin was found to be contaminated with vasoactive agents such as vasoactive intestinal polypeptide and cholecystokinin (88). With the availability of synthetic secretin and the knowledge that fasting arterial blood levels of secretin in man (40-250 pg/ml) (14,16,34) and dog (<10 pg/ml) (123) markedly increase in the circulation following duodenal acidification and meal ingestion (200-600 and 55 pg/ml, respectively),

the effects of this peptide on the postprandial activity of both vascular and visceral smooth muscle can be more accurately assessed.

Cholecystokinin

The name "cholecystokinin" (CCK) was first mentioned by Ivy and Oldberg in 1928 when they reported that a small intestinal mucosal extract stimulated gall bladder evacuation in response to lipid in the small intestine (112). In 1943, Harper and Raper suggested the name "pancreozymin" (PZ) for a small intestinal extract which stimulated enzyme-rich pancreatic secretions (98). The active principle in these extracts was subsequently identified as a single peptide consisting of 33 amino acids (CCK-33) with both gall bladder stimulating and pancreatic enzyme secretagogue properties (160,161). A larger 39 amino acid form was later isolated from the intestine (159). The hormone is now commonly referred to as cholecystokinin because the gall bladder stimulating action was first described. Recently, it has been shown that a predominant form of intestinal CCK is in the octapeptide form (CCK-8) (182,183,184).

CCK is a member of the gastrin family of gastrointestinal peptides and contains the identical carboxy-terminal 5 amino acids as gastrin (119,147,233). As a consequence, CCK possesses some overlapping biological actions with gastrin, and can serve as a weak agonist, or competitive antagonist to gastrin-mediated actions. The seventh amino acid from the carboxyl terminus is a sulphated tyrosyl residue which is essential for CCK-33 and CCK-8 to maintain full potency in most biological systems (119,120,147). Thus, the minimal active fragment of CCK is the carboxyl terminal sulphated heptapeptide.

The circulating half-life of CCK in both man and dog has been reported to be approximately 2.5 minutes (218). Although the metabolism of CCK is not clearly delineated, various investigations have suggested that the renal cortex (142) and the liver (225) are responsible for the inactivation of CCK. Also, it has been suggested that the hormone is inactivated in the peripheral circulation by a substance released from the gall bladder (2,36,89).

CCK-containing endocrine cells have been identified principally in the mucosa of the duodenum and jejunum with little distribution in the ileum (28,176). The major forms demonstrated in the intestine are the 8 and 33 amino acid forms. CCK-33 is primarily contained within endocrine cells of the mucosa (I cell), while CCK-8 is primarily localized within enteric nerves, with a smaller distribution found within endocrine cells (26,183). Intraluminal fat appears to be the most potent stimulant of CCK release from the intestine (126,149).

As with gastrin and secretin, many biological actions have been ascribed to CCK (119,147,233). However, many of the responses were obtained with high doses of the substance, thus implying that the effects may not represent a physiological action for CCK. According to Johnson (119), the accepted physiological actions for this hormone include: stimulation of gall bladder motility, stimulation of enzyme-rich pancreatic secretions, potentiation of secretin-stimulated pancreatic volume and bicarbonate output, inhibition of gastric emptying, and a trophic influence on the exocrine pancreas.

Most studies on the vascular actions of CCK in the intestine have employed partially purified forms of the hormone extracted from the intestinal mucosa. Intravenous infusion of partially purified CCK in the cat

(range = 0.5-11.3 U/kg/hr) has been demonstrated to markedly increase blood flow through the superior mesenteric artery (72,73,74,191). By this route of administration, CCK was shown to have minimal effects on aortic pressure, cardiac output, and gastric, renal, and femoral blood flow (73). Thulin and Olsson (220) reported that caval or portal infusion of pure, natural CCK-33 (0.56.5 U/kg/min) markedly affected splanchnic vascular resistance as evidenced by significant increases in hepatic artery, superior mesenteric artery, and portal vein blood flows in the dog.

Local intraarterial infusion of partially purified CCK (1 U/kg, 0.045 mg/ml, 1-2.5 U, or 3-12 U/kg/hr) into the feline intestine has been shown to significantly decrease superior mesenteric vascular resistance (44,76), decrease vascular resistance in the gastric circulation (136), and produce a significant dilation in the jejunum (9,10). It has been reported that the vasodilatory effect of CCK in the cat intestine was short-lasting due to a mechanical impedance of blood flow produced by CCK-stimulation of intestinal smooth muscle activity (76). In the study by Chou, Hsieh, and Dabney (48), local intraarterial infusion of 17% pure CCK into both digestive (duodenum and jejunum) and non-digestive organs preferentially decreased vascular resistance in the canine duodenum and jejunum (concentration requirement = 2.5 mU/ml). Following data comparison with the reported cardiovascular adjustments and blood concentrations of CCK following a meal, the authors suggested that CCK could contribute to postprandial intestinal hyperemia (48).

Relatively few attempts have been made to study the cardiovascular actions of synthetic CCK in the gastrointestinal tract. Using the synthetic octapeptide of CCK (CCK-8), portal or caval infusion between

0.02-0.22 μg/kg/min (219), or local intraarterial infusion at 0.1 μg/kg/min (19) has been shown to increase total splanchnic and local intestinal blood flow, respectively. Concomitant with the vascular effect was a marginal effect on systemic blood pressure (219) and a significant increase in intestinal oxygen consumption (19). Also, the dilator response in the dog was atropine sensitive (19). Additionally, it has been reported that CCK-8 dilates gastric submucosal arterioles following infusion into the celiac axis of cats (95).

The stimulatory action of CCK on visceral smooth muscle was initially considered by Ivy and Oldberg (112). Since then, it has been corroborated that CCK exerts a direct stimulatory effect on gall bladder smooth muscle (101). In man, it has been reported that intravenous infusion of 10% pure CCK (1,2,or 4 U/kg/hr) stimulated the basal motor activity of the duodenum and jejunum (96). Caval or portal infusion of either pure, natural CCK-33 or synthetic CCK-8 (0.5-6.5 U/kg/min) stimulated canine intestinal peristalsis (219,220). Local infusion of CCK-8 (minimal effective dose of 0.2 μg) into the vascularly perfused canine intestine dose-dependently stimulated phasic contractions along the circular axis of isolated intestinal segments. It was suggested that the contractile response was mediated entirely through a neurogenic mechanism with subsequent release of acetylcholine at the myoneural junction (206).

The contention of a neurally-mediated mechanism to CCK-8 stimulation of intestinal smooth muscle is corroborated by the in vitro work of Vizi et al. (230,231,232) and Hedner et al. (101,102) on ileal longitudinal smooth muscle strips. Yet, natural CCK ($5x10^{-9}M$) only contracted circular smooth muscle strips obtained from the opossum duodenum while being minimally effective on opossum duodenal longitudinal muscle strips.

Also, both duodenal circular and longitudinal muscle strips of the cat were unresponsive to CCK (1).

Assessment of the vascular and visceral smooth muscle actions ascribed to CCK requires careful interpretation because multiple forms of the hormone exist in the gut. CCK-33 has been suggested to function as a pro-hormone, thus it is possible that smaller fragments of the hormone circulate through the body and function as the biologically active forms (183). Also, a contributing action on either vascular or visceral smooth muscle activity by gastric inhibitory polypeptide (GIP) may be indicated for early investigations since GIP was isolated from CCK mucosal extracts (23,24,25).

Accordingly, estimates of fasting and fed plasma levels of CCK have varied. Schlegel et al. (198,199) found that CCK increased from a fasting level of 222 to 480 pg/ml after feeding in man, while Thompson et al. (218) demonstrated changes from 700 to 1200 pg/ml. Others find that fasting levels of CCK in the peripheral blood of man rise from 50-100 pg/ml to 30-40 ng/ml after ingestion of a fatty meal (99,100). In the dog, it has been reported that portal venous serum levels of CCK markedly rise from 120 to 250 ng/ml following acid perfusion of a jejunal loop (181).

Gastric Inhibitory Polypeptide

Gastric inhibitory polypeptide (GIP) belongs to the secretin family of gastrointestinal peptides. GIP is a linear chain polypeptide containing 43 amino acid residues. The peptide was isolated by Brown and co-workers in 1970 (23,24,25) from a side fraction in the purification process of cholecystokinin. Due to its antagonistic effect on acid se-

cretion by the oxyntic gastric mucosa (169), it was designated as "gastric inhibitory polypeptide." Since this polypeptide is structurally similar to secretin, they possess overlapping biological activities (119,147, 233). There is evidence that a second larger form of GIP is released into the circulation along with the 43 amino acid form (24,195).

Little information exists on the metabolism of GIP. The circulating half-life of porcine GIP is 21 minutes in human plasma (24). Investigations by O'Dorisio et al. (166) suggest that the kidneys play an important role in the removal of this peptide from the circulation.

GIP is said to be restricted to mucosal endocrine cells (K-type) with the greatest concentration of the peptide located in the duodenum and jejunum. Lower concentrations have been found in the gastric antrum and ileum (27,165,174). The peptide has been shown to be released into the circulation by a carbohydrate-, lipid-, or protein-containing meal (119,147,233). It has been suggested that vagal nerve activity is not significantly involved in the release of GIP from mucosal endocrine cells (217). During the rise in plasma levels of GIP following oral glucose administration, plasma levels of insulin markedly rise (119,147,233). This suggests that GIP may play a role in the enteroinsular axis of luminal glucose induction of insulin release from the endocrine pancreas (a glucose-dependent insulinotropic action).

Many biological actions have been ascribed to this peptide. These include: an inhibition of gastric acid secretion, stimulation of intestinal secretions, inhibition of gastric motor activity, stimulation of insulin release from isolated pancreatic islet cells, and enhancement of pancreatic insulin release in the presence of a mild hyperglycemia (24,119,147,233). Although primarily identified as an inhibitor

of gastric acid secretion, studies by Maxwell et al. (145) revealed that the circulating levels of GIP attained following a meal are probably inadequate to produce a significant inhibition on gastric acid secretion. Others have supported this contention since plasma levels of GIP that exceeded levels seen after a meal were necessary to inhibit pentagastrin-stimulated acid release (86). Currently, the physiological role for GIP appears to revolve around its demonstrated insulinotropic action (13).

The vascular actions of GIP in the splanchnic circulation have been minimally explored. In 1978, Fara and Salazer (75) demonstrated that intravenous infusion of GIP produced a dose-dependent increase in superior mesenteric blood flow which was long-lived. With doses ranging from 0.45 to 1.8 μg/min, mesenteric vascular resistance decreased from an initial drop of 14.7 to a maximal drop of 56% in the cat. Systemic blood pressure was not altered during the infusion period.

As with blood flow, the effects of GIP on gastrointestinal motility have been minimally investigated. It was initially reported by Brown et al. (24) that this polypeptide has an inhibitory effect on gastric motor activity in the body and antrum. Recently, Fara and Salazer (75) reported that during intravenous infusion of GIP (1.8 μg/min) in the cat, spontaneous jejunal motility, when present, was totally abolished.

Specific radioimmunoassays for GIP are still being developed. Depending on the assay used, postprandial blood levels have been reported to be as low as 34 pmol/l (195) and as high as 300 pmol/l (156). Others have demonstrated that circulating blood levels of GIP are elevated to 0.4 - 1.2 ng/ml following meal ingestion (58,129,145). Infusion of GIP at 1.0 μg/kg/hr in the dog increases serum levels of the peptide

no further than 1 ng/ml (169), a level which is compatible with previous findings (58,129,145).

Vasoactive Intestinal Polypeptide

Vasoactive intestinal polypeptide (VIP) was isolated from hog small intestinal extracts by Said and Mutt in 1970 (194). VIP contains 28 amino acids and is structurally similar to the secretin family of peptides. Because of this likeness, VIP shares similar biological activities with secretin (119,147,233).

It was originially thought that VIP was localized within intestinal endocrine-like cells. However, recent investigations have suggested that this polypeptide is only found within the enteric nervous system spanning the gastrointestinal tract (12,115,133,134,135). A dense concentration of VIP-containing nerve fibers and cell bodies are located within the celiac-superior mesenteric and inferior mesenteric sympathetic ganglia (107).

Based on systemic versus portal infusion of VIP, Said and Mutt initially suggested that the liver was responsible for the inactivation of the peptide (194). Further investigation has supported the hepatic route (59,124,153).

VIP has been shown to be released into the circulation by the following stimuli: intravenous calcium infusion (60), electrical stimulation of the vagus (196), mechanical stimulation of the intestinal mucosa (62,69), stimulation of high threshold vagal nerve fibers, pelvic nerve stimulation (69), and intraduodenal instillation of HCl, fat, or ethanol, but not amino acids, glucose, saline, or meal ingestion (197). Furthermore, a marked pathophysiological release of VIP into the venous

circulation of the intestine has been reported during intestinal ischemia and reduced cephalic mesenteric arterial flow (3,155), and reperfusion of the ischemic bowel (3,154).

Numerous biological effects are seen following rapid intravenous injections of VIP. The effects on smooth muscle are widespread and include: peripheral, splanchnic, and pulmonary vessel dilation, positive inotropic effect on the heart, increases in cardiac output, relaxation of tracheal and bronchial smooth muscle, relaxation of gastrointestinal smooth muscle, and increases in salivary and intestinal blood flow (233). The physiological actions ascribed to this gut peptide have not been clearly defined. According to Walsh (233), investigations conducted by Fahrenkrug et al. (69) provides good evidence that neurally released VIP from the gastrointestinal tract may be involved in the physiological regulation of gastric smooth muscle relaxation, and increases in intestinal blood flow observed after physiological manipulation of the gastrointestinal tract.

The vasodilatory property of VIP was initially observed by Said and Mutt where intraarterial infusion of 100 μg/ml of the extract markedly increased femoral blood flow and decreased systemic blood pressure. Intravenous infusion produced a sustained hypotension (194). In the isolated canine jejunal loop, intraarterial infusion or injection of VIP, attaining arterial concentrations between 0.5 ng/ml and 5 μg/ml, produced a dose-dependent vasodilation of the mesenteric vascular bed which was atropine and propranolol resistant (121). Close intraarterial infusion of VIP into the feline splanchnic circulation, attaining plasma concentrations ranging from 0.15 to 4.6 μmol/L, markedly increased gastric, jejunal, and colonic blood flow (63).

However, Thulin and Olsson (221) reported that in the dog, caval or portal infusion of VIP (0.03-0.45 μg/kg/min) markedly increased hepatic artery and portal venous blood flow without significant effect on superior mesenteric blood flow. Concomitant with these vascular effects was a decrease in systemic blood pressure. Likewise, intravenous infusion of VIP in the dog (175 ng/min) produced significant decreases in both absorptive site and total intestinal blood flow attendant with significant decreases in systemic blood pressure (143). For those studies using caval and portal routes of infusion (194,221), the caval route proved to be more effective in eliciting vascular responses to VIP.

In vitro studies on the vascular actions of VIP are few. Hellstrand and Jarhult (104) have shown that VIP (0.001-1 μg/ml, minimal effective dose = 0.05 μg/ml) produces a dose-dependent reduction in spontaneous contractions of the rat portal vein.

Most *in vivo* studies on visceral smooth muscle activity have consistently shown that VIP relaxes gastrointestinal smooth muscle in the lower esophageal sphincter (180), upper stomach (68), antral smooth muscle (157), gall bladder (193,223), and the rectum and cecum (173). Eklund and co-workers (63) reported that intraarterial infusion of VIP into the splanchnic circulation of the cat produced a profound relaxation of the gastric muscularis propria. Yet, at the same plasma concentrations (0.2 to 4.6 μmol/L), VIP contracted the colonic musculature. In isolated canine jejunal loops, short-lasting intraarterial injection or prolonged intraarterial infusion of the peptide, attaining arterial concentrations between 25-500 pg/ml, dose-dependently relaxed jejunal circular smooth muscle. However, at arterial concentrations greater than 500 pg/ml, a biphasic response was observed, i.e., relaxation followed by

contraction (122).

Results of in vitro studies are difficult to asses since either an inhibitory or contractile response is elicited following VIP administration. Investigations by Jaffer et al. (113) and Cohen and Landry (52) demonstrated that VIP (10^{-9}-10^{-7}M) was capable of producing a contractile response on guinea pig ileal longitudinal muscle strips, guinea pig duodenal longitudinal muscle strips (113), and rabbit jejunal longitudinal muscle strips (52). Responses to VIP were abolished by tetrodotoxin (113) or partly antagonized by atropine (52). However, in opossum duodenal muscle strips, 10^{-7}M VIP has been shown to raise tension in longitudinal, yet, reduce tension in circular smooth muscle strips (1). On the contrary, studies by Johns (116) and Cocks and Burnstock (51) demonstrated that VIP (0.01-1 µg/ml) produced an inhibitory effect and a slow relaxing effect on guinea pig taenia coli muscle strips.

Although radioimmunoassays for VIP have been developed, only a few are sensitive enough to permit the measurement of the peptide in normal human plasma. Median basal VIP concentrations have been reported to be 1.3 (29), 1.7 (70), and 7.3 (135) picomoles/liter. Problems with cross-reactivity at the amino terminus appears to be an important consideration (55). However, due to the potent inactivation of the peptide during passage through the liver, these reported levels of VIP may be irrelevant for physiological studies in the intestine. Following intraduodenal instillation of HCl, fat, or ethanol, peripheral plasma venous concentrations of VIP in man increase from a basal level of 4.3 to 9.8, 7.5, and 12.6 pmol/L, respectively (197). In the pig, intraduodenal instillation of HCl increases portal venous plasma concentrations of VIP from an average basal level of 17.2 to a peak of 36 pmol/L, while fat

increases VIP concentrations from a basal level of 11.5 to a peak of 20.3 pmol/L (197).

Due to the distribution of this peptide within intrinsic intestinal nerves, assessment of possible physiological regulatory roles is complicated since following VIP release, the peptide may act locally and be inactivated before attaining vasoactive levels in the circulation. Thus, measured levels of VIP in systemic arterial and venous blood may not be significant indicators of the importance of this peptide.

Finally, evidence has accumulated that supports the contention that endogenous release of VIP from intestinal nerves is responsible for the intestinal vasodilation and relaxation of gastric smooth muscle observed following physiological manipulation of the intestinal mucosa and elicitation of autonomous nervous effects known to be mediated by non-adrenergic, non-cholinergic nerve fibers in the intestine (62,69).

Substance P

Substance P is an endogenous gut and brain peptide composed of 11 amino acids. Substance P was first detected by von Euler and Gaddum in 1931 in alcoholic extracts of equine brain and intestine (65). Preparation of the extract yielded a powder with the same activity, named "preparation P." Subsequently, the name was changed to substance P which is the currently accepted name. Purification of the extract by Chang and Leeman (37) and Studer, Trzeciak, and Lergier (211) revealed that substance P was an undecapeptide possessing great potency both as a hypotensive and visceral smooth muscle stimulating agent.

A widespread distribution of the peptide is found within the mammalian gastrointestinal tract. The greatest concentration of immunore-

active substance P appears to be located in the duodenum, while the majority of extractable immunoreactive substance P is found within both circular and longitudinal smooth muscle investments of the intestine. Very little substance P is extractable from the intestinal mucosa (158). Substance P immunoreactivity is localized to neural elements in the intestinal tract that form dense networks around the ganglionic cell bodies of both myenteric and submucosal plexi. In addition, some nerve fibers have been reported to run along smooth muscle fibers alone (163,168,213).

In addition to its location in nerves, substance P is also found in endocrine-like cells in the epithelial layers of both small and large intestine with a slight predominance for the proximal small intestine (213). The peptide is distributed within duodenal and colonic mucosal cells subsequently identified to be enterochromaffin cells which also contain serotonin (5-HT) (163,212).

Both the liver and kidneys have been suggested to be responsible for the inactivation of substance P. Hallberg and Pernow (97) demonstrated that the infusion rate of substance P through the portal vein had to be raised 31 times in comparison to the dose infused through the femoral vein in order to produce a similar arterial hypotension. Similar findings were obtained by Lembeck and Hettich (139), thus suggesting that the liver has a great capacity to inactivate circulating substance P. Other investigations indicate that the kidneys play a role in the inactivation process since there was a significant gradient between arterial and renal venous immunoreactive substance P during intravenous infusion of the peptide (35,239). Also, a factor within intestinal smooth muscle has been shown to effectively inactivate substance P (94,171). Recently, Johnson and Erdos (117) have shown that cultured human vascular endothe-

lial cells contain a substance P inactivating enzyme. The enzyme appears to be located on the surface of the cell, quite similar to the location of kinninase II responsible for conversion of angiotensin I into angiotensin II.

Little knowledge has been accumulated concerning the release of substance P from the gastrointestinal tract into the circulation. In 1964, Pernow and Wallenstein (172) postulated that the heightened intestinal motility observed after direct application of a hypertonic glucose solution to the jejunal mucosa was, in part, mediated by substance P since the intestinal wall content of the peptide was shown to simultaneously increase during the motility response. Later studies by Uvnas-Wallenstein (222) demonstrated that substance P could be released into the antral lumen of cats by electrical stimulation of the vagus, antral perfusion with acetylcholine, and intravenous injections of either acetylcholine or epinephrine.

Many biological actions have been attributed to substance P. The smooth muscle actions include: a systemic hypotension, peripheral, pulmonary, coronary, and splanchnic vasodilation, bronchconstriction, intestinal smooth muscle-stimulating activity, and increases in capillary permeability associated with pain and local edema formation (66,119,147,233). Yet, the physiological role for substance P is still speculative. Considerable evidence has been accumulated which suggests that substance P may function as a neurotransmitter or neuromodulator of synaptic transmission in the central nervous system and peripheral autonomic nervous system (233).

It has been reported that the biologically active region of substance P is contained within the carboxy-terminal hexapeptide since frag-

ments containing this region retain nearly full potency for stimulating the guinea pig ileum (190,243). The amino terminal end of the peptide appears to be critical for the neural actions of substance P (167).

Von Euler and Gaddum were the first ones to describe the hypotensive properties of substance P. They suggested that the fall in arterial pressure induced by this peptide in atropinized rabbits was due to a peripheral vasodilation (65,80).

Relatively few studies have been conducted on the effects of substance P in the splanchnic vascular bed. Intravenous infusion of either synthetic (threshold dose = 0.6-1.8 ng/min/kg) (97) or extracted (10 U/kg) (139) substance P into the dog has been shown to markedly increase blood flow in both hepatic and superior mesenteric arteries. In one case (97), dose-dependent increases in hepatic and superior mesenteric arterial blood flow was demonstrated. Extracted substance P (139) and higher infusion rates of synthetic substance P (97) were found to produce significant systemic hypotension. Likewise, local intraarterial infusion of synthetic substance P, at a minimum effective infusion of 0.6 ng/min/kg, was shown to dose-dependently increase superior mesenteric arterial blood flow in the pig. At higher infusion rates, systemic arterial blood pressure was significantly reduced (200).

