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REVIEW

Profile of Neurohumoral Agents on Mesenteric and Intestinal Blood Flow in Health and Disease

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Summary

The mesenteric and intestinal blood flow is organized and regulated to support normal intestinal function, and the regulation of blood flow is, in part, determined by intestinal function itself. In the process of the development and adaptation of the intestinal mucosa for the support of the digestive processes and host defense mechanisms, and the muscle layers for propulsion of foodstuffs, a specialized microvascular architecture has evolved in each tissue layer. Compromised mesenteric and intestinal blood flow, which can be common in the elderly, may lead to devastating clinical consequences. This problem, which can be caused by vasospasm at the microvascular level, can cause intestinal ischaemia to any of the layers of the intestinal wall, and can initiate pathological events which promote significant clinical consequences such as diarrhea, abdominal angina and intestinal infarction. The objective of this review is to provide the reader with some general concepts of the mechanisms by which neurohumoral vasoactive substances influence mesenteric and intestinal arterial blood flow in health and disease with focus on transmural transport processes (absorption and secretion). The complex regulatory mechanisms of extrinsic (sympathetic-parasympathetic and endocrine) and intrinsic (enteric nervous system and humoral-endocrine) components are presented. More extensive reviews of platelet function, atherosclerosis, hypertension, diabetes mellitus, the carcinoid syndrome, 5-hydroxytryptamine and nitric oxide regulation of vascular tone are presented in this context. The possible options of pharmacological intervention (e.g. vasodilator agonists and vasoconstrictor antagonists) used for the treatment of abnormal mesenteric and intestinal vascular states are also discussed.

Key words

Absorption – Blood flow – Carcinoid syndrome – Diabetes mellitus – Diarrhoea – 5-Hydroxytryptamine – Ischaemia – Intestine – Nitric oxide – Pathophysiology – Physiology – Platelets – Secretion

1. Introduction

The intestine provides the body with fluids and nutrients, and functions as an important host defense barrier. It has evolved specific functions (digestion, absorption and secretion) with a concomitantly adapted blood circulation. In health, the intestinal vascular system supplies the intestine with critical substrates (e.g. oxygen and nutrients) for metabolism and removal of toxic by-products (e.g. heat and metabolites). Ischaemia lasting longer than four hours produces severe damage to nerves, epithelial cells and smooth muscle (Gannon and Perry 1989), pathological events resulting in high morbidity and mortality, especially among aged patients (Gallavan *et al.* 1989). Thus intestinal ischaemia has serious clinical significance and remains a challenging problem.

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The significance of mesenteric and intestinal blood flow and the mechanisms which control and regulate this flow, are now becoming understood. The control of this blood flow takes place mainly in the arteries and is partially mediated by both extrinsic and intrinsic neurohumoral pathways. The tone of the vascular smooth muscle, smooth muscle contraction (Horowitz *et al.* 1996) and hence the mesenteric blood flow, is regulated in a complex fashion, both in health and disease. The general physiology of smooth muscle activity (Horowitz *et al.* 1996), mesenteric and intestinal blood flow (Jodal and Lundgren 1989, Bastidas *et al.* 1995, Crissinger and Granger 1995) and transport processes (Holtug *et al.* 1996), in addition to the pathophysiology of intestinal non-occlusive ischaemia (Granger *et al.* 1989, Bastidas *et al.* 1995, Crissinger and Granger 1995) and diarrhoea (Hansen and Skadhauge 1995) has recently been reviewed.

The birth of intestinal neuroendocrinology dates back to 1902 with the discovery of secretin, and to 1922 with the first therapeutic application of insulin in the treatment of diabetes mellitus (Gaginella 1993). The discovery of neuroendocrine substances (hormones and peptides), which act as neurotransmitters, neurocrine, paracrine and autocrine mediators, and affect many intestinal functions has been of major importance in the understanding of intestinal vascular control mechanisms. The pathogenesis of non-occlusive mesenteric ischaemia has been of interest since the syndrome was described in the 1950's. Reflex splanchnic vasoconstriction may contribute to the pathogenesis of mesenteric ischaemia, and angiography has demonstrated localized spasm or diffuse vasoconstriction of the entire mesenteric vasculature.

This review presents the general concepts and mechanisms by which neurohumoral substances affect arterial mesenteric and intestinal blood flow in health, and in disease states. This review will present results primarily from experiments involving the small intestine both because of the complexity of the physiology as well as the great breadth of experimental data available. Information on two important vasoactive agents, 5-hydroxytryptamine (5-HT) and nitric oxide (NO) is presented in order to illustrate the signal transduction pathways by which these effectors act as modulators. Finally, reviews of platelet function, atherosclerosis, hypertension, diabetes mellitus and the carcinoid syndrome and their effects on intestinal blood flow and mucosal function will be reviewed.