A direct effect on intestinal vascular smooth muscle has been suggested for substance P since peptide-induced vasodilation was not attenuated or abolished by atropine in the dog (139) or by serotonergic and alpha-adrenergic blockade, and local nerve blockade in the pig (200). Corroborating this contention are the findings of Hellstrand and Jarhult (104) where synthetic substance P (0.01-6.3 μg/ml) dose-dependently contracted the <u>in vitro</u> rat portal vein without attenuation during phen-

tolamine or atropine blockade.

The effects of substance P on intestinal smooth muscle activity was, likewise, initially described by von Euler and Gaddum. They demonstrated that their extract could contract a previously atropinized isolated intestinal segment (65). Early investigations suggested that extracted substance P was a potent stimulant of intestinal motor activity (83, 170) due to a direct effect on intestinal smooth muscle (170).

It has been demonstrated that either intravenous infusion of 600-1000 U extracted substance P into humans (140), or local intraarterial infusion of synthetic substance P (1-10 μg/animal/min) into pigs (200) stimulated intestinal peristaltic and segmental movements and intestinal tone. A spasmogenic effect in the rat stomach, spanning fundus to pylorus (7), and a stimulation of tonic activity of the canine corpus and antrum of the stomach (152), was produced by an infusion of substance P at 5 μg/kg and 5-500 ng/kg, respectively. In the dog, a contraction of the ileum was produced with elevated doses (152). In cats, intravenous infusion of synthetic substance P (exceeding doses of 17 pmol/kg/min) produced a powerful and selective stimulatory effect on the distal colon as evidenced by forceful mass contractions and increases in basal muscle tone (105). Additionally, substance P produced a powerful spasmogenic effect in longitudinal smooth muscle (108), while in circular smooth muscle a rhythmic, peristaltic contraction occured (109).

These gastrointestinal motor responses to substance P appeared to be mediated through local nerves or through a direct effect of the peptide on intestinal smooth muscle since atropine (7,105,152), alpha- and beta-adrenergic blockade, anti-serotonergics, anti-histaminics, or verapamil (7) could not abolish or attenuate the stimulatory responses

induced by substance P infusion.

In vitro studies have demonstrated that substance P stimulates the contractile activity of several different muscles. Administration of extracted substance P (10^{-8}-10^{-7}M) selectively increased the tonic activity of guinea pig corpus, fundus, and ileal muscle strips (151). Cocks and Burnstock (51) demonstrated that synthetic substance P (0.1-10 µg/ml) produced a contraction of taenia coli muscle strips obtained from the guinea pig.

Yau (244) has provided evidence to support the growing contention of a possible physiological regulatory role for substance P on intestinal motility. Synthetic substance P was shown to dose-dependently increase the mechanical contraction of guinea pig ileal longitudinal muscle strips which was atropine, tetrodotoxin, and lioresal resistant. The effective dose (ED_{50}) of $1x10^{-9}$ gm/ml fell within the normal circulating levels reported for substance P (164,177). Corroborating this contention is the observation that synthetic substance P (1.5 to $7.5x10^{-10}$M) dose-dependently enhanced the contractile response of intestinal smooth muscle to transmural nerve stimulation (103). This implicates a possible prejunctional regulatory role for substance P on neurotransmission along with a direct effect on intestinal smooth muscle (7,105,152,244).

Postprandial blood levels of substance P have not been reported in the literature. Yet, many of the previous studies on intestinal smooth muscle suggest that the stimulatory action on motility can be considered physiological due to the extremely low concentrations of the peptide needed to induce the response. In 1973, Powell et al. (177) initially demonstrated a radioimmunological technique allowing for the measurement of brain substance P-immunoreactivity. Later, Nilsson et al. (164) de-

monstrated for the first time the presence of substance P-like immunoreactivity in the bloodstream. Using this assay, it was determined that 12 hour fasting plasma levels of the peptide in man averaged 180 pmol/l. For the dog, 12 hour fasting plasma levels were reported to be, on the average, 88 pmol/l (164).

A hormonal role for substance P on vascular or visceral smooth muscle should be carefully considered since the majority of the peptide has been demonstrated to be found within intrinsic intestinal nerves. Consequently, the peptide may function as a local neurotransmitter and/or neuromodulatory agent.

CHAPTER III

STATEMENT OF THE PROBLEM

Many peptides have been extracted, isolated, and characterized from the gastrointestinal tract. Among the actions reported for these endogenous substances are effects on intestinal vascular or visceral smooth muscle. However, few reports describe the simultaneous effects of these peptides on both types of muscle. Thus, assessment of the vasoactivity is hampered since it is known that changes in intestinal motility can produce passive alterations in vessel caliber. In addition, the possible physiological regulatory roles these peptides may have on intestinal blood flow and motility during the postprandial state is not clearly understood, and deserves further exploration.

Following the intake of food, marked alterations in motility occur in the stomach and intestine. Also, when chyme is present in these organs, their blood flow is increased. These events are mediated by changes in the activity of both visceral and vascular smooth muscle. To implicate gastrointestinal peptides as hormones which contribute to these changes, it must be shown that at blood levels no higher than those occurring postprandially, these substances do affect intestinal and/or vascular smooth muscle. Testing this possibility was the objective of this investigation.

CHAPTER IV

EXPERIMENTAL METHODS

Surgical Preparation of the Canine Ileum

Adult mongrel female dogs (18-24 kg), fasted for 24 hours, were anesthetized with sodium pentobarbital (30 mg/kg), intubated, and placed on positive pressure ventilation. A midline incision was made in the wall of the abdomen extending caudally from the umbilicus. An ileal segment of small intestine was isolated by locating the ileocecal junction, and proceeding backward into the ileum for approximately 20 cm. A segment of ileum, supplied by a single segmental artery, weighing between 25-34 grams and approximately 12 cm in length, was exteriorized and placed on a platform constructed up to the same level as the exteriorized segment. The mesentery was cut on both sides of the artery to exclude collateral circulation. The segmental artery perfusing the segment was isolated from the overlying mesentery, segmental veins, and periarterial nerves. Care was taken to minimize damage to these nerves. This exteriorized ileal segment was wrapped in a plastic film to maintain moisture and a thermometer was fixed against the wall of the ileum. A heat lamp was used to maintain a temperature between 36-38°C.

One femoral vein and both femoral arteries were isolated, and the vein immediately cannulated with PE 320 tubing for supplemental anesthesia. After all surgical procedures were completed, 30 minutes were allowed for hemostasis to occur. Following the intravenous administration of sodium heparin as an anticoagulant, one femoral artery was cannulated with PE 190 tubing directed toward the abdominal aorta for the measurement of systemic arterial pressure. The second femoral artery

was cannulated with PE 320 tubing for the establishment of an extracorporeal circuit. This circuit included a Sigmamotor pump (Model T8SH) which delivered blood from the cannulated femoral artery to the ileal segmental artery. Following cannulation, blood was pumped through the circuit until it reached the tip of a 17 gauge steel catheter. Interposed within this circuit were two small segments of latex rubber tubing. The first one was placed within the Sigmamotor pump and was the site of peptide infusion. The second one was placed just proximal to the steel catheter, from which ileal perfusion pressure was measured through a needle-tipped catheter. The segmental artery supplying the exteriorized ileal segment was cut and cannulated with the 17 gauge steel catheter. Blood was pumped at constant flow through the segment at a flow rate which produced an initial perfusion pressure equal to or 10-20 mm Hg less than systemic arterial pressure. Both perfusion and systemic pressure were measured by Statham pressure transducers (Model Pb 23Gb) and recorded on a Hewlett-Packard direct-writing oscillograph.

An incision was made at both ends of the exteriorized segment. Through one incision a thin-walled balloon, having an unstressed volume of 50 ml of water and attached to a Levin tube (#20), was inserted into the ileum and gently pulled through the segment until the tip of the balloon was observed through the second incision. This portion of the Levin tube (within the balloon) contained several holes for the injection and withdrawal of water. The other end of the Levin tube was connected to a Statham pressure transducer (Pb 23Gb) through a three-way stopcock for the measurement of ileal intraluminal pressure. The stopcock was used for the injection and withdrawal of water using a 30 cc glass syringe. Both ends of the balloon were securely tied to the ends of the

segment by umbilical tape. Two pieces of umbilical tape were securely tied around the adjacent ileal segments to prevent bleeding. The adjacent segments were cut away from the exteriorized segment and returned to the abdominal cavity. Care was taken not to restrict the venous return of the exteriorized segment. The preparation is schematically shown in Figure 1.

The balloon was filled with water (37°C) until a resting intraluminal pressure of between 2 and 4 mm Hg was recorded. This pressure was referred to as "zero volume" pressure. Aliquots of water (37°C) were slowly injected through the three-way stopcock to increase, stepwise, the balloon volume by 5, 10, 15, and 20 ml. Intraluminal and perfusion pressure were allowed to become steady at each step before increasing the balloon volume. This procedure was repeated several times until reproducible values of intraluminal pressure were obtained at each balloon volume. After reproducible results were obtained, it was assumed that the compliance of the ileal segment had reached a steady state. The entire 20 ml of volume was then withdrawn. Ileal wall compliance (C) was determined by measuring the changes in intraluminal pressure that were produced by given increments in luminal volume: $C = \Delta V/\Delta P$. When the intraluminal pressure showed small regular variations as a result of rhythmic contractions of the ileum, the value of the intraluminal pressure was taken at the valley of the wave. If contractions of the ileum produced wide and spike-like variations in luminal pressure, the experiment was terminated and the data not used. Since arterial blood was pumped through the ileal segment at a constant rate of flow, changes in perfusion pressure reflected similar changes in ileal vascular resistance.

Figure 1. Diagramatic representation of the exteriorized ileal segment. Shown are the placement of ligatures, thin-walled balloon, three-way stopcock, and Levin tube for the infusion and withdrawal of water and the recording of ileal intraluminal pressure.

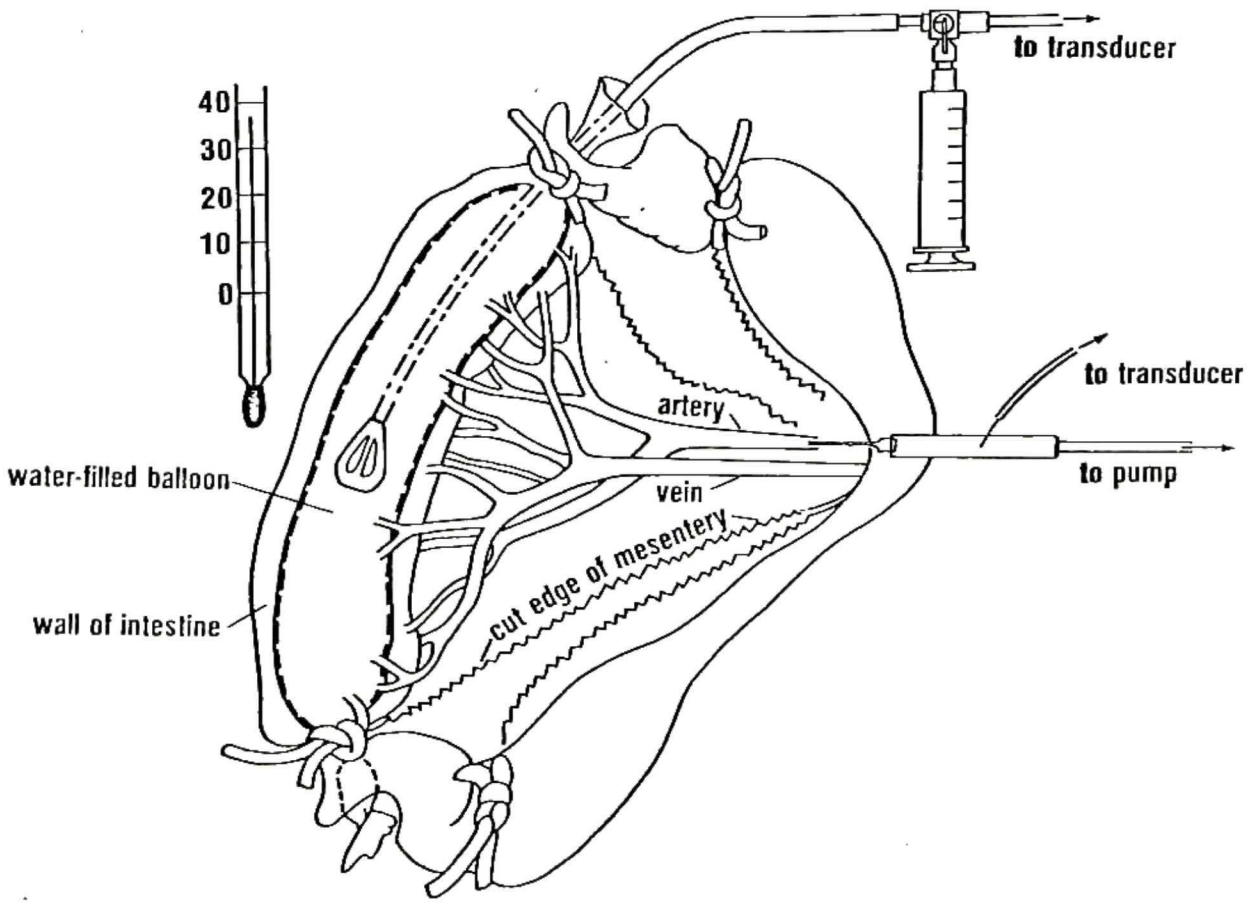
40
30
20
10
0
to transducer
to transducer
to pump
artery
vein
water-filled balloon
wall of intestine
cut edge of mesentery

Figure 1

Intraarterial Infusion of Gastrointestinal Peptides

The investigation consisted of four steps per peptide studied: 1) A control period; 2) Intraarterial infusion of an isotonic sodium chloride containing solution containing a gastrointestinal peptide at a concentration calculated to produce blood levels mimicking those reported in the literature as postprandial blood levels; 3) A post-infusion control period; 4) Intraarterial infusion of an isotonic solution of the same peptide at a concentration calculated to produce blood levels higher than those reported as postprandial blood levels. A Harvard Apparatus infusion/withdrawal pump (Model 940) was used for the infusion of the test peptides. The duration of the infusion periods were, on the average, between 20-25 minutes.

After a control compliance was achieved (as described above) and a control heart rate taken, one of the test peptides was infused into the ileal perfusion circuit (behind the pump). Ileal perfusion pressure and intraluminal pressure were monitored and time allowed for steady state pressures to be attained. After steady state pressures were attained, ileal wall compliance was determined as described above. Heart rate was also monitored during the infusion period. After the final distension and subsequent removal of the entire 20 ml of volume, perfusion pressure and intraluminal pressure were allowed to return to steady state levels. When the steady state was achieved, infusion of the test peptide was terminated. After stopping the infusion, ileal perfusion and intraluminal pressure were monitored and allowed to reach a new steady state. After steady state control pressures were attained, a control compliance was again taken. Then, infusion of the same peptide at a concentration

calculated to produce higher blood levels than those reported as postprandial was infused into the perfusion circuit. Perfusion pressure and intraluminal pressure were again monitored and allowed to reach steady state levels. After attainment, the identical protocol was followed as described for the other infusion rate. Mean systemic arterial pressure and heart rate were continuously monitored throughout control and experimental periods. Experimental results were compared against their respective controls. In saline control experiments, a second infusion of saline was used in lieu of infusion of the peptides studied.

After stopping the infusion of the higher dose of the test peptides, the experiment was terminated and blood flow to the ileal segment was measured by collecting the blood flowing from the ileal perfusion circuit into a graduated cylinder for one minute. The ileum was next cut away from its vasculature and the intraluminal balloon removed. A longitudinal incision was made along the anti-mesenteric border to inspect the mucosa and the segment weighed on an Ohaus-300 top loading balance. Mean blood flow to the ileal segment (ml/min/gm) was calculated by dividing the blood flow to the segment (ml/min) by the ileal weight (gms). Mean blood flows for all of the peptides studied are listed in Table 1. The animals were sacrificed by an intravenous overdose injection of sodium pentobarbital.

Postprandial blood levels of the peptides studied were obtained from the reported literature values for the dog. Blood levels from the literature were taken from arterial plasma or serum samples for all peptides studied except VIP, where portal venous plasma levels were used. The actual first-pass blood concentration of the peptide attained in the ileum was calculated by dividing the infusion rate of the particular

Table 1. Summary of the gastrointestinal peptides studied. Listed are the infusion rates, mean blood flows, calculated first-pass blood concentrations, and reported post-prandial blood concentrations for the six peptides studied.

Table 1

EXPERIMENT	NO.	INFUSION RATE	MEAN BLOOD FLOW (ml/min/gm ileum)	FIRST-PASS BLOOD CONCENTRATION	POSTPRANDIAL BLOOD CONCENTRATION
SALINE	6	0.2ml/min	0.76	--	
GASTRIN	7	1.42pM/min	0.59	76.1pM	85.8pM
GASTRIN	7	14.2pM/min	0.56	761pM	
SECRETIN	7	.216pM/min	0.50	16pM	18pM
SECRETIN	7	21.6pM/min	0.52	1.5nM	
CHOLECYSTOKININ OCTAPEPTIDE	7	438pM/min	0.49	34.4nM	36.5nM
GASTRIC INHIBITORY POLYPEPTIDE	7	3.24pM/min	0.50	191pM	196pM
GASTRIC INHIBITORY POLYPEPTIDE	7	32.4pM/min	0.48	1.91nM	
VASOACTIVE INTESTINAL POLYPEPTIDE	7	.596pM/min	0.56	36pM	36pM
VASOACTIVE INTESTINAL POLYPEPTIDE	7	5.96pM/min	0.56	360pM	
SUBSTANCE P	7	.74pM/min	0.56	47pM	* ?
SUBSTANCE P	7	7.4pM/min	0.56	470pM	
SUBSTANCE P	7	74pM/min	0.56	4.7nM	

*INFUSIONS BASED ON 12 HOUR FASTING LEVELS OF 88pM SUBSTANCE P IN THE CANINE.

peptide studied (as previously determined) by the average blood flow to the ileum. It was found that the calculated first-pass blood concentration very closely mimicked the reported postprandial blood level for each of the peptides studied. The infusion rates used, calculated first-pass blood concentrations, and reported postprandial blood concentrations for all of the peptides studied are listed in Table 1. For substance P, postprandial blood concentrations were not available. Thus, the infusion rate was based on 12 hour fasting blood levels reported in the literature.

The gastrointestinal peptides used in the present study were all pure, synthetically-derived compounds, purchased from either Boehringer-Mannheim (Indianapolis, Indiana) or Peninsula Laboratories (San Carlos, California). They are listed below along with their respective molecular weights. Synthesis was based on the porcine structure of the gut peptide unless otherwise noted.

The gastrointestinal peptides were dissolved in physiological saline and infused through a PE 90 needle-tipped catheter into the ileal perfusion circuit behind the perfusion pump. All solutions were infused at a volume flow rate of 0.2 ml/minute.

BOEHRINGER-MANNHEIM	m.w.	#amino acids
Gastrin I (human, nonsulphated)	2098.49	17
Secretin	3055.87	27
Cholecystokinin-octapeptide (CCK-8, sulphated)	1142.31	8
Vasoactive Intestinal Polypeptide (VIP)	3326.26	28
PENINSULA LABORATORIES		
Gastric Inhibitory Polypeptide (GIP)	5104.44	43
Substance P (SP) (equine)	1347.80	11

Presentation, Calculation, and Analysis of Data

The results are displayed (Figures 3-15) by plotting the mean steady state ileal intraluminal pressure and perfusion pressure against the corresponding ileal balloon volume. The compliance of a hollow organ is defined as the volume change per unit pressure change: $C = \Delta V/\Delta P$. Thus, the slope of the intraluminal pressure-volume curve is inversely proportional to the ileal wall compliance. Wall compliance was calculated by dividing the highest volume introduced into the intraluminal balloon (20 ml) by the difference between intraluminal pressures recorded at 0 and 20 ml balloon volumes. An increase in ileal wall compliance was indicative of a decrease in ileal wall rigidity and was considered to reflect a decrease in wall tension. The slope of the perfusion pressure-volume curve was directly proportional to the rise in resistance produced by the increment in ileal balloon volume. Ileal motility was further analyzed by comparing ileal intraluminal pressure in un-distended gut segments ("zero volume") before, and after peptide infusion.

The effect of the peptides on the ileal vasculature was determined by comparing ileal perfusion pressure immediately before starting local intraarterial infusion, with the steady state perfusion pressure recorded during peptide infusion in un-distended ("zero volume") ileal segments. Since arterial blood was pumped through the ileal segment at a constant rate of flow, changes in perfusion pressure produced by peptide infusion were indicative of proportional changes in ileal vascular resistance. Since ileal vein pressure was not measured, actual calculation of vascular resistance was not possible. Thus, any changes

in vascular resistance were inferred from changes in perfusion pressure. Heart rates and mean systemic arterial blood pressure were monitored during the infusion period and compared against their pre-infusion controls.

The data collected were statistically analyzed with a factorial analysis of variance (FANOV). Differences between treatment group means were determined by the method of Duncan (57); a p value < 0.05 was considered to reflect a statistically significant difference. Values for all vascular pressures and wall compliances tested (mean ± SEM) are located in Appendix One.

CHAPTER V

EXPERIMENTAL RESULTS

Characterization of Ileal Motility and Vascular Resistance During the Measurement of Compliance

A tracing of the variables necessary to simultaneously assess ileal wall compliance and vascular resistance is shown in Figure 2. As the intraluminal balloon volume was increased, stepwise, from 0 to 20 ml, both perfusion pressure and intraluminal pressure were observed to concurrently rise. During each addition of a 5 ml aliquot of water, (indicated by arrows), perfusion pressure rose transiently, then plateaued to a steady state value.

The stepwise distension of the ileum produced rhythmic contractions of the ileal segment. These were coincident with and the cause of the wavy fluctuations in the luminal pressure tracing. Although often observed at the 5 ml volume step, these waves were usually evident by the 10 ml volume step. Mean perfusion and intraluminal pressures were recorded when the steady state was achieved at each increment in luminal volume.

Withdrawal of the entire 20 ml of volume from the balloon (indicated by arrow in Figure 2) produced potent segmental contractions of the ileal segment. These contractions were evidenced by the spike-like rise and fall in the luminal pressure tracing. Concurrent with the segmental contractions, fluctuations in the perfusion pressure tracing were observed, indicating a fluctuating vascular resistance during this period. The potent spike-like contractions have been previously noted by Chou and Dabney (42) who called these movements "after-kicks."

Figure 2. Ileal perfusion pressure and intraluminal pressure during the measurement of ileal wall compliance. Arrows indicate infusion or withdrawal of water from the intraluminal balloon. The balloon volume was increased in 5 ml steps to a final volume of 20 ml. The entire 20 ml was withdrawn in one step.

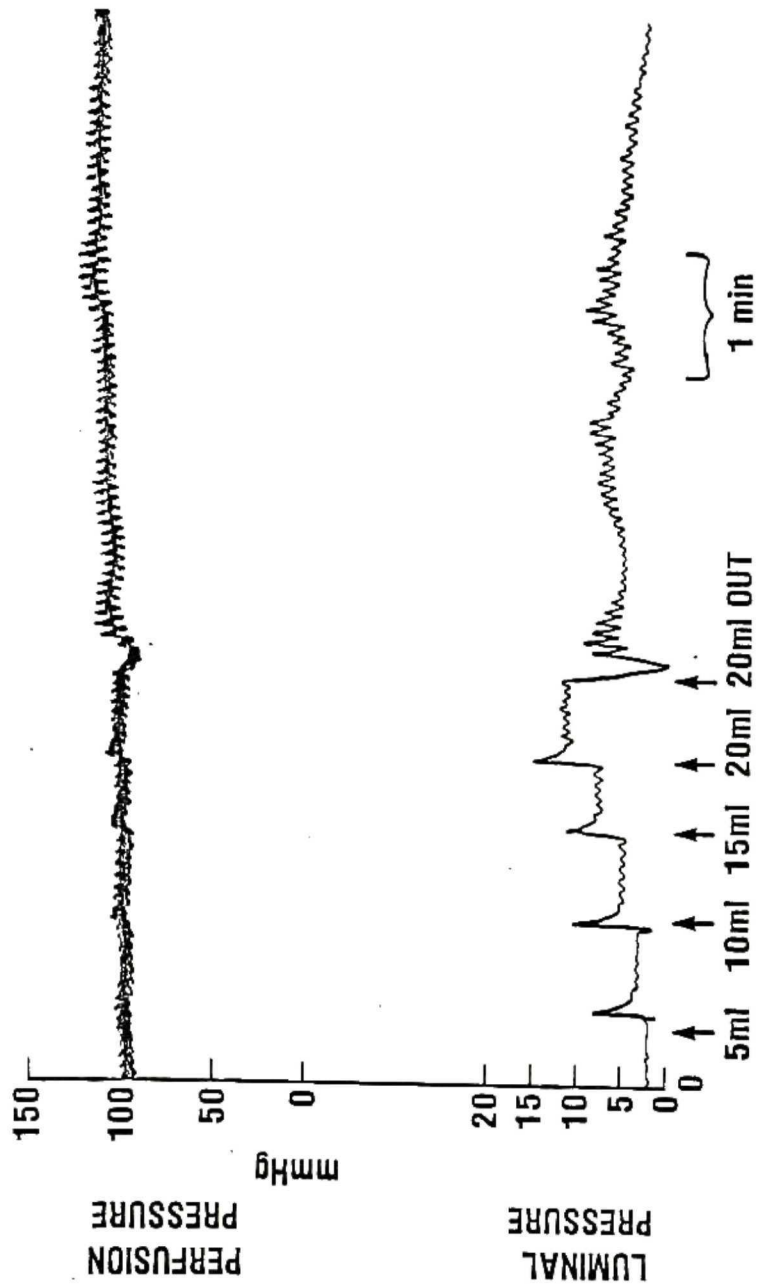
PERFUSION PRESSURE
150
100
50
0
mmHg
LUMINAL PRESSURE
20
15
10
5
0
5ml
10ml
15ml
20ml
20ml OUT
1 min

Figure 2

Progressive stretching of the ileum produced marked changes in both ileal wall compliance and vascular resistance (as evidenced by changes in perfusion pressure). As the ileum was repeatedly distended, its compliance progressively increased, until after several stretchings, it became constant (steady state). From twenty randomly selected experiments it was calculated that, on the average, the ileum had to be stretched seven times before wall compliance became constant (6.9 ± .24, mean ± SEM). As ileal wall compliance was progressively increasing, ileal vascular resistance was simultaneously decreasing (as evidenced by a declining perfusion pressure), until a steady state was attained by the fifth distension (4.8 ± .16, mean ± SEM).

Intraarterial Infusion of Gastrointestinal Peptides into the Canine Ileum

In all experiments conducted, systemic arterial blood pressure and heart rate were not significantly altered during local intraarterial infusion of the gastrointestinal peptides. Table 2 lists the actual heart rates and aortic pressures recorded during the course of experimentation. The incremental change in perfusion pressure during luminal distension (i.e., the slope of the curve of perfusion pressure vs. ileal balloon volume) was significantly altered only during infusion of substance P at 74 pM/min (Figure 15).

The effects of local intraarterial infusion of saline on ileal wall compliance and vascular resistance are shown in Figure 3. In this, and all subsequent figures, the arrow indicates the initiation of infusion of the test agent. The dotted lines represent pressure changes observed at "zero volume" after test agent infusion. The infusion of saline at a volume flow rate of 0.2 ml/min did not significantly alter perfusion pressure at "zero volume." As a consequence, ileal vascular resistance was not affected by saline infusion. Likewise, ileal wall compliance was not significantly altered from the control value. Intraluminal pressure at "zero volume" was identical for both control period and saline infusion.

The effects of local intraarterial infusion of synthetic gastrin on ileal wall compliance and vascular resistance are shown in Figure 4. Infusion of gastrin (1.42 pM/min), attaining a first-pass blood concentration of 76.1 pM (postprandial = 85.8 pM), did not significantly alter perfusion pressure at "zero volume." Therefore, ileal vascular resistance was not affected by infusion of gastrin mimicking postprandial

Table 2. Peptide effects on heart rate and aortic pressure. Experimental values are compared against the respective control values.

Table 2

	HEART RATE (beats per minute)	AORTIC PRESSURE (mmHg)
CONTROL	145	122
SALINE	145	122
CONTROL	155	135
POSTPRANDIAL GASTRIN	155	136
CONTROL	155	133
10 × POSTPRANDIAL GASTRIN	155	133
CONTROL	135	124
POSTPRANDIAL SECRETIN	135	125
CONTROL	135	124
100 × POSTPRANDIAL SECRETIN	135	123
CONTROL-POSTPRANDIAL	150	127
CHOLECYSTOKININ-OCTAPEPTIDE	155	130
CONTROL	135	120
POSTPRANDIAL GASTRIC INHIBITORY POLYPEPTIDE	135	122
CONTROL	140	120
10 × POSTPRANDIAL GASTRIC INHIBITORY POLYPEPTIDE	140	122
CONTROL	145	127
POSTPRANDIAL VASOACTIVE INTESTINAL POLYPEPTIDE	145	126
CONTROL	150	125
10 × POSTPRANDIAL VASOACTIVE INTESTINAL POLYPEPTIDE	150	125
CONTROL	155	120
SUBSTANCE P (.74pM/min)	155	119
CONTROL	155	119
SUBSTANCE P (7.4 pM/min)	155	118
CONTROL	155	119
SUBSTANCE P (74 pM/min)	155	118

Figure 3. Effects of saline infusion (0.2 ml/min) on intraluminal pressure, ileal wall compliance, and perfusion pressure.

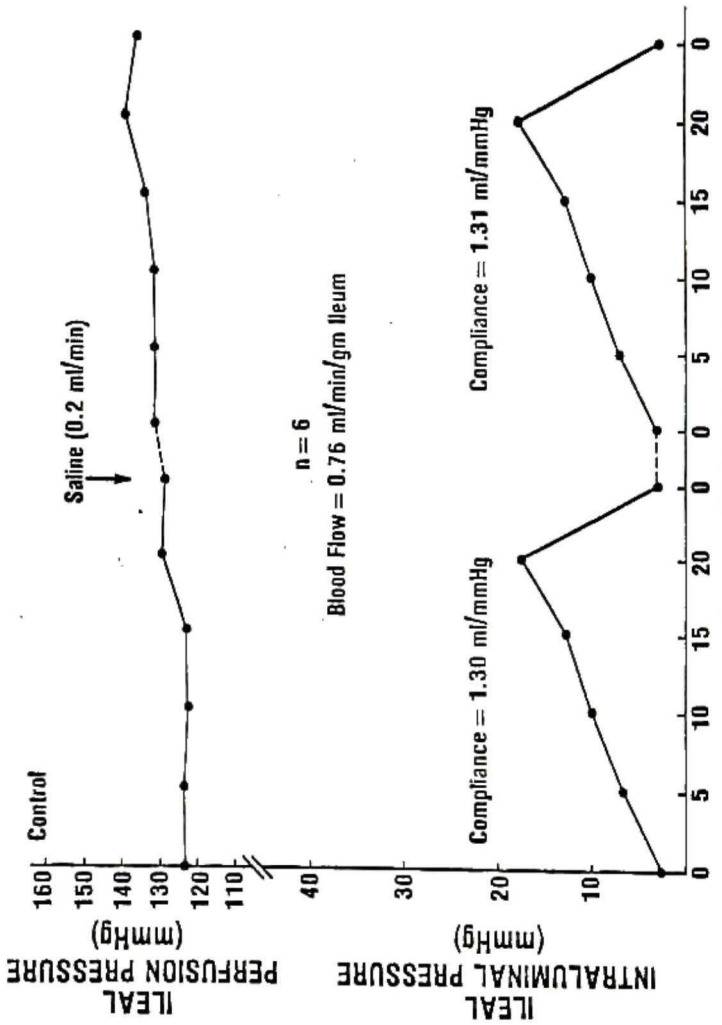
ILEAL PERFUSION PRESSURE (mmHg)
160
150
140
130
120
110
Control
Saline (0.2 ml/min)
n = 6
Blood Flow = 0.76 ml/min/gm Ileum
40
30
20
10
ILEAL INTRALUMINAL PRESSURE (mmHg)
Compliance = 1.30 ml/mmHg
Compliance = 1.31 ml/mmHg
0 5 10 15 20 0 0 5 10 15 20 0
ILEAL BALLOON VOLUME (ml)

Figure 3

Figure 4. Effects of postprandial blood levels of synthetic gastrin (76.1 pM) on intraluminal pressure, ileal wall compliance, and perfusion pressure.

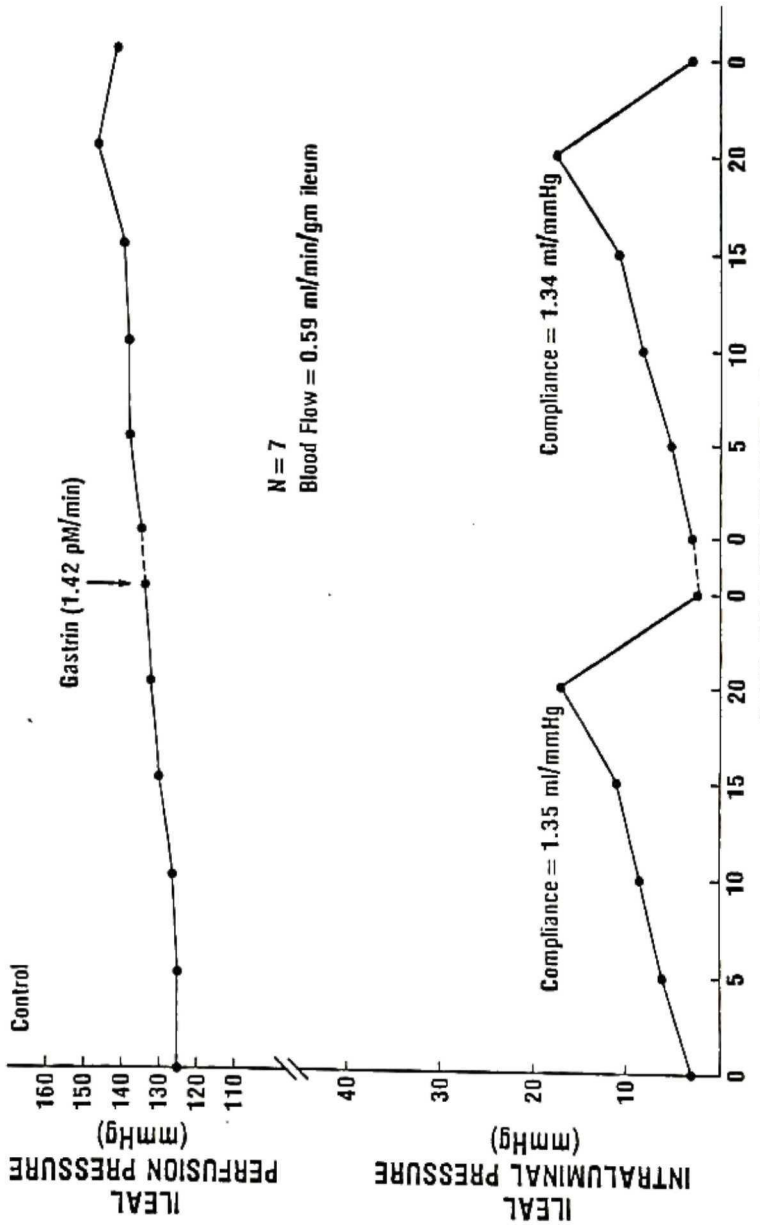
Control
Gastrin (1.42 pM/min)
N = 7
Blood Flow = 0.59 ml/min/gm ileum
Compliance = 1.35 ml/mmHg
Compliance = 1.34 ml/mmHg
ILEAL PERFUSION PRESSURE (mmHg)
ILEAL INTRALUMINAL PRESSURE (mmHg)
ILEAL BALLOON VOLUME (ml)
160
150
140
130
120
110
40
30
20
10
0
5
10
15
20
0
0
5
10
15
20
0

Figure 4

blood concentrations. Ileal wall compliance was likewise, not significantly altered during the infusion period. Intraluminal pressure at "zero volume" was not significantly changed from the control value.

Infusion of gastrin at 14.2 pM/min (Figure 5), attaining a first-pass blood concentration of 761 pM (postprandial = 85.8 pM), did not significantly alter "zero volume" perfusion pressure. Thus, ileal vascular resistance was not modified by this dose of gastrin. Ileal wall compliance and "zero volume" intraluminal pressure were not significantly different from their respective control values.

The effects of local intraarterial infusion of synthetic secretin on ileal wall compliance and vascular resistance are shown in Figure 6. Infusion of secretin (0.216 pM/min), attaining a first-pass blood concentration of 16 pM (postprandial = 18 pM), did not significantly alter "zero volume" perfusion pressure. Thus, ileal vascular resistance was not altered by infusion of secretin mimicking postprandial blood concentrations. However, this infusion rate of secretin significantly increased ileal wall compliance from a control of 1.15 to 1.36 ml/mm Hg. This indicates that ileal wall tension was decreased by blood levels of secretin mimicking postprandial blood concentrations. Although wall compliance was significantly increased, intraluminal pressure at "zero volume" was not significantly changed from the control value.

Infusion of secretin at 21.6 pM/min (Figure 7), attaining a first-pass blood concentration of 1.5 nM (postprandial = 18 pM) significantly lowered ileal perfusion pressure at "zero volume" from a control of 148 to 132 mm Hg. Thus, ileal vascular resistance was significantly decreased by 100x postprandial blood levels of secretin. Latency for secretin-induced vasodilation was, on the average, 60 seconds after pep-

Figure 5. Effects of 10x postprandial blood levels of synthetic gastrin (761 pM) on intraluminal pressure, ileal wall compliance, and perfusion pressure.

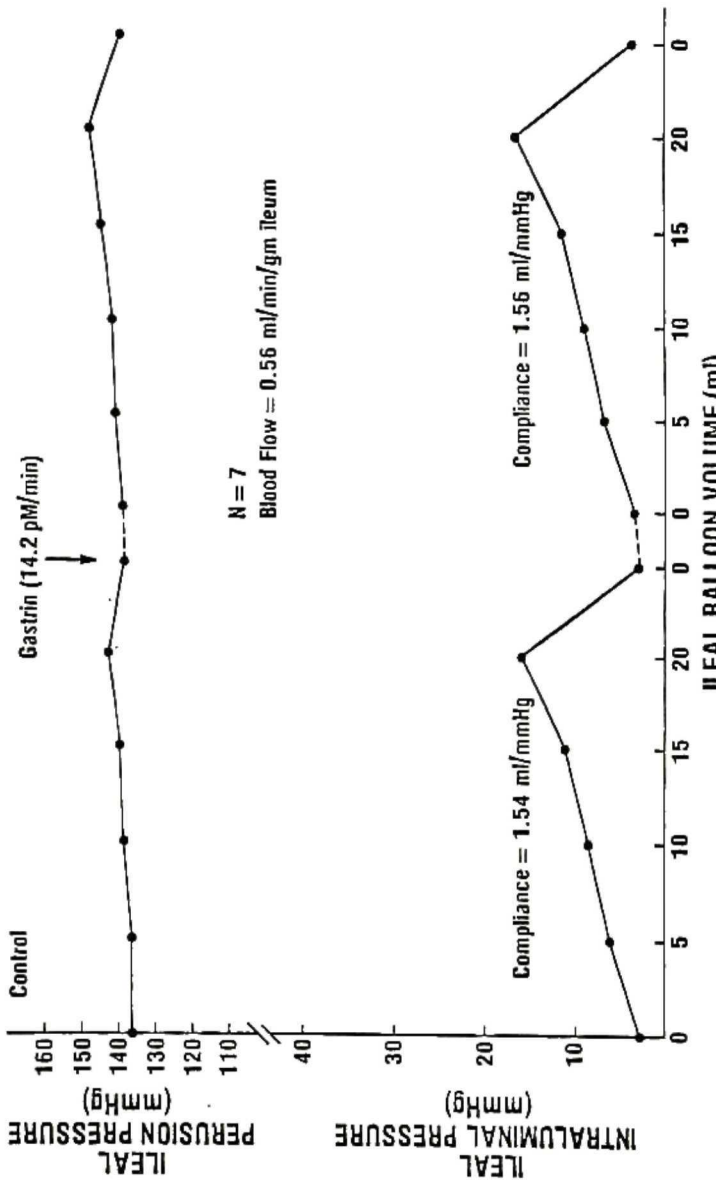
Control
Gastrin (14.2 pM/min)
N = 7
Blood Flow = 0.56 ml/min/gm Ileum
Compliance = 1.54 ml/mmHg
Compliance = 1.56 ml/mmHg
ILEAL PERFUSION PRESSURE (mmHg)
ILEAL INTRALUMINAL PRESSURE (mmHg)
ILEAL BALLOON VOLUME (ml)
160
150
140
130
120
110
40
30
20
10
0
5
10
15
20
0
0
5
10
15
20
0

Figure 5

Figure 6. Effects of postprandial blood levels of synthetic secretin (16 pM) on intraluminal pressure, ileal wall compliance, and perfusion pressure.

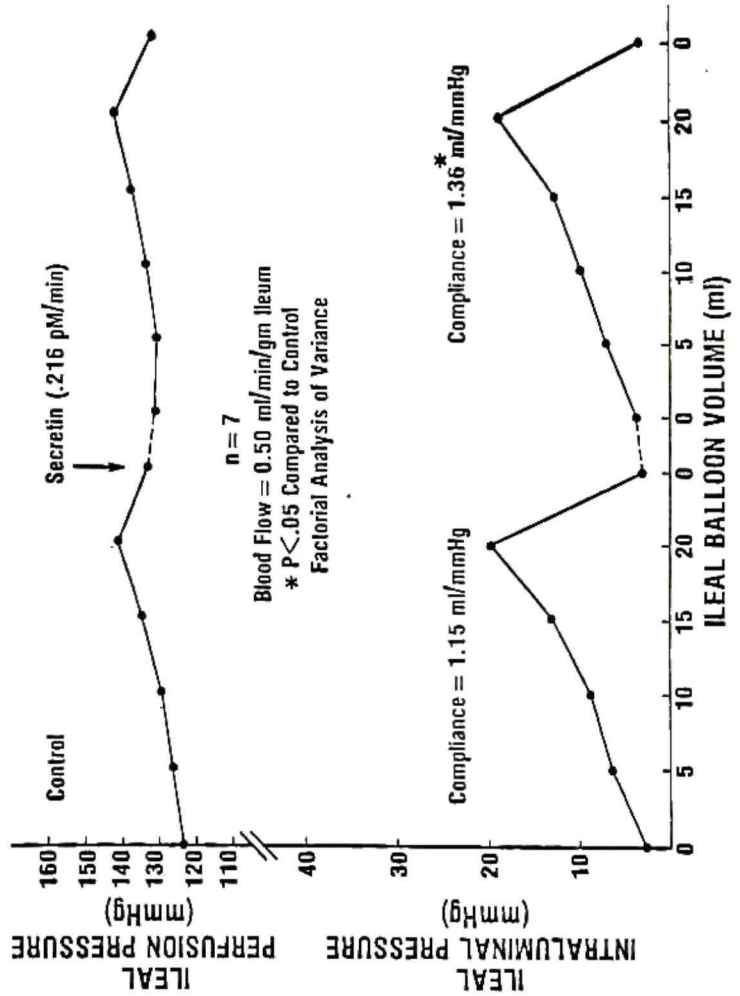
ILEAL PERFUSION PRESSURE (mmHg)
160
150
140
130
120
110
Control
Secretin (.216 pM/min)
n = 7
Blood Flow = 0.50 ml/min/gm Ileum
* P<.05 Compared to Control
Factorial Analysis of Variance
ILEAL INTRALUMINAL PRESSURE (mmHg)
40
30
20
10
Compliance = 1.15 ml/mmHg
Compliance = 1.36* ml/mmHg
0 5 10 15 20 0 0 5 10 15 20 0
ILEAL BALLOON VOLUME (ml)

Figure 6

Figure 7. Effects of 100x postprandial blood levels of synthetic secretin (1.5 nM) on intraluminal pressure, ileal wall compliance, and perfusion pressure.