2. Vascular morphology and anatomical organization

The organization and structure of the intestinal and mesenteric blood vessels is specialized to accommodate the individual requirements of the muscle layers and the mucosal epithelium. The intestinal arterial blood supply is organized as two circuits in series with one another, as extramural and intramural blood vessels. There is, however, substantial interspecies variation in this organization (Gannon and Perry 1989, Chou 1989), but some common features are similar across species. In general, arterial vessels originate from a superior mesenteric artery, and venous drainage parallels the arterial blood supply. This superior mesenteric artery divides within the mesentery into jejunal and ileal branches which in turn interconnect *via* arcades with straight branches (vasa recta) towards the intestinal wall. The presence of anastomotic connections at several levels, both between large vessels and smaller submucosal vessels provides the bowel with protection from hypoxia and ischaemia in the face of decreased blood flow. Neonatal intestine, with an immature and incompletely developed collateral circulation is at greater risk for ischaemic injury than adult intestine (Crissinger and Granger 1995).

The mesenteric artery divides into branches (pre-1A) that penetrate the outer muscular layer of the intestinal wall and supply the major distributing plexus within the submucosa (Gannon and Perry 1989). The intestinal arteries begin as first-order vessels (1A). Luminal pressure in these vessels has been measured at about 50 mm Hg. 1A vessels penetrate both muscle layers and course along the outer surface of the submucosa, giving rise to second-order arterioles (2A). The myenteric and submucosal nerve plexuses have separate, sparse, and largely planar capillary networks. The capillaries of these autonomic plexuses are continuous, thereby functionally connecting them. Third-order arterioles (3A) from the submucosal pass directly into the tips of the mucosal villi. At the tip of the villi they then divide into an arcade of vessels that feed the net-like subepithelial capillary system located on all sides of the tongue-shaped villus. Capillaries merge into venules in the center of each villus, which then emerges adjacent to the supplying arteriole. The villus capillaries lie approximately 1 mm from the basal membrane of the epithelial cells. There is a separate arteriolar and capillary network that supplies the intestinal crypts. In rodents, subepithelial capillaries lie in very close proximity to arterioles creating a countercurrent exchange system not present in humans (Chou 1989). The muscle layers of the intestinal wall receive blood flow from an independent network supplied from branches of 1A and 2A vessels (Lundgren 1984, Ooms and Degryse 1986, Gannon and Perry 1989).

The walls of the small arteries consist of an outer tunica adventitia, a tunica media and an inner tunica intima. The adventitia contains nerve cells and axons that do not penetrate the media. The media of these vessels is comprised of multiple layers of helically arranged smooth muscle cells which forms a contractile apparatus. In pre-1A arteries there are approximately six layers of smooth muscle cells, while in smaller 1A arteries, there may be as little as one layer of smooth muscle. The inner tunica intima, which covers the luminal surface of these vessels, is comprised of endothelial cells and their basal lamina. The intima is fenestrated, allowing contact between endothelial cells and smooth muscle cells (Mulvany and Aalkjaer 1990). There are, however, significant inter-species differences in the density of nerve fibers and varicosities, the depth of innervation in the medial layer of the vessel, the distance between the neural varicosities and smooth muscle cells, and the amount of tissue between varicosities and the smooth muscle cells (Hirst 1989). Intestinal mucosal capillaries are

fenestrated and as such allow transcapillary passage of molecules smaller than 3 nm. In addition, a variety of intercellular junctions contributes to molecular exchange and communication between adjacent endothelial cells.

3. Measurement of blood flow

The relationship between intestinal blood flow and function can best be explored by studying local perfusion within the mucosa and muscularis separately, rather than measuring total blood flow to the intestine. Many methods have been described for measuring fractional blood flow, and mucosal perfusion in particular (Lundgren 1984, Shepherd and Kiel 1989). The modern era of measuring intestinal blood flow began with the development of the radioactive microsphere technique in the 1970's and was followed by the laser-Doppler flowmeter technique, which was introduced in the early 1980's. Currently a wide variety of techniques are available for the measurement of total and fractionated intestinal blood flow. Techniques include measurement of venous outflow, electromagnetic flowmetry, pulsed and duplex Doppler flowmetry measuring total organ blood flow. Fractional blood flow is determined using microspheres, aminopyrine, iodoantipyrine or hydrogen gas clearance, inert gas washout, laser Doppler velocimetry and *in vivo* microscopy techniques. Unfortunately, no individual method fulfills all of the criteria required of an "ideal" method (safe, noninvasive, continuous, quantitative, accurate, reproducible, capable of measuring intramural distribution, absence of effects on blood flow, and clinically applicable) (Crissinger and Granger 1996). Nonetheless, each of these methods provides some information which has brought about a better understanding of the relationships between vasospasm, tissue hypoxia, ischaemia and intestinal dysfunction (Crissinger and Granger 1996).