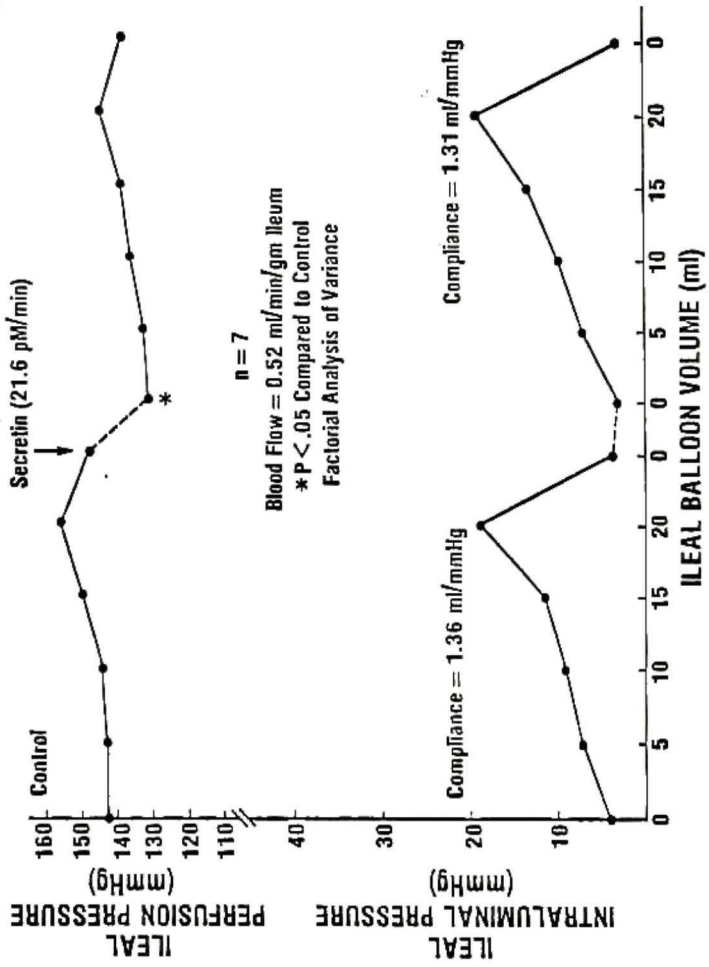
Control
Secretin (21.6 pM/min)
n = 7
Blood Flow = 0.52 ml/min/gm Ileum
*P < .05 Compared to Control
Factorial Analysis of Variance
ILEAL PERFUSION PRESSURE (mmHg)
ILEAL INTRALUMINAL PRESSURE (mmHg)
Compliance = 1.36 ml/mmHg
Compliance = 1.31 ml/mmHg
ILEAL BALLOON VOLUME (ml)

Figure 7

tide infusion (range = 45-90 seconds). Intraluminal pressure at "zero volume" was not significantly altered nor was ileal wall compliance further increased during the infusion of this higher dose of secretin. After termination of the infusion, ileal perfusion pressure quickly returned to pre-infusion levels.

The effects of local intraarterial infusion of synthetic cholecystokinin-octapeptide (CCK-8) on ileal wall compliance and vascular resistance are shown in Figure 8. Infusion of CCK-8 (438 pM/min), attaining a first-pass blood concentration of 34.4 nM (postprandial = 36.5 nM), did not significantly alter ileal perfusion pressure at "zero volume." Thus, ileal vascular resistance was not affected by an infusion of CCK-8 mimicking postprandial blood levels. However, ileal wall compliance was significantly decreased from a control of 1.19 to 0.88 ml/mm Hg, reflecting an increase in ileal wall tension at postprandial blood levels of the peptide. At "zero volume," intraluminal pressure was markedly elevated from a control of 4.5 to 21.5 mm Hg. Potent spike-like variations in intraluminal pressure (peaking, on the average, at 45 mm Hg) preceeded the final steady state "zero volume" pressure of 21.5 mm Hg. Latency for CCK-8-induced elevation in intraluminal pressure was 45 seconds on the average, after beginning CCK-8 infusion (range = 30-60 seconds). Higher blood levels of CCK-8 were not possible to assess due to the marked alterations in ileal luminal pressure observed.

Inspection of CCK-8-induced changes in perfusion pressure and intraluminal pressure at "zero volume" revealed that for a 370% increase in intraluminal pressure, a corresponding increase in perfusion pressure of only 2.7% was observed. The data seem to indicate that the increased intraluminal pressure generated by CCK-8 was not significantly trans-

Figure 8. Effects of postprandial blood levels of synthetic cholecystokinin-octapeptide (CCK-8) (34.4 nM) on intraluminal pressure, ileal wall compliance, and perfusion pressure.

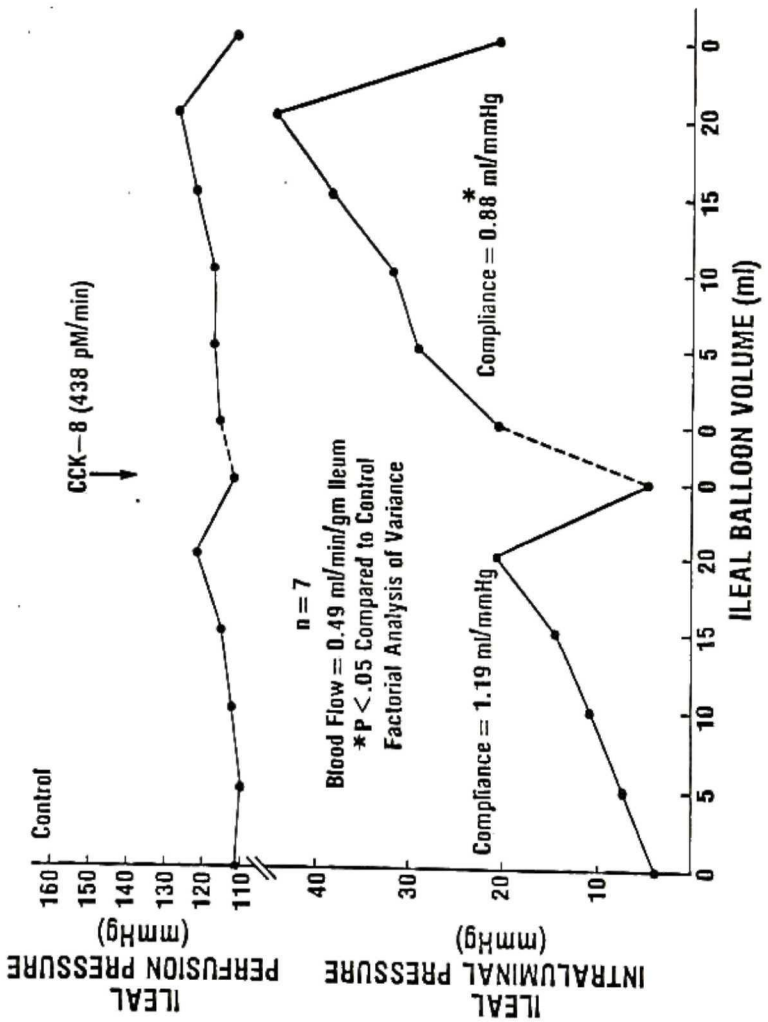
ILEAL PERFUSION PRESSURE (mmHg)
160
150
140
130
120
110
Control
CCK–8 (438 pM/min)
n = 7
Blood Flow = 0.49 ml/min/gm Ileum
*P < .05 Compared to Control
Factorial Analysis of Variance
ILEAL INTRALUMINAL PRESSURE (mmHg)
40
30
20
10
Compliance = 1.19 ml/mmHg
Compliance = 0.88* ml/mmHg
0 5 10 15 20 0 0 5 10 15 20 0
ILEAL BALLOON VOLUME (ml)

Figure 8

mitted to the vasculature.

The effects of local intraarterial infusion of synthetic gastric inhibitory polypeptide (GIP) on ileal wall compliance and vascular resistance are shown in Figure 9. Infusion of GIP (3.24 pM/min), attaining a first-pass blood concentration of 191 pM (postprandial = 196 pM), did not significantly alter "zero volume" perfusion pressure. Therefore, ileal vascular resistance was not affected by an infusion of GIP which mimicked postprandial blood levels. However, this infusion rate of GIP significantly increased ileal wall compliance from a control of 1.75 to 2.02 ml/mm Hg. This indicates that ileal wall tension was decreased by blood levels of GIP mimicking postprandial blood concentrations. Although wall compliance was increased, intraluminal pressure at "zero volume" was not significantly altered from the control value.

Infusion of GIP at 32.4 pM/min (Figure 10), attaining a first-pass blood concentration of 1.91 nM (postprandial = 196 pM), significantly lowered ileal perfusion pressure at "zero volume" from a control of 118 to 104 mm Hg. Thus, ileal vascular resistance was significantly decreased by 10x postprandial blood levels of GIP. Latency for GIP-induced vasodilation was 60 seconds on the average, after beginning GIP infusion (range = 45-90 seconds). Intraluminal pressure at "zero volume" was not significantly altered nor was ileal wall compliance further increased by this higher infusion rate of GIP. After termination of the infusion, ileal perfusion pressure quickly returned to pre-infusion levels.

The effects of local intraarterial infusion of synthetic vasoactive intestinal polypeptide (VIP) on ileal wall compliance and vascular resistance are shown in Figure 11. Infusion of VIP (0.596 pM/min), attaining a first-pass blood concentration of 36 pM (postprandial = 36 pM),

Figure 9. Effects of postprandial blood levels of synthetic gastric inhibitory polypeptide (GIP) (191 pM) on intraluminal pressure, ileal wall compliance, and perfusion pressure.

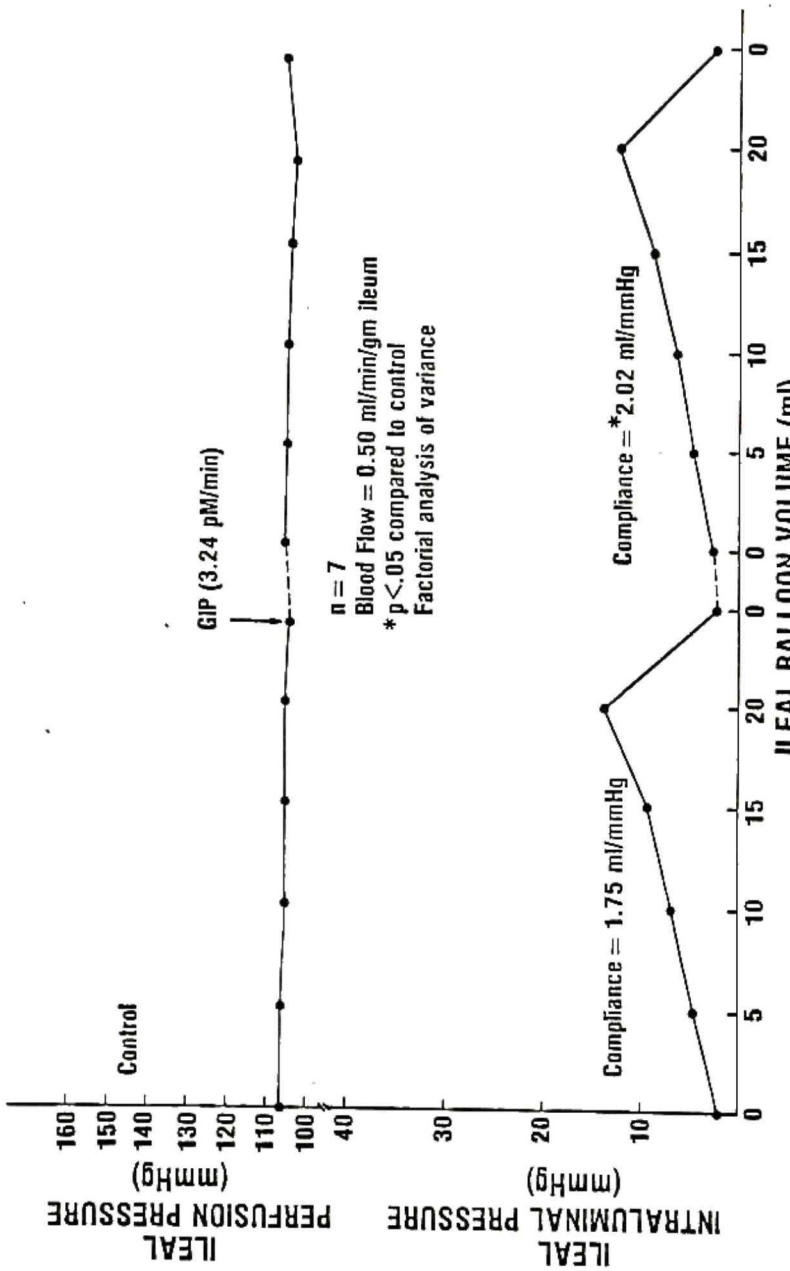
ILEAL PERFUSION PRESSURE (mmHg)
160
150
140
130
120
110
100
40
Control
GIP (3.24 pM/min)
n = 7
Blood Flow = 0.50 ml/min/gm ileum
*p<.05 compared to control
Factorial analysis of variance
ILEAL INTRALUMINAL PRESSURE (mmHg)
30
20
10
Compliance = 1.75 ml/mmHg
Compliance = *2.02 ml/mmHg
0 5 10 15 20 0 0 5 10 15 20 0
ILEAL BALLOON VOLUME (ml)

Figure 9

Figure 10. Effects of 10x postprandial blood levels of synthetic gastric inhibitory polypeptide (GIP) (1.91 nM) on intraluminal pressure, ileal wall compliance, and perfusion pressure.

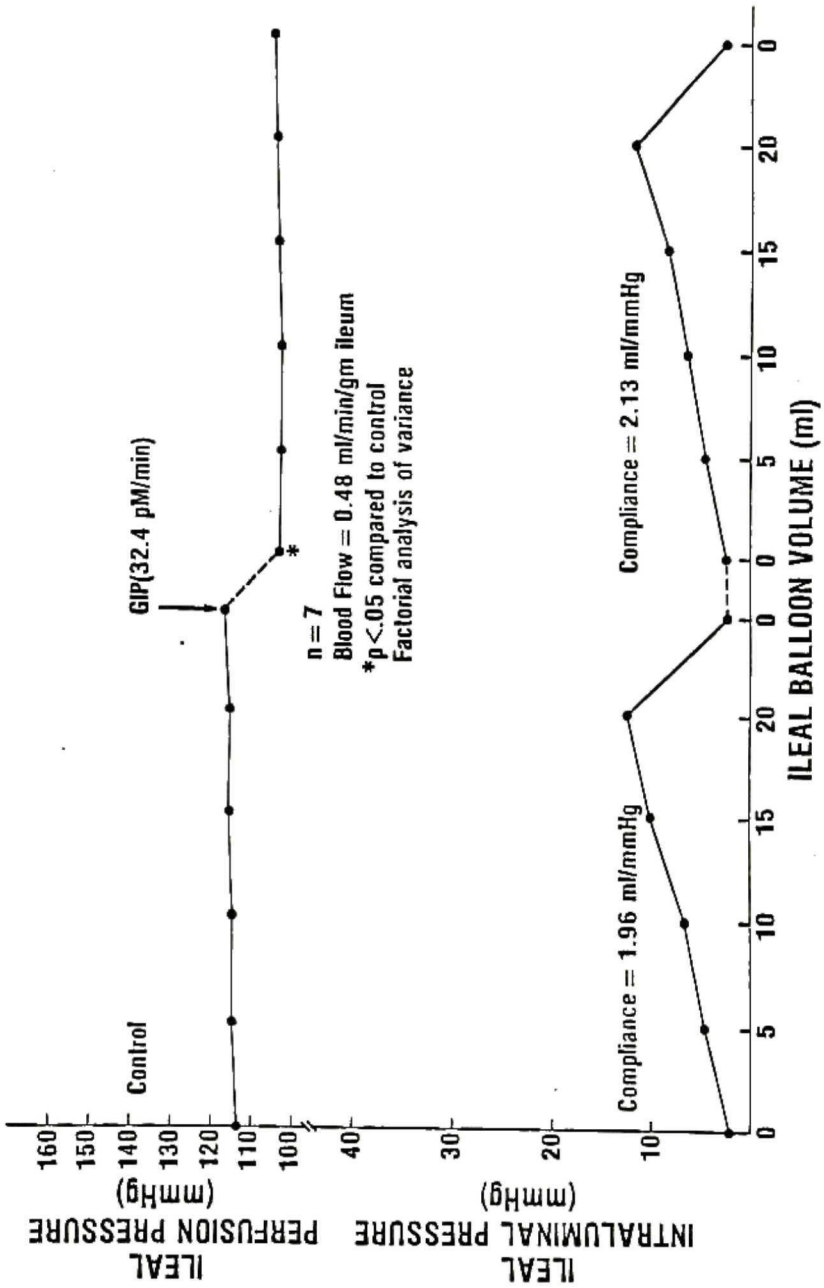
ILEAL PERFUSION PRESSURE (mmHg)
ILEAL INTRALUMINAL PRESSURE (mmHg)
ILEAL BALLOON VOLUME (ml)
Control
GIP(32.4 pM/min)
n = 7
Blood Flow = 0.48 ml/min/gm ileum
*p <.05 compared to control
Factorial analysis of variance
Compliance = 1.96 ml/mmHg
Compliance = 2.13 ml/mmHg

Figure 10

Figure 11. Effects of postprandial blood levels of synthetic vasoactive intestinal polypeptide (VIP) (36 pM) on intraluminal pressure, ileal wall compliance, and perfusion pressure.

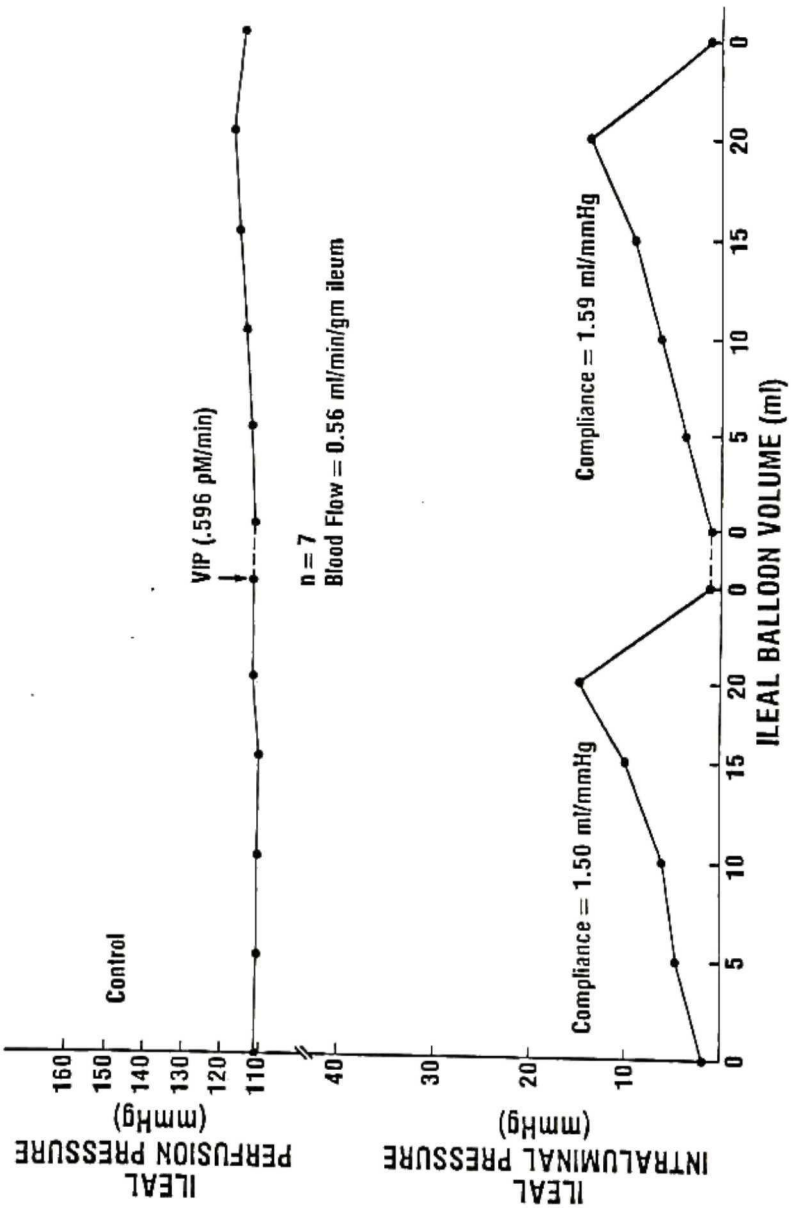
ILEAL PERFUSION PRESSURE (mmHg)
160
150
140
130
120
110
40
30
20
10
Control
VIP (.596 pM/min)
n = 7
Blood Flow = 0.56 ml/min/gm ileum
ILEAL INTRALUMINAL PRESSURE (mmHg)
Compliance = 1.50 ml/mmHg
Compliance = 1.59 ml/mmHg
0 5 10 15 20 0 0 5 10 15 20 0
ILEAL BALLOON VOLUME (ml)

Figure 11

did not alter "zero volume" perfusion pressure. Therefore, ileal vascular resistance was not affected by infusion of VIP mimicking postprandial blood concentrations. Intraluminal pressure at "zero volume" and ileal wall compliance were not significantly affected by postprandial blood levels of VIP.

Infusion of VIP at 5.96 pM/min (Figure 12), attaining a first-pass blood concentration of 360 pM (postprandial = 36 pM), did not significantly alter perfusion pressure at "zero volume." Thus, ileal vascular resistance was not significantly altered by 10x postprandial blood levels of VIP. Also, intraluminal pressure at "zero volume" and ileal wall compliance were not significantly different from their respective controls during the infusion of this higher dose of VIP.

Substance P was infused into the ileum at three different rates: 0.74, 7.4, and 74 pM/min. Infusion of substance P at 0.74 pM/min (Figure 13), attaining a first-pass blood concentration of 47 pM (postprandial unknown), significantly lowered ileal perfusion pressure at "zero volume" from a control of 109 to 100 mm Hg. Thus, ileal vascular resistance was significantly decreased by a blood level of 47 pM substance P. The latency for substance P-induced vasodilation was 45 seconds on the average, after beginning peptide infusion (range = 30-60 seconds). Intraluminal pressure at "zero volume" and ileal wall compliance were not significantly changed from their respective controls during the infusion period. After infusion termination, ileal perfusion pressure quickly rose to pre-infusion levels.

Infusion of substance P at 7.4 pM/min (Figure 14), attaining a first-pass blood concentration of 470 pM, significantly lowered ileal perfusion pressure at "zero volume" from a control of 121 to 86.5 mm Hg.

Figure 12. Effects of 10x postprandial blood levels of synthetic vasoactive intestinal polypeptide (VIP) (360 pM) on intraluminal pressure, ileal wall compliance, and perfusion pressure.