Flow and distribution

Under normal conditions, the small intestine receives about 10 % of the cardiac output and contains 10 % of the total blood volume (Donald 1983, Lundgren 1984). About 80 % of blood in the mesenteric circulation passes to the submucosal and mucosal layers, this flow providing critical support to vital transport processes (Hirst 1989). Villus blood flow accounts for about 30 %, the crypts 25 % and the submucosa 25 % of the total intestinal blood flow (Donald 1983, Gannon and Perry 1989). The blood flow in the small arteries under normal conditions is about 1 ml/min/g, but can be increased to about 2.5 ml/min/g. This increase in flow occurs primarily to the mucosa, which will accommodate much as 90 % of the total intestinal blood flow (Lundgren 1984).

Blood flow measurement can be markedly affected by experimental or physiological conditions. The type of surgical procedure and/or the anaesthetic used during the experimental procedures may greatly influence results obtained when measuring intestinal blood flow. For example, laparotomy may cause the intestinal blood flow to be reduced by approximately half during the first 15 min of the procedure. Blood flow may be redistributed to the muscularis following manipulation of the intestine. Both total flow and distribution of flow returns to normal after 60 min. These changes have been attributed to activation of the angiotensin-vasopressin system (Granger *et al.* 1989).

Intraoperative blood flow may be under or overestimated because of the affects of anaesthesia. Anaesthetics and their adjuvants alter intestinal haemodynamics both through direct vascular effects and indirectly, through effects on cardiac dynamics and on neurohumoral control mechanisms. Used in doses required to attain surgical anaesthesia, pentobarbital, chloralose-urethane, halothane, isoflurane and enflurane all cause a decrease in intestinal blood flow, while ketamine and morphine increase blood flow. Meperidine, fentanyl, alphaxalone and alphadolone do not seem to change blood flow significantly (Granger *et al.* 1989, Gootman *et al.* 1990).

4. Control mechanisms

The mechanisms responsible for the control of mesenteric and intestinal blood flow are multiple and often interdependent. Regulation of intestinal blood flow depends almost entirely on the arteriolar smooth muscle tone, and its regulation by both local (intrinsic) and systemic (extrinsic) factors. Extrinsic regulation may be neural, humoral or both, while intrinsic regulation primarily involves the intramural nervous system, endothelial signal transduction, and hormone and/or paracrine secretion of peptides or non-peptide vasoactive substances (Rogers and David 1995). The type of physiological response produced depends upon the species and the region of the intestine being studied, and upon the digestive state of the subject.

The intestinal blood flow is autoregulated primarily by metabolic and myogenic feedback mechanisms, thus enabling the development of pressure-flow relationships seen under normal conditions as well as the "escape" phenomenon and the reactive hyperaemia seen in certain pathological states. The myogenic determinants of flow appear to dominate in the small intestine (Crissinger and Granger 1995). Extrinsic signals modify the local release of neurotransmitters and thereby alter the responsiveness of the vascular smooth muscle cells. The nervous system autoregulates mucosal blood flow by controlling the contractile state of the small (resistance) arterioles that constitute the submucosal arteriolar network (Jodal and Lundgren 1989, Mulvany and Aalkjaer 1990).

Under resting conditions, mucosal perfusion is mainly regulated by the vasodilators NO and prostaglandin I_2 (PGI_2), and by the potent vasoconstrictor endothelin (all produced by the endothelium), and by the intrinsic sympathetic tone, mediated by norepinephrine (NE) (Salzman 1995). Alterations of circulating and local concentrations of neurohumoral substances alter the local blood flow and thereby intestinal function, including mucosal transport processes. Finally, arterial baro- and chemoreceptors, cardiopulmonary receptors, and afferents from muscle receptors influence blood flow and blood volume (Donald 1983, Lundgren 1984, Kreulen and Keef 1989).

The nervous system

Neuronal regulation of intestinal haemodynamics is present immediately after birth,

although the efficiency of these processes is age-dependent during the early postnatal period (Nowicki 1989). The neuronal control mechanisms primarily involve autonomic reflexes, and include functionally interconnected but morphologically identical intrinsic and extrinsic nerve fibers (Hirst 1989). Vessels are innervated by both vasoconstrictor and vasodilator fibers, considered sympathetic and parasympathetic, respectively. Submucosal neurons are the primary vasomotor subservers and as such modulate intestinal blood flow and transport processes. Excitation of these neurons leads to vasodilatation, i.e. increased blood flow to the mucosa (Ooms and Degryse 1986, Vanner 1993, Surprenant 1994). Fine fibers ascend from a dense plexus that surrounds the arterioles of the crypt region, enter the villi and then extend into the capillary network underlying the basolateral membrane (Lundgren 1984, Ooms and Degryse 1986). The sympathetic nervous influence over the intestinal microcirculation overshadows that of the other determinants of intestinal blood flow. The sympathetic nerves carry adrenergic vasoconstrictor fibers, and non-adrenergic, non-cholinergic (NANC) vasodilatory fibers (Lundgren 1984). These originate in the reticular substance of the medulla and lower third of the pons, also known as the vasomotor center of the brain. This area of the pons is in turn influenced by several areas higher in the pons, mesencephalon and diencephalon. The vasomotor center consists of a vasoconstrictor area (C-1), a vasodilator area (A-1) and an integrating, reflex sensory area (A-2). Many afferent nerve fibers arise in the gut (intrinsic) (Guyton 1991). These afferent nerves (C fibers) are primarily capsaicin- and lidocaine-sensitive and serve as luminal sensors which regulate local blood flow by releasing neurohumoral substances. The activation of these nerves by mechanical or thermal stimuli, ischaemia and hypoxia results in increased local blood flow (Chou and Alemayehu 1993). These afferent fibers transmit signals to the medulla *via* the vagus nerve, initiating reflex signals which return to the intestinal vasculature, creating a neuronal feedback loop controlling blood flow and mucosal function, and altering other physiological functions of the gut (Guyton 1991).

Sympathetics

Small arteries and the submucosal arterioles of the small intestine in particular are tonically extrinsically innervated by postganglionic sympathetic adrenergic neurons (Hirst 1989). In addition, an extensive sympathetic network innervates the myenteric and submucosal plexus as well as the intestinal epithelium and crypts. These neurons are not myelinated, and are located almost exclusively in the tunica adventitia. There is great variation in nerve density depending on the size of the vessel, and the age and species of the animal studied (Bevan *et al.* 1980, Jodal and Lundgren 1989). Ongoing basal sympathetic adrenergic vasoconstriction is mediated by nerves originating from the paravertebral ganglia, thereby linking the extrinsic nerves to the intrinsic system of autonomic reflexes (Jodal and Lundgren 1989, Surprenant 1994). Norepinephrine, adenosine triphosphate (ATP) and neuropeptide Y (NPY) are the sympathetic neurotransmitters which are co-located and stored in synaptic vesicles (Jodal and Lundgren 1989, Surprenant 1994). They are (as are most other neurotransmitters) released by exocytosis and inactivated predominantly by uptake. This neuromuscular transmission takes place at discrete points on the smooth muscle membrane (Luff *et al.* 1987). NE and other adrenergic agents function by the activation of distinct receptors. Activation of additional receptors can either facilitate (e.g. nicotinic drugs) or reduce (e.g. muscarinic drugs) NE release. The complement of smooth muscle receptors varies with species, age, vessel size, and the location along the length of a vessel (Bevan *et al.* 1980, Jodal and Lundgren 1989). Other circulating and locally released agents affect the state of these neurotransmitter-receptors by modifying their state (affinity, binding, efficacy) through "cross-talk" mechanisms (Schmid *et al.* 1983). For example, since 5-HT accumulates in adrenergic nerves, it can be released from these nerves as a false transmitter together with NE, thereby modulating the response to NE (Verbeuren *et al.* 1983).

Neurogenic excitatory junction potential resulting in vasoconstriction of submucosal arterioles is caused by NE release onto prejunctional α_2-adrenoreceptors, thereby depressing the release of ATP, which causes vasoconstriction acting on P_{2x}-purinoreceptors (Surprenant 1994). NE causes a biphasic response after binding to the adrenergic receptors on resistance artery smooth muscle. Initially, flow redistributes to and within the mucosa, with increased flow to the villi and decreased flow to the crypts. These flow changes cause an unchanged absorption rate in the villi and a reduced secretion rate in the crypts, which results in hyperaemia and an increase in the net absorption rate. This initial vasodilatation is followed by a steady-state constriction. Both α_1- and α_2-adrenoreceptors participate in these responses. NPY also causes vasoconstriction and potentiates response to NE (Jodal and Lundgren 1989).

Parasympathetics

The parasympathetic fibers travel predominantly with vagus nerves. There are three varieties of parasympathetic neurons, all with acetylcholine (Ach) as the primary (Jodal and Lundgren 1989). While direct application of Ach on vascular smooth muscle causes vasoconstriction, release of Ach by neurons is generally associated with vasodilation (Hwa *et al.* 1994). The muscarinic receptors are located on the endothelium. Their activation leads to the release of NO from endothelial cells. NO diffuses to the vascular smooth muscle and causes relaxation and vasodilation. In addition, nearly all vasodilator neurons are immunoreactive to vasoactive intestinal polypeptide (VIP), suggesting that VIP may be a second messenger to Ach in the parasympathetic relaxation mechanism (Surprenant 1994). However, although Ach functions experimentally as a potent vasodilator, there does not seem to be much functional cholinergic innervation of intestinal blood vessels (Kreulen and Keef 1989, Hirst 1989, Mulvany and Aalkjaer 1990) (Figs 1 and 2).