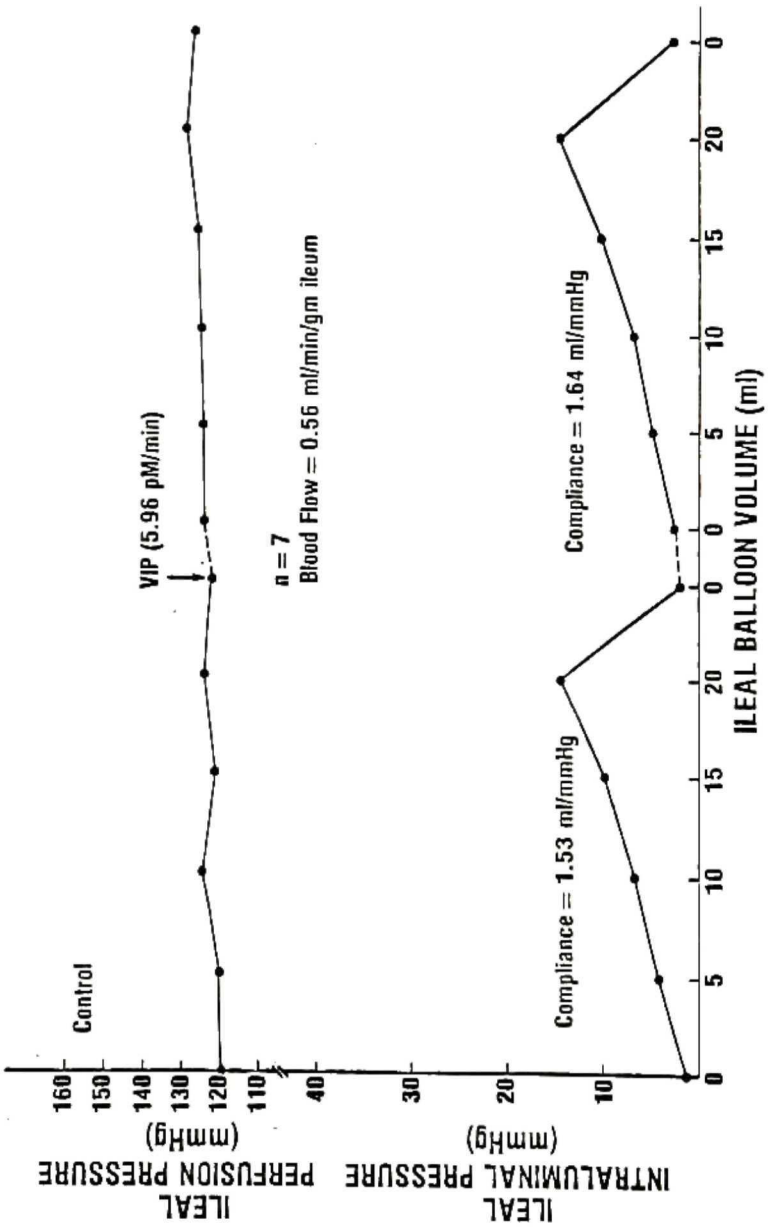
ILEAL PERFUSION PRESSURE (mmHg)
160
150
140
130
120
110
40
30
20
10
Control
VIP (5.96 pM/min)
n = 7
Blood Flow = 0.56 ml/min/gm ileum
ILEAL INTRALUMINAL PRESSURE (mmHg)
Compliance = 1.53 ml/mmHg
Compliance = 1.64 ml/mmHg
0 5 10 15 20 0 0 5 10 15 20 0
ILEAL BALLOON VOLUME (ml)

Figure 12

Figure 13. Effects of 47 pM blood levels of synthetic substance P (SP) on intraluminal pressure, ileal wall compliance, and perfusion pressure.

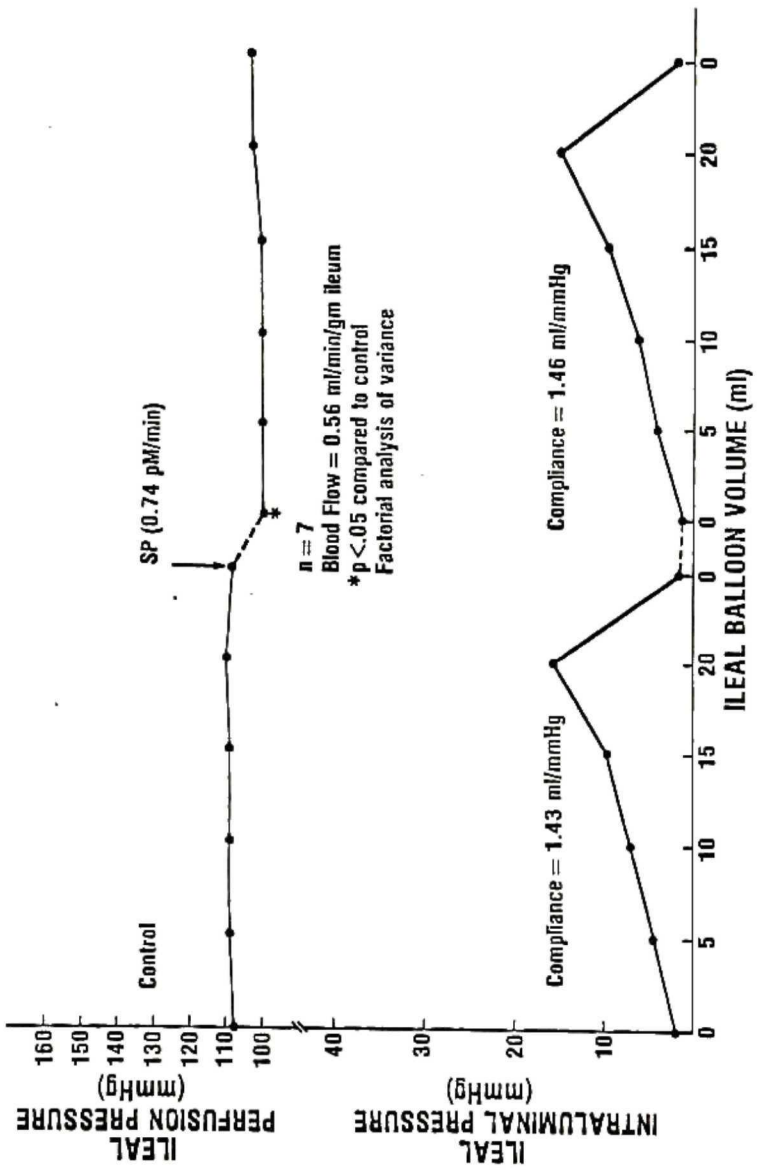
ILEAL PERFUSION PRESSURE (mmHg)
160
150
140
130
120
110
100
Control
SP (0.74 pM/min)
n = 7
Blood Flow = 0.56 ml/min/gm ileum
*p <.05 compared to control
Factorial analysis of variance
40
30
20
10
ILEAL INTRALUMINAL PRESSURE (mmHg)
Compliance = 1.43 ml/mmHg
Compliance = 1.46 ml/mmHg
0 5 10 15 20 0 0 5 10 15 20 0
ILEAL BALLOON VOLUME (ml)

Figure 13

Figure 14. Effects of 470 pM blood levels of synthetic substance P (SP) on intraluminal pressure, ileal wall compliance, and perfusion pressure.

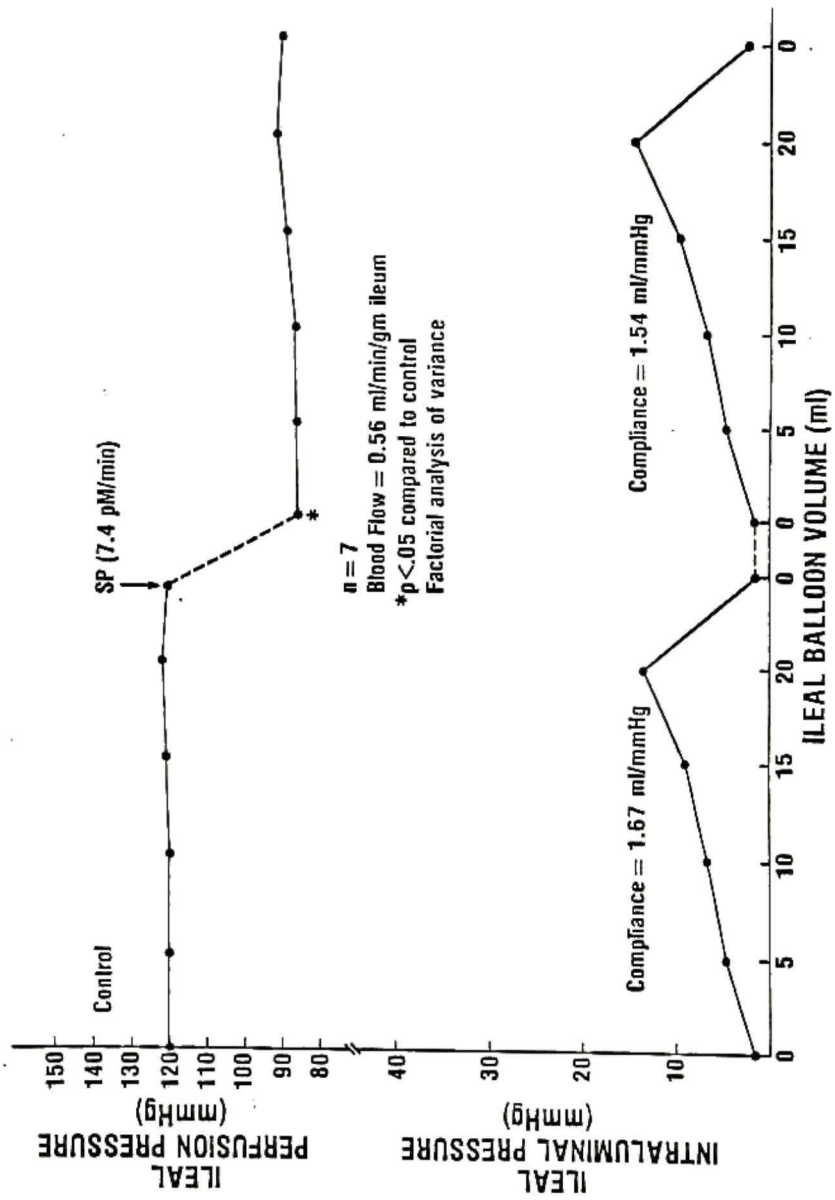
ILEAL PERFUSION PRESSURE (mmHg)
150
140
130
120
110
100
90
80
40
30
20
10
Control
SP (7.4 pM/min)
*
n = 7
Blood Flow = 0.56 ml/min/gm ileum
*p<.05 compared to control
Factorial analysis of variance
ILEAL INTRALUMINAL PRESSURE (mmHg)
Compliance = 1.67 ml/mmHg
Compliance = 1.54 ml/mmHg
0
5
10
15
20
0
0
5
10
15
20
ILEAL BALLOON VOLUME (ml)

Figure 14

The percentage drop in perfusion pressure observed was 28.5%. A comparison to the drop of 8.3% seen with the lower infusion rate (0.74 pM/min), indicated that a dose-dependent vasodilation of the vasculature was occuring. However, intraluminal pressure at "zero volume" and ileal wall compliance were not significantly affected during this infusion period. After infusion termination, ileal perfusion pressure quickly returned to pre-infusion levels.

Infusion of substance P at 74 pM/min (Figure 15), attaining a first-pass blood concentration of 4.7 nM, significantly lowered ileal perfusion pressure at "zero volume" from a control of 149 to 76 mm Hg. This implied that the ileal vasculature was still responsive to this infusion rate of substance P. The percentage drop in perfusion pressure observed averaged 49%. Latency for substance P-induced vasodilation was identical to that observed at the previous two infusion rates. In addition, ileal wall compliance was significantly decreased from a control of 1.75 to 1.00 ml/mm Hg, reflecting a peptide-induced increase in ileal wall tension at a blood concentration of 4.7 nM. Intraluminal pressure at "zero volume" was, as well, significantly elevated from a control of 2.1 to 6.5 mm Hg. In all cases, peptide-induced vasodilation preceeded alterations in ileal intraluminal pressure.

Figure 15. Effects of 4.7 nM blood levels of synthetic substance P (SP) on intraluminal pressure, ileal wall compliance, and perfusion pressure.

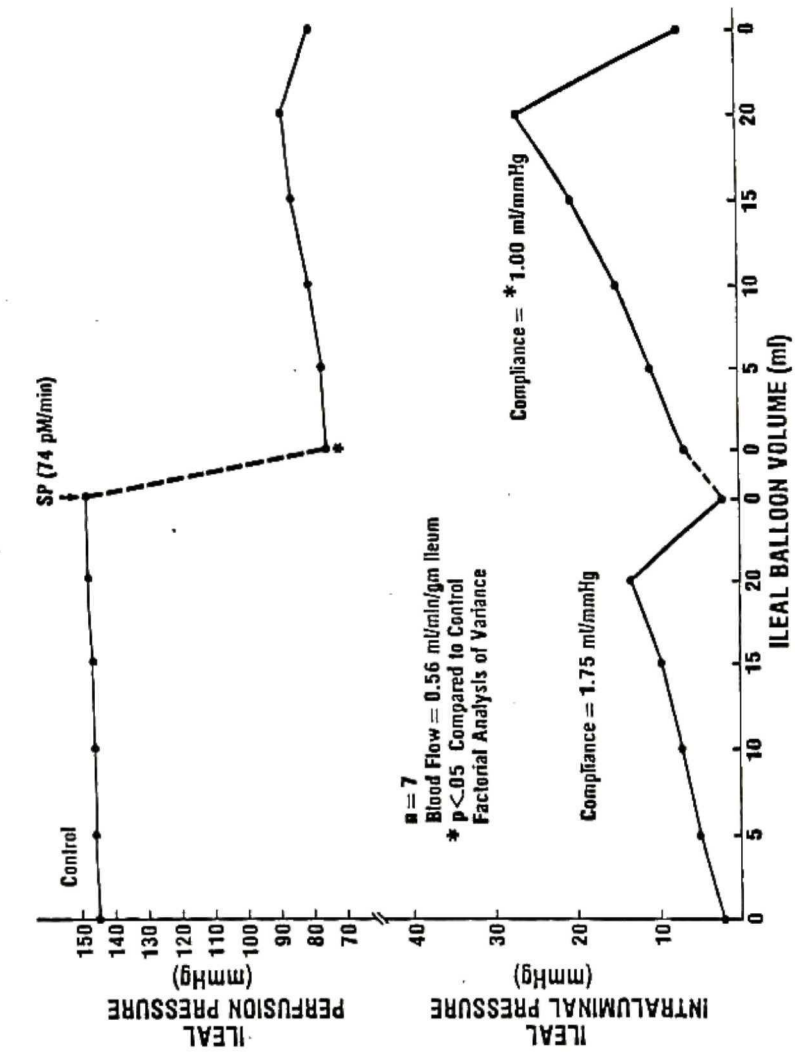
ILEAL PERFUSION PRESSURE (mmHg)
150
140
130
120
110
100
90
80
70
40
Control
SP (74 pM/min)
*
n = 7
Blood Flow = 0.56 ml/min/gm Ileum
* p<.05 Compared to Control
Factorial Analysis of Variance
ILEAL INTRALUMINAL PRESSURE (mmHg)
30
20
10
Compliance = 1.75 ml/mmHg
Compliance = *1.00 ml/mmHg
0
5
10
15
20
0
5
10
15
20
0
ILEAL BALLOON VOLUME (ml)

Figure 15

Pattern of Dilation of the Vasculature by Substance P at either 7.4 or 74 pM/min

In 6 out the 7 animals examined, substance P-induced vasodilation consisted of two drops in perfusion pressure, separated by a transient steady state plateau, at either 7.4 or 74 pM/ min. Figure 16 depicts the effect of this peptide on ileal perfusion pressure (the arrow indicates the point of infusion). Although this figure shows the effect of substance P at 74 pM/min, the same phenomenon was also observed at the the infusion rate of 7.4 pM/min. The vasodilation observed at the lowest infusion rate (0.74 pM/min) consisted of only a single drop in perfusion pressure.

Local infusion of substance P (7.4 pM/min) significantly lowered ileal perfusion pressure 16% on the average (compared to pre-infusion pressure), until an initial steady state plateau was reached. The duration of the initial dilation plateau was not consistent from experiment to experiment. Figure 16 depicts one of the longer duration periods recorded. Subsequently, a second significant drop in perfusion pressure (on the average of 27% and compared to the initial dilation plateau) was observed until a new steady state plateau was attained.

Local infusion of substance P (74 pM/min) significantly lowered ileal perfusion pressure 34% on the average (compared to pre-infusion pressure), until an initial steady state plateau was attained. Again, a subsequent second significant drop in perfusion pressure occured (compared to the initial dilation plateau), dropping 42% on the average, until a new steady state plateau was attained

Figure 16. Pattern of the alteration in ileal perfusion pressure produced by synthetic substance P (SP) at 74 pM/min.

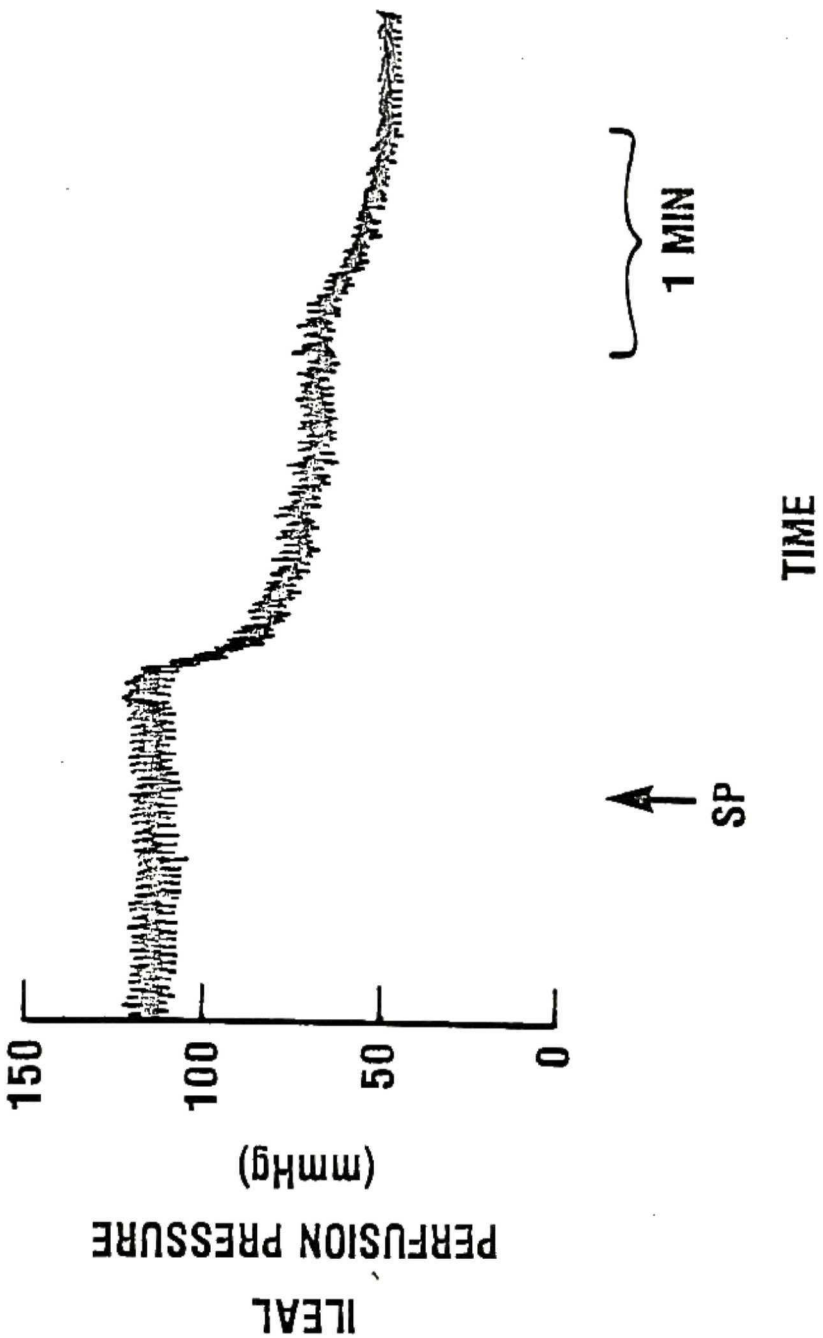

Figure 16

Summary of Effects of Peptides on Ileal Wall Compliance, Ileal Perfusion Pressure, Heart Rate, and Aortic Pressure

The effects of postprandial and 10 or 100x postprandial blood levels of the gastrointestinal peptides, and three different infusion rates of substance P on ileal wall compliance, ileal perfusion pressure, heart rate, and aortic pressure are shown in Table 3. The calculated first-pass blood concentration for each peptide is given in the table.

Table 3. Summary of peptide action in the ileum. Shown are the effects of peptide infusion on ileal wall compliance, ileal perfusion pressure, heart rate, and aortic pressure. The calculated first-pass blood concentration is given for each peptide.

Table 3

HORMONE OR PEPTIDE	FIRST-PASS BLOOD CONCENTRATION	ILEAL WALL COMPLIANCE	ILEAL PERFUSION PRESSURE	HEART RATE	AORTIC PRESSURE
SALINE		↔	↔	↔	↔
GASTRIN	76.1 pM	↔	↔	↔	↔
GASTRIN	761 pM	↔	↔	↔	↔
SECRETIN	16 pM	↑	↔	↔	↔
SECRETIN	1.5 nM	↔*	↓	↔	↔
CHOLECYSTOKININ OCTAPEPTIDE	34.4 nM	↓	↔	↔	↔
GASTRIC INHIBITORY POLYPEPTIDE	191 pM	↑	↔	↔	↔
GASTRIC INHIBITORY POLYPEPTIDE	1.91 nM	↔*	↓	↔	↔
VASOACTIVE INTESTINAL POLYPEPTIDE	36 pM	↔	↔	↔	↔
VASOACTIVE INTESTINAL POLYPEPTIDE	360 pM	↔	↔	↔	↔
SUBSTANCE P	47 pM	↔	↓	↔	↔
SUBSTANCE P	470 pM	↔	↓	↔	↔
SUBSTANCE P	4.7 nM	↓	↓	↔	↔

* denotes no further increase in wall compliance.

CHAPTER VI

DISCUSSION

Numerous peptides have been isolated and characterized from the gastrointestinal tract, and many have been suggested as "candidate hormones" with physiological significance (119,147,233). The three established gastrointestinal hormones: gastrin, secretin, and cholecystokinin (CCK), were isolated and characterized in the 1960's. A fourth peptide, gastric inhibitory polypeptide (GIP), may soon be shown to completely fulfill the requirements which characterize G.I. hormones as well.

Among the actions reported for these endogenous peptides are effects on either intestinal vascular or visceral smooth muscle. Yet, few reports describe the simultaneous effects these substances have on both types of muscle. Thus, accurate assessment of the vasoactivity of a particular peptide in the mesenteric circulation may potentially be hampered since it is known that alterations in intestinal motility can affect local blood flow. Furthermore, the possible physiological regulatory roles these endogenous peptides may have on mesenteric blood flow and motility during the digestive state is not clearly defined.