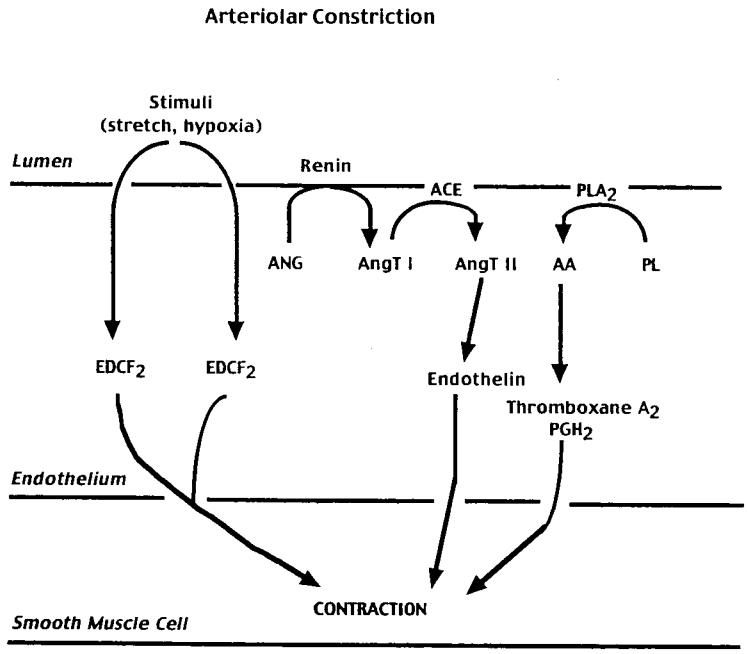

Fig. 1. *Mechanism of endothelium-dependent arterial vasoconstriction. EDCF: endothelium-derived contracting factor, Ang: angiotensinogen, AngT: angiotensin, ACE: angiotensin converting enzyme, AA: arachidonic acid, PLA_2: phospholipase A_2, PL: phospholipid.*