Therefore, the main objective of this investigation was to ascertain the simultaneous effects of postprandial blood levels of six gastrointestinal peptides on both intestinal vascular and visceral smooth muscle through the simultaneous assessment of intestinal vascular resistance and intestinal wall compliance. This investigation was designed to indicate which of these peptides might be involved in the alterations in intestinal blood flow and motility coincident with the digestive process.

Characterization of Ileal Motility and Vascular Resistance During the Measurement of Compliance

This investigation revealed that during the stepwise increase in luminal volume, a concurrent increase in ileal luminal and perfusion pressure was observed as clearly shown in Figure 2. Since blood flow to the ileal segment was maintained constant, changes in perfusion pressure imply directionally similar changes in ileal vascular resistance. As is also shown in Figure 2, the pattern of change in perfusion pressure paralleled the pattern of change in intestinal motility. Thus, it is suggested that these changes in vascular resistance are secondary to primary changes in intestinal smooth muscle tone.

During progressive stretching of the ileum, its compliance steadily increased as evidenced by a lower luminal pressure at any given volume. During this progressive rise in compliance, ileal vascular resistance was observed to simultaneously decrease. These observations during the measurement of compliance are qualitatively similar to those reported by Chou and Dabney in 1967 (42) when they initially described the experimental method employed in the current investigation.

On rare occasion, it was observed that ileal perfusion pressure would decrease rather than increase during the stepwise increase in luminal volume. This phenomenon was initially reported by Chou (40) where he speculated that the finding was the result of a "rearrangement" of the intramural vessels. Based on the findings of Thomas et al. (216) and Burton et al. (32), Chou postulated that "rearrangement may produce a decrease in the gnarliness of the intramural vessels, i.e., a decrease in the degree of contortion."

The pronounced motility which was induced following the with-

drawal of the entire luminal volume (20 ml), appears to be due to a peristaltic reflex, as previously reported by Chou (40). He mentions that a similar type of motility pattern occurs during the emptying phase of the peristaltic reflex, and since the withdrawal of water from the luminal balloon is mechanically equivalent to emptying of the intestinal content during digestion, this contention is plausible.

Inspection of Figures 6 and 9 allows for the suggestion that the method for the measurement of intestinal wall compliance is a more sensitive index of intestinal wall tension than is assessment through measurement of intraluminal pressure alone. For example, secretin and GIP increased ileal wall compliance without having significant effect on "zero volume" intraluminal pressure.

Control values for ileal wall compliance (Figures 3-15) varied from 1.15 (Figure 6) to 1.96 (Figure 10), with a mean of 1.50 ml/mm Hg (Figure 11). However, control compliance for a given series is in part a function of the weight of the ileal segment, i.e., wall compliance would be greater for a series where the mean weight of the segment was greater than that for a corresponding series. Thus, depending upon the weight of the ileal segment used per series, the value for ileal wall compliance could deviate away from a mean of 1.50 ml/mm Hg (29.8 gms), to the lowest value of 1.15 (27.3), or to the highest value of 1.96 (34.1). Linear regression analysis (ileal weight vs. calculated control compliance) yields a correlation coefficient factor of $r = 0.73$ with $y = 0.057x - 0.2$. It follows that there is a close association between the size of the ileal segment used, and its calculated control wall compliance.

Intraarterial Infusion of Gastrointestinal Peptides

Infusion of the six gastrointestinal peptides utilized in this study did not affect systemic arterial blood pressure or heart rate (Tables 2 and 3) and thus, any effects produced on vascular or visceral smooth muscle during the infusion period was likely to have resulted from an activation of local mechanisms. Furthermore, since intraarterial infusion of saline, the diluent for the peptides, did not affect ileal vascular resistance or wall compliance (Figure 3), any effects seen in these parameters can be ascribed to effects of peptide infusion.

Gastrin

Intraarterial infusion of synthetic gastrin (1.42 pM/min), mimicking reported postprandial blood levels of the hormone (Table 1), did not affect either ileal vascular resistance at "zero volume" or intestinal wall compliance (Figure 4). Subsequent infusion of gastrin at 14.2 pM/min, resulting in a calculated first-pass blood level 10x greater than postprandial (Table 1), again, was without effect on vascular resistance and wall compliance. Thus, it is suggested that postprandial blood levels of gastrin do not affect the activity of intestinal vascular or visceral smooth muscle.

Early investigations conducted in the mesenteric circulation employed mucosal extracts of gastrin, where either subcutaneous injection (31) or local intraarterial infusion (44) produced increases in superior mesenteric blood flow. However, since these results were obtained with only a relatively pure extract, the dilatory action ascribed for gastrin may have been partly due to contaminants present in the gastrin extracts.

Further studies using the synthetic pentapeptide analogue, penta-

gastrin, demonstrated that intraarterial infusion at 3 μg/kg/min could increase superior mesenteric blood flow in the cat by 50% (76), selectively produce a vasodilatory effect in the canine duodenum and jejunum (at blood concentrations between 25-50 ng/ml) (48), and increase both intestinal blood flow and oxygen consumption in the dog (0.5 μg/kg/min) (19).

The disparity in the findings between these studies and the present investigation may be the result of the different dosage employed for study. In the present study, an infusion rate of either 2.97 or 29.7 ng/min was used. This dosage is at least two to three orders of magnitude lower than the dosages used by Fasth et al. (76) in the cat, and Bowen et al. (19) in the dog. It is possible that at these higher infusion rates, a sufficient concentration of the peptide is present at the receptor site to evoke a response.

As reported by Chou, Hsieh, and Dabney (48), although the vasodilatory effect of pentagastrin was selective for the digestive organs alone (implying a possible postprandial role on vascular smooth muscle), the increment in the blood concentration necessary for inducing this response was approximately 400-900x greater than reported postprandial blood levels of gastrin (160-200 pg/ml, expressed as the heptadecapeptide equivalent, and based on a molecular weight of 2098, equal to 85.8 pM). Although it was mentioned that the vasoactive potency of the endogenous forms, G-17 and G-34, could be greater than the potency of pentagastrin, the large difference between the reported postprandial blood levels and the concentration requirements necessary for intestinal dilation are not supportive of a physiological action on vascular smooth muscle.

In the present investigation, using the same administration as used by Chou et al. (48), neither calculated blood level of gastrin

achieved, 160 pg/ml (mimicking postprandial) or 1.6 ng/ml (10x postprandial) dilated the ileal vasculature. Therefore, the present study corroborates the suggestion by these investigators that gastrin is not a physiological vasodilator in the mesenteric circulation. Additionally, Richardson (188) and Wormsley et al. (241) showed that doses of pentagastrin, which stimulated gastric acid secretion, did not dilate the jejunal vasculature in the cat. These findings strengthen the suggestion that gastrin is not a physiological dilator in the intestine since these doses stimulated gastric acid output, a well established physiological action for gastrin, without an accompanying vasodilation. However, it has been suggested that gastrin may be a physiological dilator in the gastric circulation (235).

It can be seen from Figures 4 and 5 that gastrin, at either postprandial or 10x postprandial blood levels, did not affect intestinal wall compliance. Thus, these data suggest that at these blood levels of the hormone, visceral smooth muscle activity, like vascular smooth muscle, is not significantly affected.

Many studies have demonstrated that either gastrin or pentagastrin alters visceral smooth muscle activity along the gastrointestinal tract. Infusion of pentagastrin (3 μg/kg/min) into the feline intestine (76), or administration of 5 or 25 μg pentagastrin into the isolated, vascularly perfused canine intestinal segment (207) has been shown to stimulate visceral smooth muscle activity. The mechanism of action in the canine intestine was suggested to be a result of pentagastrin-mediated release of acetylcholine from postganglionic cholinergic nerves (207). This suggestion supported previous findings of an indirect action of gastrin and pentagastrin on smooth muscle (76,90,91).

These findings differ from those obtained in the current study, which indicate that gastrin does not affect visceral smooth muscle in the canine ileum. Disparity in the findings may be attributable to a species difference (cat vs. dog (76)), differences in doses employed (11.4 µg/min (76), and 5 or 25 µg (207) vs. 2.97-29.7 ng/min), type of agent infused (pentagastrin vs. gastrin), or experimental design. It is significant to note that in the study by Stewart and Burks (207) where pentagastrin was found to be stimulatory in the canine intestine, an artificially perfused (Krebs-bicarbonate) intestinal segment was employed.

In vitro work on intestinal smooth muscle (6,141,230,231,232) supports the findings obtained in the cat intestine (76) and the isolated canine intestine (207) of an indirect, neurogenically-mediated action of pentagastrin on visceral smooth muscle. However, like the other investigations (76,207), higher concentrations of pentagastrin were used in these in vitro experiments in comparison to the blood concentrations of gastrin attained in the present study (Table 1).

Supporting the findings from the present investigation are results obtained in isolated human duodenal, jejunal, and ileal muscle strips (150), and both circular and longitudinal muscle strips of the cat duodenum (1). Most significant is the observation that the doses of gastrin or pentagastrin used (150,1) were much higher than the doses of synthetic gastrin used in the exteriorized canine ileum. Yet, in both cases, intestinal smooth muscle was unresponsive.

Secretin

Intraarterial infusion of synthetic secretin (0.216 pM/min), mimicking reported postprandial blood levels of the hormone (Table 1), did not affect ileal vascular resistance at "zero volume." However, although "zero volume" luminal pressure was unaffected, intestinal wall compliance significantly increased indicating a decrease in the rigidity of the ileal wall (Figure 6). Subsequent infusion of secretin at 21.6 pM/min, attaining calculated blood levels which were 100x greater than postprandial, significantly decreased ileal vascular resistance at "zero volume." However, during the infusion period, intestinal wall compliance did not increase further (Figure 7). It can be seen from Figures 6 and 7 that following the hormone-induced increase in wall compliance (Figure 6), the subsequent control compliance after peptide infusion (Figure 7) was not different from that obtained during secretin infusion. This observation suggests that the compliance of the intestinal segment had reached a maximum during secretin infusion mimicking postprandial blood levels (Figure 6) and that subsequent infusion of the higher dose of secretin (Figure 7) was without further effect on visceral smooth muscle.

Since the significant elevation in intestinal wall compliance induced by postprandial blood levels of secretin remained elevated throughout subsequent infusion of higher doses of secretin, it is suggested that the postprandial effect of secretin on visceral smooth muscle produces a maximal response on wall compliance which is long-lasting. However, it was only at 100x postprandial blood levels (Figure 7), that a significant decrease in vascular resistance was observed. Therefore,

this indicates that visceral smooth muscle of the ileum is more sensitive to secretin than is vascular smooth muscle. The dilatory effect of secretin appears to be due to activation of local mechanisms since the onset of action was rapid, and following infusion termination, vasodilation quickly dissipated.

The present findings, suggesting that postprandial blood levels of secretin are not vasoactive in the mesenteric circulation, are in accord with the data obtained by Chou et al. (48) in the canine duodenum and jejunum where the concentration requirement of natural secretin necessary for inducing vasodilation was markedly higher than the reported increment in serum concentration of the hormone attained following a meal. Furthermore, this dose range of secretin decreased vascular resistance in other non-digestive organs known not to be hemodynamically altered during the postprandial state (48).

The vasoactive range of secretin in the canine duodenum and jejunum was reported to be between 1750-2750 pg/ml (based on 0.004 mU/pg) (48), while in the canine ileum, 4.58 ng/ml (Figure 7, Table 1). The difference in vasodilatory potency observed in these two studies may be due to an organ-dependent phenomenon (duodenum and jejunum vs. ileum), or due to the nature of the preparation used (extracted vs. synthetic). Although their secretin was considered "pure" (48), possible contamination by vasoactive peptides (VIP and CCK) cannot be ruled out since the form employed was extracted (19,88,95). Many of the previous studies in the mesenteric circulation used the natural form of the hormone (4,10,31,72,-74,76,82,85,95,189). Thus, the vasodilatory action ascribed to secretin may have involved a significant contributing effect by vasoactive contaminants.

In an attempt to quantitate the potency of natural vs. synthetic secretin in the intestinal circulation of the cat, Ross demonstrated that 1 μg of synthetic secretin appeared to be slightly more than twice as potent as 1 U (unit) of natural secretin (191). Assuming that this relation holds true for the canine mesenteric circulation also, many of the previously described vasodilatory effects of natural secretin (10,31,72, 74 82,85,191) may have been produced at blood levels which are far above the reported postprandial blood levels of this hormone.

In some studies, the vasodilatory effect of secretin may have partly been produced from an action of the hormone on systemic arterial blood pressure and/or the heart. Ross suggested that secretin may have a direct effect on the heart since elevations in systemic arterial pressure induced by secretin infusion were attended by simultaneous increases in heart rate and cardiac output (191). Similarly, Goodhead et al. (85) demonstrated an elevated cardiac output concomitant with secretin-mediated increases in duodenal blood flow. Therefore, it cannot be excluded that part of the increase in intestinal blood flow ascribed to secretin (85,191) was due to an elevated cardiac output and a subsequent elevated perfusion to the mesenteric circulation.

In the present study, infusion of secretin at either 0.216 or 21.6 pM/min, did not alter aortic pressure or heart rate (Tables 2 and 3). Thus, the dilatory action ascribed for secretin at 100x postprandial blood levels is suggested to be due to activation of local mechanisms in the ileal vascular bed.

The findings reported by Richardson (189) are interesting to assess. In the cat, it was demonstrated that intraarterial infusion of natural secretin resulted in blood levels of the hormone between 100

and 260 pg/ml. It was then suggested if physiological blood levels of secretin (presumably postprandial) in the cat were similar to those reported in man (postprandial blood level = 200-600 pg/ml) (14,16,34), the observations of an increase in the functional exchange vessel surface area and elevations in intestinal blood flow produced by secretin infusion could reflect a functionally significant action for the hormone. However, it is not clearly defined whether postprandial blood levels of secretin in the cat mimic the levels attained in man following meal ingestion.

As previously mentioned, early investigations employing extracted, partially purified secretin (4,9,10,31,48,72,74,76,82,85,95,189,191) were found to result in significant vasodilation and/or increases in intestinal blood flow in various animals. However, due to the strong suggestion of contamination by vasoactive agents (88), the exact vasoactive potency of secretin alone was probably not known. On this point, Guth and Smith (95) observed that although intraarterial infusion of natural secretin, (at a minimal effective dose of 0.066 U/kg/min) dilated gastric submucosal arterioles, infusion of similiar doses of synthetic secretin was found to have no effect on the submucosal arterioles. Consequently, they suggested that the vasodilatory property of natural secretin (at least for their study) was most likely due to a contaminant. They suggested that vasoactive intestinal polypeptide (VIP) may be the contaminating peptide since, in the elution process for the extraction of secretin, VIP comes off in the fraction just after secretin. Further support for the contaminating peptide contention of natural secretin comes from the investigation by Bowen et al. (19). These investigators found that infusion of synthetic secretin at 0.03 or 0.3 μg/kg/min produced no sig-

nificant effect on mesenteric blood flow in the dog.

It is interesting to note that both the study by Bowen et al. (19) and the current study in the canine ileum, employed synthetic secretin, used the same route of administration, and used the canine mesenteric circulation as the target site. Yet, differences in the vasoactivity in the intestine is seen depending upon which study is examined. In comparison with the infusion rates of either 660 pg/ml or 66 ng/min (Figures 6 and 7), mimicking postprandial and 100x postprandial blood levels, respectively, the studies by Bowen's group (19) employed infusion rates of either 555 ng/min or 5.55 µg/min. Intraarterial infusion of secretin at 66 ng/ml resulted in a significant decrease in ileal vascular resistance as evidenced by a drop in ileal perfusion pressure (Figure 7). Yet, Bowen's doses, which exceed this dilatory dose, did not increase intestinal blood flow.

This discrepancy between findings may be attributed to the method employed to explore the hemodynamic effects of secretin in the mesenteric circulation. Bowen's group used natural flow preparations with an electromagnetic blood flow transducer placed around the superior mesenteric artery. In the current study, constant flow preparations were employed with changes in ileal perfusion pressure measured from a cannulated ileal segmental artery. Thus, the present study infers changes in vascular resistance while their study measures changes in blood flow through the whole small bowel.

Although not delineated in the present investigation, the vasodilatory action of secretin appears to be mediated by activation of local mechanisms since it occured within 60 seconds after infusion and following infusion termination, quickly dissipated. A similar sugges-

tion has been made by Chou, Hsieh, and Dabney (48). Ross (191) has reported vasodilation in the superior mesenteric artery after local denervation or ganglionic blockade, thus suggesting a direct dilatory action for secretin. Likewise, it has been suggested that the vasodilatory effect may be secondary to a hormone-induced metabolic effect on parenchymal cells (74) or due to the release of gut wall serotonin (8,9).

The present findings of secretin-induced increases in intestinal wall compliance (Figures 6 and 7), reflecting a decrease in ileal wall tension, are in agreement with the studies of Chey et al. (39), and Ramirez and Farrar (179) where either extracted or synthetic secretin was shown to produce an inhibition of spontaneous motor activity in the duodenum of both man and dog.

Not only is this inhibitory effect on non-vascular smooth muscle obtained in the intestine, but also demonstrated in the stomach and gastric antrum (38,39,56). The mechanism of action in the stomach appears to be due to a direct effect since the onset of action was immediate and could occur in vagally denervated gastric pouches (38,56).

If one makes the assumption, based on previous vascular studies where 1 U of natural secretin was roughly equivalent to 500 ng of the synthetic form (191), then the previous studies (38,39,56,179) employed secretin in doses ranging between 500-2500 ng/kg. Therefore, the inhibitory responses ascribed for secretin on gastrointestinal smooth muscle could have potentially occured at blood levels far exceeding those currently employed. The sensitivity of both intestinal vascular and visceral smooth muscle to secretin has not been critically evaluated. Yet, it was shown in the present study that visceral smooth muscle is indeed more sensitive to secretin than vascular smooth muscle.

Although no further increase in wall compliance was observed during secretin infusion at 21.6 pM/min (Figure 7), the present findings do not disagree with previous studies demonstrating inhibitory actions on gastrointestinal motility (38,39,56,179) at secretin blood levels suggested to be higher than the ones used in the canine ileum for the following reason. In the present study, it is suggested that postprandial blood levels of secretin (Figure 6) evokes a maximal long-lasting response on intestinal smooth muscle (maximal increase in wall compliance). Thus, at higher blood levels of secretin no response could be elicited (Figure 7). If the higher concentration of secretin would have been infused first, an increase in wall compliance would be predicted.

Although postprandial blood levels of secretin increased intestinal wall compliance, the slope of the curve of perfusion pressure vs. ileal balloon volume was not significantly altered (Figure 6). Thus, a potential indirect dilatory effect of secretin (passive), mediated by hormone-induced decreases in extravascular compression was not evident. Yet, at 100x postprandial blood levels, although compliance did not change, vascular resistance did (Figure 7), indicating that alterations in extravascular pressure were not responsible for the hormone-induced dilation.

In a similar study conducted by Fasth et al. (76) in the adrenalectomized feline intestine, local infusion of natural secretin was shown to significantly increase intestinal blood flow without affecting small intestinal motility. Since the dosage of secretin was not available, comparisons are difficult to assess. Yet, it was suggested that the insensitivity of visceral smooth muscle to secretin was most likely due to the absence of secretin-induced release of adrenal medullary catechol-

amines. Previous studies using the structurally similar hormone, glucagon, indicated that the inhibitory effect on small intestinal motility was mediated by glucagon-induced release of adrenal catecholamines attendant with increases in systemic arterial blood pressure (77).

However, it is suggested that unlike local infusion into the feline intestine, similar infusions of secretin into the exteriorized canine ileum does not appear to result in a stimulation and release of adrenal catecholamines (and subsequent relaxation of intestinal smooth muscle) since ileal wall compliance was demonstrated to increase without alteration in systemic arterial blood pressure during secretin infusion (Figure 6, Table 2).

Although the mechanisms of action of secretin-mediated increases in ileal wall compliance has not been ascertained, two possible mechanisms come to mind: 1) a direct effect on visceral smooth muscle, and 2) an indirect action mediated through the intrinsic neural innervation of the intestine.

Cholecystokinin

Intraarterial infusion of synthetic CCK-8 (438 pM/min) mimicking reported postprandial blood levels of the hormone (Table 1) did not affect ileal vascular resistance at "zero volume." However, luminal pressure at "zero volume" was significantly increased and intestinal wall compliance significantly decreased during the infusion period. Subsequent infusion at levels mimicking 10x postprandial blood levels was not possible to assess due to the marked and wide spike-like variations in ileal intralumial pressure. The effect of CCK-8 on visceral smooth muscle activity is rapid since, on the average, latency for hormone-induced increases in luminal pressure was 45 seconds after infusion initiation. Thus, it is suggested that CCK-8 affects the postprandial activity of visceral, but not vascular smooth muscle.

Strengthening the contention that CCK-8 is not a direct vasodilator in the canine ileum are the unpublished observations recorded during CCK-8 infusion at rates approximately mimicking 100x lower reported postprandial blood levels (first-pass blood concentration = 0.43 nM). It was observed in all of the dogs tested (n=4), that infusion of CCK-8 was without effect on ileal perfusion pressure at "zero volume" while similar effects on both luminal pressure and intestinal wall compliance were produced as previously described for the larger dose. The lack of a vasoactive effect is most interesting to assess since previous studies in the intestine employing CCK-8 (19,95,219) produced vasodilatory effects in the splanchnic circulation.

Although no vascular dilating action was observed during the infusion period, it is odd that the marked decrease in intestinal wall

compliance produced by CCK-8 (increase in wall tension) did not increase the amount of extravascular compression exerted upon local intramural vessels. Thus, it can be seen that the degree of increase in perfusion pressure during luminal distension (the slope of the curve of perfusion pressure vs. ileal balloon volume) was not different from that observed during the control period (Figure 8). If CCK-8 was without any vasodilatory effect, then one would expect to observe a marked increase in perfusion pressure at "zero volume" and throughout the remainder of the distending period since in the less compliant state, more of the generated intraluminal pressure should be transmitted to the local vasculature. However, as seen in Figure 8, CCK-8-mediated increases in intraluminal pressure at "zero volume" amounted to 370%, while simultaneously measured perfusion pressure only rose 2.7%. The data shows that the increase in intraluminal pressure generated by CCK-8 infusion was not significantly transmitted to the vasculature, and supports the idea of a masked vasodilatory effect of CCK-8 at this infusion rate.