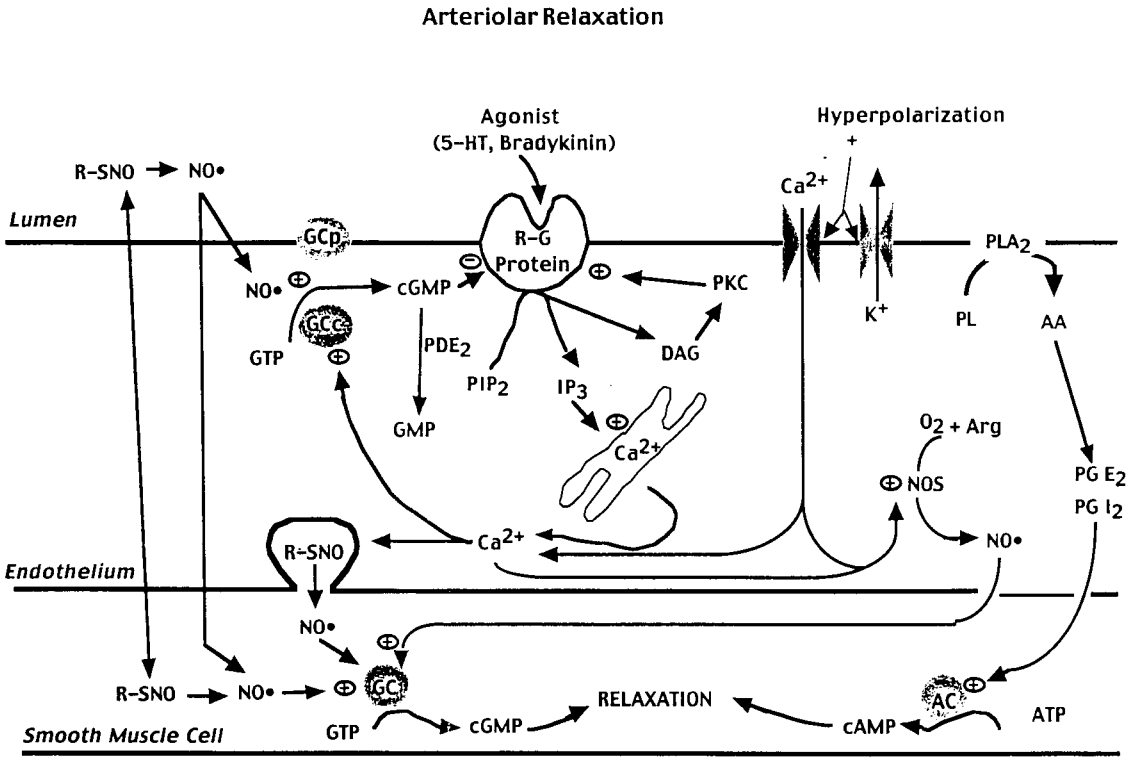

Fig. 2. *Mechanism of arterial relaxation. Relaxation of vascular smooth muscle is predominantly the result of endothelium-derived factors. GC: guanyl cyclase, R: receptor, G: G protein, PKC: protein kinase C, DAG: diacyl glycerol, PDE: phosphodiesterase, R-SNO: nitrosothiols, PIP_2: phosphoinositolphosphate, IP_3: inositol triphosphate, Arg: arginine, PGI_2: prostacyclin, PGE_2: prostaglandin E_2, NO: nitric oxide.*

Neuroeffector transmission

Luminal chemical stimuli of the mucosa produce a local reflex vasodilatation in the submucosal vascular network that results in an increase of mucosal blood flow. Non-neuronal (e.g. release of 5-HT from enterochromaffin cells) as well as neuronal mechanisms are also involved in this reflex (Vanner 1993, Surprenant 1994). This neurogenic transmission is characterized by presynaptic regulation of the transmitters released from the perivascular neural varicosities and the reuptake by activation of presynaptic receptors. In general, two kinds of neural signals dominate in the mesenteric and intestinal vasculature, fast and slow signals. Both appear to be modulated by more than one neurotransmitter. These substances include, but are not limited to NE, Ach, bradykinin, adenosine, ATP, prostaglandins, calcitonin gene-related peptide (CGRP), vasopressin, NPY, somatostatin (SOM), enkephalins, VIP and substance P (SP) (Kreulen and Keef 1989, Hirst 1989, Mulvany and Aalkjaer 1990).

Table 1
Dominating effect of endogenous neurohumoral agents on mesenteric and intestinal arterioles

Constrictor	*Dilator*	*Unknown*
Angiotensin I, II, and III	Acetylcholine	Bombesin
Antidiuretic hormone	Adenosine	Chromogranin A
Calcium	Adenosine diphosphate	Chromogranin B
Dopamine (high dose)	Adenosine triphosphate	Cryptins
Endothelin-1	Bradykinin	Duodenal cholecystokinin-releasing peptide
Epinephrine	Calcitonin gene-related peptide	Duodenal secretin-releasing peptide
Leukotrienes	Carbon dioxide	Enteroglucagon
Motilin	Cholecystokinin	Galanin
Neuromedin U	Dopamine (low dose)	Gastrin-releasing polypeptide
Neuropeptide Y	Gastric inhibitory peptide	Glucagon-like peptides
Norepinephrine (high dose)	Gastrin	Guanylin
Oxytocin	Glucagon	Incretins
Peptide YY	Glucocorticoids	Monitor peptide
Phenylephrine	Glucose-dependent insulinotropic peptide	Neuromedin B
Potassium	Histamine	Neuromedin C
Prostaglandin B_2, D, F_1, H_2	Hydrogen	Pancreastatin
Serotonin (high dose)	Insulin	Pancreatic polypeptide
Somatostatin	Kalikrein	Sorbin
Thromboxane A_2	Magnesium	Trefoil peptides
Vasopressin	Neuromedin N	
	Neurotensin	
	Nitric oxide	
	Nitroglycerin	
	Norepinephrine (low dose)	
	Opiates (enkephalins)	
	Pituitary adenylate cyclase-activating polupeptide	
	Prostacyclin	
	Prostaglandin E and I	
	Secretin	
	Serotonin (low dose)	
	Sodium	
	Substance P	
	Thrombin	
	Thyreotropin-releasing hormone	
	Uridine triphosphate	
	Vasoactive intestinal polypeptide	
	Xenin	
	Xenopsin	

These substances stimulate the submucosal nerve plexuses, and cause both cholinergic and non-cholinergic vasodilatation of submucosal arterioles (Chou and Alemayehu 1993). All of the local neuronal pathways are tetrodotoxin (TTX)-sensitive and are located either in the submucosal plexus or the mucosa layer. This reflex vasodilatation appears to result from activation of inhibitory synaptic nerves from sympathetic and myenteric ganglia and from synaptic activity in VIP-containing submucosal neurons. These VIP-containing neurons are afferent (sensory) in nature and innervating both the submucosal arterioles and the mucosa. They provide an afferent limb to the mucosa as well as efferent vasodilator limb to the arterioles. The efferent vasodilator neurons are predominantly cholinergic (of the type containing the calcium-binding protein calretinin), acting on muscarinic receptors. They also project to the mucosa, where they act as efferent secretomotor fibers or as afferent chemosensory and mechanosensory fibers (Vanner 1993, Surprenant 1994) (Table 1).