Supporting the masked dilatory idea is the calculation that the percentage of intraluminal pressure transmitted from the ileal lumen to the vasculature was markedly decreased during the infusion period (Δ perfusion pressure / Δ luminal pressure x 100). Taking absolute pressures at both 0 and 20 ml volume for both the control and infusion period (Figure 8), one finds that during the control period, 66% of the intraluminal pressure was transmitted to the ileal vasculature. Yet, during CCK-8 infusion, only 49% was transmitted. Therefore, It is reasonable to suggest that dilation of the vasculature was opposing a passive constriction induced by an elevated tension in visceral smooth muscle. Indeed, similar observations have been made by many others (5,15,21,22,43)

where acetylcholine, a potent intestinal vasodilatory agent was shown to have its dilating action masked due to a stimulation of intestinal smooth muscle activity and resultant increases in luminal pressure.

Although the present data suggests a masked vasodilatory action of CCK-8, supporting data suggests that the hormone fragment in itself, is not a direct vasodilatory substance in the canine ileum. Evidence is obtained through comparison with the results obtained during substance P infusion at 74 pM/min, where both a marked vasodilatory effect (as evidenced by a significant decrease in "zero volume" perfusion pressure of 49%) and a concurrent significant decrease in intestinal wall compliance (43%) was demonstrated (Figure 15). The vascular dilating action of substance P was always observed to preceed the rise in ileal intraluminal pressure at "zero volume." In fact, the intestinal smooth muscle stimulating action of substance P (and subsequent passive constriction of the vasculature at higher points of distension of the segment) was observed after ileal perfusion pressure reached the steady state. Therefore, if CCK-8 had a direct vasodilatory effect in the canine ileum, it seems likely that it would have been evident during the initial infusion period.

It is possible that CCK-8 induces an active hyperemia of the visceral smooth muscle layer which opposes the passive constriction of the vasculature imposed by the elevated tension in visceral smooth muscle. In support of this suggestion are the observations reported by Chou and Grassmick (46) in the exteriorized canine duodenum, jejunum, and ileum. Using radio-labeled microspheres, they measured the distribution of total wall flow between mucosa-subucosa and muscularis-serosa compartments during various manipulations of the intestinal segments. The authors sug-

gested that an active hyperemia, similar to that seen in exercising skeletal muscle, occurs in the muscularis of the intestinal wall during intestinal contractions. They observed that physostigmine (potentiating acetylcholine) decreased blood flow only to the mucosa-submucosa layer. Apparently, blood vessels in the muscularis-serosa layer escaped the mechanical compression of an increase in wall tension. Thus, active hyperemia of the muscle layer, along with a direct vascular effect of acetylcholine, counteracted the passive constricting action of a fall in vascular transmural pressure produced by elevations in luminal pressure.

Infusion of CCK-8 into the canine intestine has been shown to release acetylcholine from postganglionic cholinergic nerves (206). Therefore, an active hyperemia of the muscle layer, similar to that reported in the canine small bowel (46) may, in part, be responsible for opposing passive constriction of the ileal vasculature mediated by CCK-8-induced increases in intestinal smooth muscle tension.

It has been suggested by Granger et al. (87) that luminal pressure elevation enhances transcapillary fluid exchange and imposes a "vascular waterfall" effect (similar to that in the upright lung) on the intestinal vasculature.

Normally, the driving pressure for flow through the intestinal vasculature is determined by the arterio-venous pressure difference, $P_a - P_v$. However, as a consequence of luminal distension (elevating intraluminal pressure, P_l), tissue pressure rises, and when it exceeds venous pressure, a passive constriction occurs at the venular level. The pressure at the collapse point is now equal to tissue pressure since veins are very compliant and do not resist collapse. Under this system of elevated luminal pressure, the driving pressure through the

vasculature would become arterial pressure minus intraluminal pressure ($P_a - P_l$). Venous outflow pressure would no longer have any influence on blood flow.

In the present study, although ileal venous pressure was not measured, a value of 20 mm Hg was inferred since turning off the blood pump produced an average pressure of 20 mm Hg in the ileal perfusion circuit (reflecting superior mesenteric venous pressure). Therefore, when luminal distension pressure (P_l) exceeded venous pressure (P_v), as evidenced in Figure 8 at the distending volumes of 5 through 20 ml during CCK-8 infusion, a "vascular waterfall" effect could have been imposed on the intramural vasculature. Consequently, the driving pressure through the microcirculation, ΔP, could have been effectively altered. For example, at the 5 ml distending volume, P_l = 29.5 mm Hg; thus, effective ΔP would be $P_a - P_l$ (118 - 29.5) or 88.5 mm Hg during CCK-8 infusion. At the control and CCK-8 "zero volume" points, ΔP is $P_a - (P_v$ or $P_l)$ (113 - 20) and (116 - 20) or 93 and 96 mm Hg, respectively. Thus, the "vascular waterfall" effect does not become apparent until the 5 ml distending volume step during CCK-8 infusion. It becomes increasingly more evident as the segment is further distended. Since blood flow (Q) was maintained constant, the progressive decrease in driving pressure (ΔP) must have reflected a decrease in ileal vascular resistance (R) during progressive distension of the ileum ($Q = \Delta P/R$).

According to Granger and co-workers (87), a myogenic activation of vascular smooth muscle at the arteriolar level may be implicated in the changes in vascular resistance. Luminal distension (increasing P_l) would tend to reduce arteriolar vascular transmural pressure, and since myogenic control appears to be important in the minute-by-minute local

control of intestinal blood flow (88), a reduction in transmural pressure would lead to a relaxation of arteriolar smooth muscle. Consequently, resistance to blood flow would decrease.

In previous investigations (10,44,48,72,73,74,76,136,191) CCK, in a partially purified form, was shown to significantly increase mesenteric and gastric blood flow without affecting flow in other non-splanchnic vascular beds. Comparison of the data with cardiovascular adjustments, intestinal function, and blood concentration of gastrointestinal hormones following meal ingestion suggested that CCK might contribute to the mesenteric hyperemia coincident with the digestive process (48, 49,72,73,74). However, since these studies used doses of CCK in "Ivy Dog Units" (U), and due to a lack of correlation of potency with the synthetic octapeptide (CCK-8), a quantitative comparison with the dose utilized in the current study in the canine ileum is not possible. Additionally, since partially purified forms of CCK were used in previous studies, a contributing vascular dilating action by contaminants cannot be ruled out. Indeed, gastric inhibitory polypeptide (GIP) was recently isolated from a side fraction in the purification process of CCK (23,24, 25) and subsequently shown to possess intestinal vasodilatory actions in both the feline (75) and canine (Figure 10) mesenteric vascular bed. Thus, GIP may have contributed significantly to the dilating action previously ascribed to CCK (10,44,48,72,73,74,76,136,191).

Few studies have employed the synthetic octapeptide of CCK. CCK-8, given by portal, caval, or intraarterial infusion, significantly increased intestinal blood flow and oxygen consumption (19,219) and dilated submucosal gastric arterioles when infused into the celiac axis (95). These findings do not agree with the present observation of a lack

of vasodilator effect in the canine ileum (Figure 8).

A quantitative comparison of doses can be attempted with the investigation by Thulin (219). From bioassay, Thulin found that 30 U were equivalent to 1 μg of CCK-8. Thus, infusion of CCK-8 was between 510 ng/min and 5.6 μg/min, with the lowest infusion rate matching the present intraarterial infusion at 500 ng/min. Caval or portal infusion of this lowest dose resulted in a significant increase in superior mesenteric blood flow as determined by an electromagnetic flowmeter. Yet, in the present study, local intraarterial infusion did not lower ileal vascular resistance as inferred from an unchanged perfusion pressure (Figure 8). Both studies did, however, give evidence of CCK-8 induced stimulation of intestinal smooth muscle activity.

Based on differences in methodology, it is possible that CCK-8-induced increases in superior mesenteric arterial blood flow was a reflection of increases in blood flow to the duodenum, jejunum, and pancreas (all three vascular beds are perfused by this artery). Yet, although the superior mesenteric artery supplies the ileum, the vasodilator potency of CCK-8 was not pronounced in this vascular bed. However, the vasodilatory effect in the gastric submucosal arteriole (95) is quite interesting since with a minimal effective dose of 0.28 ng/min, CCK-8 dilated the corpus vasculature. Hence, a possible physiological role may be supported here.

Much evidence has accumulated which supports the contention that gastrointestinal hormones (especially CCK) may be involved in the postprandial hyperemia of the duodenum and jejunum (18,48,50,72,73,74,110). Various mechanisms of action of CCK-mediated hyperemia in the duodenum and jejunum have been recently suggested. The mechanisms implicated

include a direct effect on vascular smooth muscle (48,88), a cholinergically-mediated dilation (19), a serotonergically-mediated dilation (8, 9), or dilation due to stimulation of parenchymal cell metabolism (74).

However, the present investigation suggests that CCK-8 is not involved in the ileal hyperemia coincident with the digestive process. Since the luminal stimulants (lipids and carbohydrates) are effectively absorbed by the time the intestinal chyme reaches the ileum, it is possible that the ileal hyperemia is resultant from the presence of some other luminal stimuli. On this point, it has been suggested that endogenous bile is important in the ileal hyperemia coincident with the digestive process (50,130). Of the major constituents (bile salts, lecithin, cholesterol, bilirubin), bile salts have been recently implicated to play a significant role in postprandial ileal hyperemia (131).

It can be seen from Figure 8 that calculated postprandial blood levels of CCK markedly increased "zero volume" luminal pressure and significantly decreased ileal wall compliance. Infusions at 6 pM/min (first-pass blood concentration = .43 nM, unpublished observations), mimicked these responses. Thus, at either blood concentration, the activity of visceral smooth muscle was markedly enhanced.

The present findings are in accord with <u>in vivo</u> studies using either natural CCK-33 (220) or synthetic CCK-8 (219) where a stimulatory effect on intestinal peristalsis was observed. They also concur with the demonstrated stimulatory effect of partially purified CCK on both gall bladder smooth muscle (101,112) and basal motor tone of the duodenum and jejunum (96). Thus, it appears that partially purified forms, the natural form of the parent hormone (CCK-33), and the synthetic carboxy-terminal fragment (CCK-8), all share the same stimulatory effect on splan-

chnic visceral smooth muscle.

Although shown to be a potent stimulant of visceral smooth muscle activity, CCK appears to have a direct effect on gall bladder smooth muscle (101), while in the small intestine, an indirect mechanism of action has been proposed (101,206,230,231,232). It has been shown in guinea pig ileal longitudinal muscle strips that CCK-8 stimulated intestinal motility through an interaction of the octapeptide with non-nicotinic receptors on postganglionic cholinergic nerves. Subsequent release of acetylcholine at the myoneural junction mediated the response (101, 230,231,232). Similar findings have been demonstrated in the isolated, vascularly perfused canine small intestine (206). Thus, it appears that CCK-8 mediates its visceral smooth muscle stimulating action through a neurogenic mechanism. However, in isolated circular and longitudinal muscle strips, natural CCK was shown to directly stimulate opossum circular muscle without affecting longitudinal muscle. Cat circular or longitudinal smooth muscle was unresponsive to CCK (1).

This disparity in findings with this study (Figure 8) and previous investigations (101,206,219,220,230,231,232) may be partly attributed to a species difference or to the specific area of the intestine studied. Investigations demonstrating a neurogenic mechanism for CCK-8, used ileal (101,206,230,231,232) or jejunal (206) segments. Direct stimulatory effects were obtained in duodenal circular smooth muscle (1). Furthermore, it is conceivable that circular and longitudinal smooth muscle of the duodenum respond differently to the same agent, or do not respond at all.

Caution should be observed when interpreting results obtained with physiological blood levels of CCK in possible regulation of intes-

tinal function. Development of sensitive and specific radioimmunoassays (RIA) for CCK have proved difficult, especially for the carboxy-terminal fragments of the hormone (233). Recent findings suggest that the predominant circulating forms of CCK are CCK-4, CCK-8, and CCK-12. These appear to be the physiologically important carboxyl fragments (183). Coupled with the fact that blood levels of CCK-8 in the present study were based on the RIA for the parent hormone (CCK-33), it is possible that postprandial blood levels of CCK-8 are different from those obtained for CCK-33.

Rehfeld and co-workers have recently shown (1982) that acidified fat emulsions increased CCK-8 concentration in a duodenal perfusate between 212-535 pM (183). Assuming that this range mimics postprandial blood levels, the first-pass blood concentration attained during CCK-8 infusion at 6 pM/min (0.43nM, unpublshed observations), falls within this range. As with the higher postprandial blood level (Figure 8), CCK-8 at this concentration failed to lower ileal perfusion pressure. Yet, a physiological regulatory role on the postprandial activity of intestinal smooth muscle is strengthened since ileal wall compliance was shown to significantly decrease during peptide infusion at 6 pM/min.

Gastric Inhibitory Polypeptide

Intraarterial infusion of synthetic gastric inhibitory polypeptide (GIP) (3.24 pM/min), mimicking reported postprandial blood levels of the peptide (Table 1), did not affect ileal vascular resistance at "zero volume." Although luminal pressure was unaltered in undistended segments, ileal wall compliance significantly increased during the infusion period, indicating that ileal wall tension had decreased. Subsequent infusion of GIP at rates which produced calculated blood levels equivalent to 10x those of postprandial (32.4 pM/min), significantly decreased ileal vascular resistance. However, intestinal wall compliance was not further increased by the higher blood level of GIP (Figure 10). It can be seen from Figures 9 and 10 that after the peptide-induced increase in wall compliance (Figure 9), the subsequent control compliance was not different from the previously measured one (Figure 10). Indeed, the value for wall compliance during low dose GIP infusion, subsequent control, and during high dose GIP infusion are all similar. Yet, they are all significantly elevated in comparison to the original control compliance. These data suggest, that like secretin (Figures 6 and 7), postprandial blood levels of GIP produces a long-lasting maximal reponse on ileal smooth muscle (maximal increase in wall compliance).

Although postprandial blood levels of GIP increased intestinal wall compliance, the slope of the curve of perfusion pressure vs. ileal balloon volume was not significantly altered (Figure 9). Thus, a potential indirect dilatory effect of GIP (passive), mediated by peptide-induced decreases in extravascular compression was not evident. Yet, at 10x postprandial blood levels, although wall compliance did not change,

vascular resistance did (Figure 10), indicating that alterations in extravascular pressure were not responsible for peptide-induced dilation. Therefore, like secretin, visceral smooth muscle activity is more sensitive to GIP infusion than is vascular smooth muscle. The vasodilatory effect of higher blood levels of GIP (Figure 10) appears to be due to activation of local mechanisms since dilation was rapid in onset, and dissipated quickly following termination of the infusion.

In comparison with the findings obtained with secretin, one can see that at postprandial blood levels (16 pM-secretin and 191 pM-GIP), secretin increased wall compliance by 18%, while GIP, by 15.5%. Furthermore, at greater blood levels (1.5 nM-secretin and 1.91 nM-GIP), ileal vascular resistance was equally decreased as evidenced by a secretin-induced decrease in perfusion pressure of 11%, and a GIP-induced decrease of 12% (Figures 6,7,9,10). Thus, these two gastrointestinal peptides share similar biological activities on vascular and visceral smooth muscle. This observation is in accord with other biological activities similarly ascribed to both peptides (119,147,233). Secretin and GIP belong to the same family of gastrointestinal peptides and porcine GIP has 9 out of the first 26 amino acids in the same position as in porcine secretin (23). Therefore, based on this similar amino acid sequence, similar actions on vascular and visceral smooth muscle could be predicted.

Extensive search of the literature (1970-1982) reveals that only one other investigation has been conducted on the effects of GIP on mesenteric hemodynamics and motility. Fara and Salazer (75) demonstrated that in the cat intestine, intravenous infusion of natural GIP produced a dose-dependent increase in superior mesenteric blood flow (threshold

dose = 0.25 μg/min). During infusions between 0.45-1.8 μg/min, mesenteric vascular resistance was shown to progressively decrease from an initial drop of 14.7 to a maximum of 56%. Since systemic arterial pressure and femoral blood flow were unaltered, a local dilating action was suggested. Furthermore, at the highest infusion rate used (1.8 μg/min), any spontaneous jejunal motility, when present, was totally abolished.

The same findings (vasodilation and relaxation of visceral smooth muscle) have been demonstrated in the exteriorized canine ileum (Figures 9 and 10). Thus, it appears that GIP, either natural or synthetic, can affect the activity of vascular and visceral smooth muscle in both feline and canine intestinal preparations. In the dog, a local regulatory mechanism for the vascular dilating action of GIP is suggested since like the cat (75), systemic arterial blood pressure was not altered, and additionally, heart rate was unchanged (Tables 2 and 3). The actual mechanism(s) of GIP-induced relaxation of vascular and visceral smooth muscle has yet to be delineated.

The present investigation in the canine ileum suggests that GIP affects the postprandial activity of visceral smooth muscle since ileal wall compliance was shown to significantly increase at a calculated blood concentration of GIP mimicking reported postprandial levels. However, a vascular dilating action of GIP at that blood level was not seen. Vasodilation appeared when the calculated first-pass blood concentration of GIP was elevated to 10x postprandial levels. Consequently, it is suggested from this study that the hemodynamic effect does not represent a physiological action for GIP.

In the cat, the threshold dose for vasodilation was 250 ng/min (75). Since this infusion rate was much higher than the one used to mim-

ic postprandial blood levels in the dog (16.5 ng/min, Figure 10), the vasodilatory action in the cat intestine probably does not represent a physiological effect of GIP. However, it cannot be excluded that postprandial blood levels of GIP in the cat are different from those reported for dog and man (75).

Although blood levels of the peptide hormones (gastrin, secretin, CCK, and GIP) are significantly elevated during the postprandial state, it is not absolutely clear whether the reported postprandial blood levels reflect the physiologically effective concentration at the target tissue site, that is, vascular or visceral smooth muscle. Thus, it is possible that the reported postprandial blood levels underestimate the hormone concentration necessary to elicit a physiological response on intestinal vascular or visceral smooth muscle.

Vasoactive Intestinal Polypeptide

Intraarterial infusion of synthetic VIP (0.596 pM/min), mimicking reported postprandial blood levels of the peptide in the portal vein (Table 1), did not affect ileal vascular resistance at "zero volume" or intestinal wall compliance (Figure 11). Subsequent infusion of the peptide at 5.96 pM/min, attaining blood levels calculated to be 10x those reported as postprandial levels had the same result (Figure 12). Thus, it is suggested that postprandial blood levels of VIP do not affect the activity of intestinal vascular or visceral smooth muscle. This lack of effect of VIP as a humoral agent allows for the suggestion of a neurally-mediated mechanism of action for the peptide on both intestinal vascular and visceral smooth muscle (62, 63,69).

Kachelhoffer and co-workers (121) demonstrated in the isolated canine jejunal loop that close intraarterial infusion of VIP (arterial concentrations = 0.5 ng/ml (.15 nM) to 5 µg/ml (1.5 µM) dose-dependently dilated the mesenteric vascular bed. They suggested that this was a direct effect since blockade with either atropine or propranolol did not attenuate the dilation. Eklund and co-workers (63) have reported that at arterial blood concentrations between 0.15 and 4.6 µM, VIP significantly increased gastric, jejunal, and colonic blood flow which was atropine resistant in the cat.

These vasodilatory actions ascribed to VIP in the canine jejunum (121) and cat splanchnic vasculature (63) most likely do not represent a physiological humoral effect of the peptide. Arterial blood levels of the peptide necessary to induce vasodilation were markedly higher than the reported postprandial blood level of 36 pM (197) which was sub-

sequently found to lack vasoactivity in the canine ileum (Figure 11).

The postprandial blood level for VIP in the present study was taken from the porcine portal vein (197). Therefore, it cannot be excluded that postprandial blood levels of VIP in the dog or cat portal vein are different from the 36 pM blood concentration measured in the pig.

In the study by Thulin and Olsson (221), caval or portal infusion of VIP (0.705-10.6 μg/min) did not significantly affect superior mesenteric blood flow in the dog. The disparity between this finding and other observations demonstrating significant vasodilation in the intestine may be attributed to the route of peptide infusion. For the studies observing splanchnic vasodilation (63,121), local intraarterial infusions were used. However, Thulin and Olsson (221) used caval or portal routes of administration. This implies that at least for portal infusions, significant vasodilatory concentrations of VIP in the superior mesenteric artery were most likely not achieved since the liver parenchyma has been shown to effectively remove circulating VIP entering the liver through the portal blood (59,124,153,194).

Intravenous infusion of VIP has been shown to decrease total segmental blood flow in the canine ileum (143). However, since infusion produced a marked systemic hypotension, it can be suggested that the decrease in intestinal blood flow was mediated by activation of the sympathetic neural axis, and subsequent vasoconstriction of the ileal vasculature.

Although the present study suggests that postprandial blood levels of VIP do not affect the activity of visceral smooth muscle in the intestine, the first-pass blood concentration of 36 pM (120 pg/ml) falls

within arterial blood concentrations (25-500 pg/ml) shown to dose-dependently relax canine jejunal circular smooth muscle (122). However, it was reported that the relaxant effect of VIP was fast and short-lasting (1 minute duration) and, consequently, the investigators suggested that this effect was not of physiological importance. The inability of the current investigation to observe fast changes in intestinal motility may be due to the different methodology used to measure changes in intestinal smooth muscle activity. Comparison of intraluminal pressures reveals that in the isolated canine jejunum (122), the intraluminal balloon may have been more sensitive to any quick changes in intestinal smooth muscle activity since control motility was considerably higher than that observed in the canine ileum. In the present study, only small changes in the luminal pressure tracing were observed. Thus, it would be theoretically easier to detect peptide-induced relaxant effects in the preparation where the control motility was higher. This suggestion is supported by the observations of Cocks and Burnstock (51) in isolated intestinal smooth muscle strips. Their results indicated that in medium and high tone preparations, VIP (0.5 μg/ml) produced a relaxant effect. However, in low tone preparations, no response could be elicited. Since control intraluminal pressure in the present study was considered low (2-4 mm Hg), failure to observe any fast, short-lasting relaxant effects is consistent with the data of Cocks and Burnstock (51).

The stimulatory effect on isolated smooth muscle ascribed to VIP (1,52,113) may be due to the high dose of the peptide used. In the canine jejunum, higher doses of VIP were shown to produce a biphasic response on intestinal smooth muscle, i.e., relaxation followed by a "rebound excitation" of smooth muscle (122). Yet, lower doses (similar

to that used in the current study) only elicited a quick, short-lasting inhibitory effect on jejunal smooth muscle.