Vasoactive neurohumoral agents

Most neurohumoral agents produced in the mucosa cause vasodilation when infused directly into the splanchnic circulation (Table 1). These agents modulate vascular tone by activating selective receptors. For example, receptors for angiotensin, bradykinin, NE and histamine have been demonstrated on endothelial cells, while receptors for bradykinin, angiotensin, platelet-derived growth factor and 5-HT, among others, have been found on vascular smooth muscle cells (Coughlin *et al.* 1984). Angiotensin II and vasopressin appear to be important physiological vasoconstrictors. They redistribute blood flow to the submucosa from the muscle and mucosa layers (Granger *et al.* 1989). Vasopressin, produced in the hypothalamus, evokes a sustained reduction in blood flow. Cholecystokinin (CCK) primarily increases mucosal blood flow by activating specific CCK receptors present on the smooth muscle cells. Enteroglucagon-like peptide, found in the intestinal epithelial "L" type cells is released in connection with a meal and may be involved blood flow regulation. Circulating epinephrine, released by the adrenal glands, causes activation of both α- and β-adrenoreceptors, whereas NE, another adrenal secretory product activates primarily α-adrenoreceptors. Gastrin's vascular effects, like those of SP, are mainly secondary to its effects on secretion. Glucagon causes a selective and sustained increase in villus blood flow. Neurotensin (NT) is found in neuronal N cells, but there does not appear to be any direct vascular innervation by neurotensin-containing nerve terminals. Neurotensin's vasodilatory effects are confined to the muscular layer. Motilin reduces mucosal blood flow. VIP, acting as a neurocrine and paracrine mediator, seems to be ubiquitous, being associated with nerves supplying most of the intestinal blood vessels. The arterial innervation by VIP-containing nerves is more prominent in the mucosa than in the submucosa. Thus stimulation of these nerves would be expected to cause a differential increase in blood flow to the mucosa through vasodilatation of these well innervated mucosal vessels. Somatostatin is present in nerves and epithelial endocrine-like cells (D cells) and acts as a paracrine vasoconstrictor in pharmacological doses. CGRP seems to act both as a neuropeptide and a circulating hormone. Polypeptide YY (PYY) redistributes blood flow from the muscularis to the submucosa and mucosa. Bradykinin's effects are both endothelium- and prostaglandin-dependent and -independent. Enkephalins (opioids) affect blood flow through neural mechanisms, although there are only a few fibers present on blood vessels. It is unknown if there are opioid receptors on vascular smooth muscles. Substance P is primarily localized to nervous tissue, often co-located with 5-HT. These fibers innervate the muscle layers, the vessels present in the submucosa and basal part of the mucosa, and the epithelial cells. SP acts directly on receptors on the smooth muscle cells (Schmid *et al.* 1983, Jodal and Lundgren 1989, Chou and Alemayehu 1993, Brand and Schmidt 1995). 5-HT and NO are discussed in later sections of this review.

5. Signal transduction pathways

Most neurohumoral substances activate distinct receptors directly located on the vascular smooth muscle cell. Contraction results when a vasoconstrictor binds to receptors on the smooth muscle cell and activates the phospholipase C (PLC). PLC increases the turnover of phosphoinositols and results in calcium influx and increased intracellular Ca^{2+} . Increased intracellular levels of Ca^{2+} promote Ca^{2+} binding to calmodulin (CAM), which then activates myosin kinase and catalyzes phosphorylation of a light-chain kinase to initiate the interaction of myosin and actin to produce contraction. Constriction is terminated following activation of the Ca^{2+}-CAM activated ATPase, the molecular pump responsible for increasing pumping Ca^{2+} from the cells. Decreased $[Ca^{2+}]_i$ alters myosin so that it is no more capable to interact with actin, allowing the relaxation. In contrast, relaxation occurs when cell surface bound receptors activate adenylate cyclase (AC). AC increases intracellular concentration of cyclic adenosine monophosphate (cAMP), which, in turn activates protein kinase A (PKA). PKA phosphorylates myosin light-chain kinase, and the inactivation of this molecule causes relaxation (Schmid *et al.* 1983) (Fig. 3).