Unlike the peptide hormones which are housed within mucosal endocrine cells, VIP is said to be solely distributed and released from the intrinsic neural innervation of the intestine (12,107,115,133,134,135). Additionally, substance P is said to be primarily localized within intestinal neural elements, with a smaller distribution within mucosal enterochromaffin cells (163,212,213). Thus, the reported postprandial blood levels for VIP and substance P may reflect an overflow of the peptides from interstitial spaces into the circulation. It follows that postprandial blood levels may underestimate the true postprandial level of VIP and substance P at the tissue level. Thus, proposal of possible physiological regulatory roles for these peptides as humoral agents is complicated. Following release, VIP and substance P may act locally as a paracrine or neurotransmitter/modulator agent, and be inactivated before attaining vasoactive levels in the circulation.

Consequently, one would expect that arterial blood concentrations of the peptides would have to be markedly elevated to mimic the neurogenically-mediated responses since mechanisms for re-uptake into the presynaptic nerves and/or local metabolizing enzymes are likely to exist at the synaptic clefts. Also, since VIP and substance P are large polar molecules, capillary permeability would be predicted to be low. Thus, following neural release, both peptides could have difficulty in gaining access to the circulation in large quantities after eliciting their biological effects. It follows that high rates of intraarterial infusion of the peptides would be necessary to attain the same concentration at the target site as that produced by local neural release.

Indeed, due to the unique distribution of VIP along the alimentary canal (peptidergic nerves), many investigators have proposed that this peptide is a neurotransmitter within the gastrointestinal tract (11,63,69,196).

Substance P

Intraarterial infusion of synthetic substance P at progressively increasing rates: 0.74, 7.4, and 74 pM/min, dose-dependently decreased ileal vascular resistance at "zero volume" as evidenced by significant drops in perfusion pressure of 8.3, 28.5, and 49%, respectively (Figures 13,14, and 15). Peptide-induced dilation remained throughout the duration of the infusion period at all doses employed. Intestinal wall compliance was not affected during substance P infusion at either 0.74 or 7.4 pM/min (Figures 13 and 14). However, during infusion at 74 pM/min, wall compliance was significantly decreased, thus inferring that ileal wall tension had significantly increased. Also, "zero volume" luminal pressure was shown to be elevated in comparison to controls (Figure 15). Therefore, it is suggested that at a first-pass blood concentration as low as 47 pM, substance P significantly affects ileal vascular smooth muscle, and continues to do so at progressively higher arterial blood concentrations of 470 pM and 4.7 nM. However, visceral smooth muscle activity was not shown to be significantly affected until a blood concentration of 4.7 nM was attained (Table 3).

Since postprandial blood levels of substance P were not available from the literature, local infusion was based on 12-hour fasting blood levels reported for the dog (Table 1). Thus, the actions of substance P in the canine ileum were assessed at arterial first-pass blood concentrations which were both lower than (47 pM), and greater than (470 pM and 4.7 nM) the reported fasting blood level of 88 pM (164). Since ileal vascular resistance was significantly decreased at a first-pass blood concentration of 47 pM, this peptide is a most potent vaso-

dilator in the canine ileum. Furthermore, it has been suggested that blood concentrations of substance P may be elevated during the postprandial state (172,222). If so, then the actions reported at the higher blood levels (470 pM and 4.7 nM) may be of physiological importance.

The present study indicates that ileal vascular smooth muscle is more sensitive to substance P than visceral smooth muscle. Vasodilation was elicited at an infusion rate of 0.74 pM/min (Figure 13). Yet, stimulation of visceral smooth muscle activity was only evident during infusion at 74 pM/min (Figure 15). It is also interesting to note that during infusion of substance P at the highest rate (74 pM/min), vascular changes always preceeded changes in visceral smooth muscle activity. Therefore, it follows that substance P dilates the canine ileal vasculature before producing any effects on intestinal smooth muscle.

During the infusion period (74 pM/min), the increment in perfusion pressure during the measurement of compliance was increased, i.e., the slope of the curve of perfusion pressure vs. ileal balloon volume was greater than that observed during the control period (Figure 15). Thus, although substance P significantly dilated the vasculature at "zero volume," peptide-induced stimulation of intestinal smooth muscle (decrease in wall compliance) significantly affected vascular resistance at the higher points of distension of the segment. Simply stated, the enhanced extravascular compression resulting from peptide-induced increases in ileal wall tension passively opposed the vascular-dilating action of substance P at higher distending volumes in the ileum. Supporting data comes from the calculation of the percent transmittance of luminal pressure to local perfusion pressure (Δ perfusion pressure / Δ luminal pressure). During the control period, 35% of the luminal pres-

sure was transmitted to the vasculature, while during the infusion period, 65% was transmitted. This effect of extravascular compression was not evident during CCK-8-induced decreases in intestinal wall compliance (Figure 8). With CCK-8, both a vascular waterfall effect and an active hyperemia of the muscle layers were suggested to be involved in the response observed for perfusion pressure. These differences observed between substance P and CCK-8 may be partly attributed to the neural release of acetylcholine produced by CCK-8 (206).

The majority of the investigations on the hemodynamic actions of substance P have been conducted in non-splanchnic vascular beds. In these beds a direct effect on vascular smooth muscle is suggested since peptide-induced vasodilation was resistant to atropine, H_1- and H_2-histamine receptor antagonists, ganglionic blockade, α- and β-adrenergic receptor antagonists, and guanethedine (66).

The present observations of a substance P-induced, dose-dependent decrease in ileal vascular resistance (Figures 13,14,and 15), concur with the findings reported by Schrauwen and Houvenaghel in the pig (200). Through local intraarterial infusion of substance P these investigators obtained a dose-dependent increase in superior mesenteric arterial blood flow with a minimal effective dose of 0.6 ng/kg/min (10.8 ng/min). Decreases in systemic arterial and increases in portal venous pressures were evident during infusions between 18-180 µg/min.

In the present study, local infusion of synthetic substance P significantly dilated the ileal vasculature at an infusion rate of 1 ng/min (0.74 pM/min, Figure 13). Thus, it appears that substance P is a more potent vasodilator in the canine than in the porcine mesenteric vascular bed. However, at the higher infusion rates employed a dispar-

ity in the vascular action of substance P is observed. In the pig, local infusion between 18 and 180 μg/min produced up to a 48% decrease in the calculated vascular resistance concomitant with a significant decrease in systemic arterial blood pressure (200). In the canine ileum, infusion of the peptide at 100 ng/min (74 pM/min, Figure 15) produced a 49% decrease in ileal perfusion pressure at constant flow without any effect on aortic pressure (Tables 2 and 3). The largest dose of substance P infused into the pig is roughly 1800x larger than the highest dose infused into the dog. This disparity in doses may be responsible for the fall in systemic arterial pressure produced by substance P in the pig.

The liver has been demonstrated to be an important site for the removal and inactivation of substance P from the circulation (66,97,139). Thus, it is possible that local intraarterial infusion of substance P into the intestine, at a sufficiently high rate, could overcome the capacity of the hepatic parenchyma to inactivate the peptide. Consequently, entrance into the systemic circulation would result in a decrease in blood pressure due to the potent vasodilatory action ascribed to this peptide (66). Therefore, at infusion rates between 18 and 180 μg/min in the pig (200), the hepatic inactivating capacity may have been overcome resulting in a spillover of the peptide into the peripheral circulation and subsequent vasodilation. The results of the present study suggest that at an infusion rate of 100 ng/min, substance P does not overcome hepatic inactivation since aortic blood pressure was not affected (Tables 2 and 3). However, at an infusion rate of 1 μg/min, substance P-induced vasodilation of the ileal vasculature was attended by a transient decrease in aortic blood pressure, on the average of 40 mm Hg (n=6, unpublished observations).

It was suggested by Schrauwen and Houvenaghel (200) that substance P-induced vasodilation in the pig intestine was direct since serotonergic, α-adrenergic, and local neural blockade could not attenuate or abolish the vasodilatory response. However, statements about its action in the canine ileum can only be general since blocking studies were not conducted. Yet, due to the rapid onset in action, and rapid return of perfusion pressure to pre-infusion control values following infusion termination (for all doses used), it seems likely that primarily, local mechanisms are involved. The dilation, however, may be either direct or indirect.

In addition, it has been shown that intravenous infusion of synthetic substance P into dogs produced similar effects on the intestinal vasculature as were seen during intraarterial infusion into the canine ileum. At threshold infusion rates of 1.2-1.8 ng/min/kg, Hallberg and Pernow (97) demonstrated a significant increase in superior mesenteric arterial blood flow which was dose-dependent as the infusion rate was increased. Flow rose rapidly and remained elevated throughout the infusion period. Also, it was noted that at threshold infusion rates between 0.6 and 0.9 ng/min/kg, hepatic arterial blood flow significantly rose and followed the same pattern of change demonstrated for the superior mesenteric artery at higher infusion rates. Thus, it appears that the hepatic artery is more sensitive to substance P than the superior mesenteric artery. Furthermore, in comparison with the blood flow changes in other vascular beds (femoral, renal, and carotid), Hallberg and Pernow demonstrated a considerable sensitivity to substance P in both carotid and splanchnic vascular beds (97). This is quite interesting since originally in 1931, von Euler and Gaddum obtained the peptide from horse

brain and intestinal extracts (65). Corroborating the suggestion of an important physiological role for substance P in the mesenteric circulation (97) is the present finding of a significant vasodilation of the ileal vasculature at an arterial blood concentration (47 pM) which is lower than the reported 12-hour fasting level (88 pM). Collectively, the vascular-dilating action of substance P in the mesenteric circulation appears to be quite similar in both the dog and pig (97,200). The peptide is a potent vasodilator with a rapid onset of action, showing little tachyphylaxis, and produces a dose-dependent vasodilation as the infusion rate is increased.

During the present investigation, ileal wall compliance was observed to significantly decrease during substance P infusion at 74 pM/min (Figure 15). The stimulatory effect on visceral smooth muscle was evident at "zero volume" where peptide infusion elicited an increase in luminal pressure from 2.1 to 6.5 mm Hg. These observations of a substance P-induced increase in visceral smooth muscle activity are in accord with the findings of Schrauwen and Houvenaghel in the pig where only larger infusion rates of the peptide (18-180 μg/min) stimulated intestinal motility (200). Therefore, it is apparent that the actions of substance P in both the canine and porcine intestine follow the same sequence of activation. There is a selective relaxation of vascular smooth muscle at lower infusion rates, followed by a stimulation of intestinal smooth muscle activity and subsequent elevation of intestinal tone at higher rates.

The stimulatory effect of substance P on intestinal smooth muscle has been demonstrated in man where the peptide was shown to markedly increase peristaltic and segmental movements of the intestine. The stimulatory action was reproduced in isolated intestinal segments (140).

The peristaltic and segmental contractions induced by substance P are suggestive of a potential role for the peptide in the movement of the intestinal content along the gastrointestinal tract. Indeed, Hellstrom and Rosell (105) demonstrated that substance P was able to exert a dose-dependent contractile effect on the distal colon of the cat. Thus, a possible role for the peptide in the defecation reflex is possible.

The enhanced basal tone produced by substance P in the cat colon correlates well with the present observation of an increase in "zero volume" luminal pressure seen after peptide infusion. Additionally, the contractile effect on the colon is similar to the decrease in intestinal wall compliance produced in the canine ileum (105). Since vascular resistance was shown to significantly fall during substance P-induced increases in ileal wall tension (Figure 15), maintainance of blood flow to the intestinal wall was ensured. Thus, the possibility of a role for the peptide in the defecation reflex is plausible. Furthermore, primary localization of substance P to intrinsic enteric nerves (163,168, 212,213) allows for the possibility of a neuromodulatory role for the peptide on intestinal smooth muscle activity. On this point, it is interesting to note than in Hirschsprung's disease, the dilated, atonic rectosigmoid colon is largely devoid of substance P and intrinsic ganglion cells (61). As a consequence, the megacolon condition interferes with the normal defecation process.

The stimulatory effects of substance P on small intestinal and colonic visceral smooth muscle appear to be direct since cholinergic, serotonergic, α-adrenergic, or local neural blockade could not attenuate or abolish the stimulatory responses (65,83,105,152,170,200). Also, a direct effect of substance P on gastric smooth muscle appears

possible since peptide-induced stimulation was shown to be resistant to atropine, α- and β-adrenergic blockade, anti-serotonergics, and both H_1- and H_2-histamine receptor blockers (7,152).

Yau (244) demonstrated that low concentrations of the peptide exerted a strong dose-dependent stimulatory effect on isolated longitudinal smooth muscle. The action was considered to be direct since atropine, tetrodotoxin, or lioresal could not attenuate the stimulatory response. Since Yau's threshold stimulatory dose of $1x10^{-9}$ gm/ml fell within the limits of reported values for normal circulating levels of substance P ($5x10^{-11}$ to $2x10^{-9}$ gm/ml) (164,177), his data provides evidence for the contention that substance P is of physiological importance in the regulation of ileal smooth muscle motility. Similarly, it has been recently suggested that substance P functions as a neuromodulator in the intestine to: 1) set the level of excitability of peripheral neurons in the inferior mesenteric ganglion, and 2) facilitate synaptic transmission along noradrenergic pathways (127).

In the present study, substance P significantly decreased ileal wall compliance (increased wall tension) at a first-pass blood concentration of 4.7 nM ($6.3x10^{-9}$ gm/ml) (Figure 15). Although this increment in the arterial blood concentration of substance P is greater than the reported plasma range in the dog ($5x10^{-11}$ to $2.1x10^{-10}$ gm/ml) (164), it is important to note that this reported plasma range of substance P may not be the physiologically important levels. These reported levels may be an underestimate of the significantly important blood levels since they were measured in dogs that were fasted for 12 hours.

The perspective of a physiological regulatory role for the peptide on intestinal blood flow is intriguing. It was shown in the present

study that at a first-pass blood concentration of 47 pM ($6.3x10^{-11}$ gm/ml), a significant dilation of the ileal vasculature was produced (Figure 13). This increment in the arterial blood concentration of substance P correlates well with the lower limit of the circulating plasma levels in the 12-hour fasted dog (164). Due to the marked sensitivity of both mesenteric vascular and visceral smooth muscle, it is suggested that substance P may exert a physiological regulatory role on both mesenteric vascular resistance and intestinal motility during the postprandial state. Although suggested to act as a humoral agent in the present study, the peptide could also act as a paracrine and/or neuromodulatory agent in the intestine. Data obtained from the current study on the effects of substance P in the canine ileum places this gastrointestinal peptide in the class of mesenteric vasodilators known to stimulate intestinal smooth muscle activity, e.g., acetylcholine, histamine, and bradykinin (203,237).

Finally, it was observed that during intraarterial infusion of substance P at either 7.4 or 74 pM/min, peptide-induced vasodilation consisted of two consecutive drops in perfusion pressure separated by a transient steady state plateau (Figure 16). It is believed that the initial dilation is a result of substance P-induced relaxation of vascular smooth muscle mediated through local mechanisms, since heart rate and systemic arterial pressure were unaltered (Tables 2 and 3). However, the second dilation may be unique. The possibility of peptide recirculation can be ruled out as being partly implicated in the second dilation because, for this to occur, the dose of substance P infused into the ileum would have to overcome the capacity of the liver to remove it from the circulation. In other experiments performed in the canine ileum

(unpublished observations), it was shown that to cause systemic arterial blood pressure to fall subsequent to peptide infusion, an infusion rate of 1 μg/min (740 pM/min) was necessary. Since the highest infusion rate employed in this study was 100 ng/min (74 pM/min), and since heart rate and systemic arterial blood pressure were unaltered, it is unlikely than any of the locally infused substance P escaped hepatic inactivation.

Three possibilities come to mind to explain the second dilation produced by substance P infusion. The first possibility is that the peptide may be sequentially stimulating two different classes of substance P-sensitive vascular receptors (similar to histamine-mediated dilation, 88,215). The second possibility is that the dilation is mediated by a substance P-induced release of some endogenous intestinal vasodilator normally sequestered within the intestinal wall. For example, the peptide could stimulate the release of acetylcholine, histamine, bradykinin, prostaglandins, or VIP. A third possibility is that the second dilation could be totally neurally-mediated. Perhaps substance P stimulates afferent nerves which consequently results in a subsequent reduction in sympathetic vasomotor tone in the ileal vasculature. Nonetheless, this observation is fascinating and deserves further study.

CHAPTER VII

SUMMARY AND CONCLUSIONS

Experiments were conducted in segments of the exteriorized canine ileum to ascertain the simultaneous effects of local intraarterial infusion of synthetic gastrointestinal peptides on both intestinal wall compliance and ileal vascular resistance.

Intraarterial infusion of saline (0.2 ml/min) did not affect ileal perfusion pressure or intestinal wall compliance.

Intraarterial infusion of synthetic gastrin (1.42 pM/min), attaining a first-pass blood concentration of 76.1 pM (postprandial = 85.8 pM), did not affect ileal perfusion pressure or intestinal wall compliance. Infusion of gastrin at 14.2 pM/min had the same result.

Intraarterial infusion of synthetic secretin (0.216 pM/min), attaining a first-pass blood concentration of 16 pM (postprandial = 18 pM), did not affect ileal perfusion pressure; however, intestinal wall compliance was significantly increased. Infusion of secretin at 21.6 pM/min significantly lowered ileal perfusion pressure without increasing intestinal wall compliance further.

Intraarterial infusion of synthetic cholecystokinin-octapeptide (CCK-8) (438 pM/min), attaining a first-pass blood concentration of 34.4 nM (postprandial = 36.5 nM), did not affect ileal perfusion pressure; however, intestinal wall compliance was significantly decreased. The effects of infusion of CCK-8 at higher doses were not possible to assess.

Intraarterial infusion of synthetic gastric inhibitory polypeptide (GIP) (3.24 pM/min), attaining a first-pass blood concentration

of 191 pM (postprandial = 196 pM), did not affect ileal perfusion pressure; however, intestinal wall compliance was significantly increased. Infusion of GIP at 32.4 pM/min significantly lowered ileal perfusion pressure without increasing intestinal wall compliance further.

Intraarterial infusion of synthetic vasoactive intestinal polypeptide (VIP) (0.596 pM/min), attaining a first-pass blood concentration of 36 pM (postprandial = 36 pM), did not affect ileal perfusion pressure or intestinal wall compliance. Infusion of VIP at 5.96 pM/min had the same result.

Intraarterial infusion of synthetic substance P (SP) (0.74 pM/min), attaining a first-pass blood concentration of 47 pM (postprandial unknown), significantly lowered ileal perfusion pressure without affecting intestinal wall compliance. Infusion of SP at 7.4 pM/min, likewise lowered ileal perfusion pressure without an effect on intestinal wall compliance. However, at the highest infusion rate of the peptide (74 pM/min), attaining a first-pass blood concentration of 4.7 nM, the significant drop in ileal perfusion pressure was now accompanied by a significant decrease in intestinal wall compliance.

Systemic arterial blood pressure and heart rate were not altered by any of the test peptides infused (Figures 3-15 and Tables 1-3).

The present set of findings suggest that:

1. Postprandial blood levels (calculated) of gastrointestinal peptide hormones (gastrin, secretin, CCK, and GIP) do not contribute to the ileal hyperemia coincident with the digestive process.
2. Secretin, CCK, and GIP do affect the postprandial activity

of ileal smooth muscle since all three peptides altered intestinal wall compliance.

3. Secretin and GIP share similar biological activities on both ileal vascular and visceral smooth muscle.
4. Postprandial blood levels of VIP do not affect the activity of either ileal vascular or visceral smooth muscle. Thus, VIP apparently does not contribute a physiologically important humoral effect on intestinal blood flow or motility.
5. The assessment of a possible physiological regulatory for substance P during the postprandial state is complicated since the peptide: a) has not been measured in the circulation during the digestive state, and b) has both a neural and cellular distribution and release within the intestine.
6. Due to the high sensitivity of both intestinal vascular and visceral smooth muscle to low doses of the peptide, substance P may serve as a physiologically important humoral agent. For substance P to exert physiologically important humoral effects on both intestinal blood flow and motility, blood levels of the peptide must be elevated during the digestive state.
7. Since intestinal wall compliance was shown to significantly increase, while luminal pressure at "zero volume" was not altered (Figures 6 and 9), the measurement of intestinal wall compliance is a more sensitive index of intestinal wall tension than is assessment through measurement of

luminal pressure alone.

8. Simultaneous infusions of these peptides, mimicking their postprandial blood levels, should be pursued in the canine ileum to delineate if the peptides work in concert, and whether there is a potentiation of their effect on the vasculature and motility during the digestive state.

APPENDIX ONE

VASCULAR PRESSURES AND WALL COMPLIANCE
All values are mean ± SEM.

Experiment	Figure Number	Perfusion Pressure "zero volume" (mm Hg)	Wall Compliance (ml/mm Hg)
Control	3	129 ± 5.2	1.30 ± .12
Saline		134 ± 5.3	1.31 ± .09
Control	4	130 ± 7.4	1.35 ± .15
Gastrin(76.1pM)		137 ± 7.1	1.34 ± .18
Control	5	138 ± 8.9	1.54 ± .19
Gastrin(761pM)		139 ± 7.9	1.56 ± .21
Control	6	135 ± 8.6	1.15 ± .07
Secretin(16pM)		132 ± 8.1	*1.36 ± .07
Control	7	148 ± 5.9	1.36 ± .10
Secretin(1.5nM)		*132 ± 5.2	1.31 ± .09
Control	8	113 ± 4.5	1.19 ± .10
Cholecystokinin Octapeptide(34.4nM)		116 ± 6.6	*0.88 ± .11
Control	9	105 ± 8.1	1.75 ± .10
Gastric inhibitory polypeptide(191pM)		107 ± 8.7	*2.02 ± .10
Control	10	118 ± 5.4	1.96 ± .18
Gastric inhibitory polypeptide(1.91nM)		*104 ± 4.6	2.13 ± .17
Control	11	113 ± 7.6	1.50 ± .14
Vasoactive intestinal polypeptide(36pM)		113 ± 7.3	1.59 ± .12
Control	12	123 ± 11.1	1.53 ± .16
Vasoactive intestinal polypeptide(360pM)		126 ± 9.9	1.64 ± .18

Experiment	Figure Number	Perfusion Pressure "zero volume" (mm Hg)	Wall Compliance (ml/mm Hg)
Control	13	109 ± 2.8	1.43 ± .17
Substance P(47pM)		*100 ± 3.1	1.46 ± .20
Control	14	121 ± 4.8	1.67 ± .20
Substance P(470pM)		*86.5 ± 3.4	1.54 ± .16
Control	15	149 ± 6.8	1.75 ± .23
Substance P(4.7nM)		*76 ± 3.7	*1.00 ± .21

* $p < 0.05$ compared to control, factorial analysis of variance

CHAPTER VIII

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