6. Digestion

Digestion is accompanied by an increase in blood flow in the submucosal arterioles (Vanner 1993) resulting in up to a 100% increase of perfusion to the intestinal mucosa. The mechanisms responsible for the hyperaemia (vasodilatation) occurring during digestion and during the postprandial period are complex, and are both species- and age-dependent (Crissinger and Granger 1995). In general, there are at least two phases in the vascular response to feeding: the anticipatory-ingestion phase and the postprandial phase. The postprandial phase is characterized by hyperaemia and increase in blood flow. Postprandially, blood flow in the superior mesenteric artery increases by as much as 60% within 5 min after completing a meal. Blood flow peaks after 30-90 min and declines slowly over the following 2–3 hours (Lundgren 1984, Granger *et al.*

1989). Postprandial hyperaemia, is generally confined to the mucosal layer, and results primarily from the change in submucosal and mucosal tissue activity. This vascular response is largely determined by the nature of the food ingested and the secondary effects of neurohumoral agents released in response to both mechanical and chemical stimuli (Donald 1983, Chou and Alemayehu 1993). Breakdown products of food (lipids, carbohydrates and proteins) together with bile (forming the chyme) are the most important luminal chemical stimuli responsible for postprandial hyperaemia. Of the three major constituents of food, lipids produce the greatest hyperaemia, compared to proteins and carbohydrates. On the other hand, carbohydrates induce the most rapid increase in mucosal blood flow (Lundgren 1984, Granger *et al.* 1989).

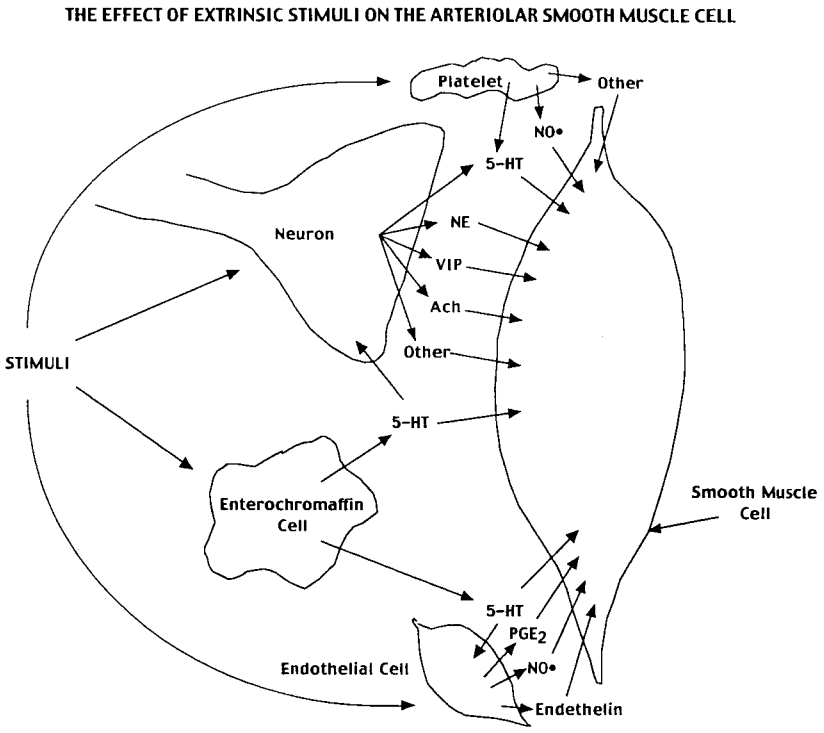

Fig. 3. *Scheme of the effects of extrinsic stimuli on the arteriolar smooth muscle cell. Local production of vasoactive mediators by adjacent neurons, endothelial cells, platelets and other vascular smooth muscle cells in response to stimuli (hypoxia, chyme etc.) alters vascular tone. 5-HT: serotonin, NE: norepinephrine, Ach: acetylcholine, VIP: vasoactive intestinal peptide, PGI_2: prostacyclin.*

Neurohumoral agents associated with chyme-induced increases in blood flow are important as endocrine, autocrine, paracrine or neurocrine mediators in both normal and disease states (Chou and Alemayehu 1993). Locally, these substances are released following stimulation by chyme. Specifically, the mucosal sensory receptors (e.g. capsaicin-sensitive C fibers) are stimulated by the chyme, and activate intrinsic nerves as well as cause the release of blood-borne vasoactive agents (e.g. 5-HT, gastrin, histamine, prostaglandins, bradykinin, VIP, NT, secretin, CCK, SP and glucagon) (Granger *et al.* 1989). It appears that 5-HT, CCK, VIP, SP, SOM and Ach, individually or in combination, contribute significantly as both circulating and locally active agents to the production of postprandial hyperaemia. Experimental data suggest that the observed postprandial mucosal hyperaemia is mediated *via* an intramural nerve reflex involving a serotonergic receptor, with VIP as the ultimate transmitter acting on the vascular smooth muscle. This hypothesis is supported by experimental data demonstrating a postprandial rise in venous plasma VIP concentration. VIP is likely released from intramural neurons that are activated by 5-HT release (Granger *et al.* 1989). CCK, secretin, gastric inhibitory peptide (GIP), gastrin and NT, however, appear to act only locally under normal conditions, but may have systemic effects only in disease states (Chou and Alemayehu 1993). While 5-HT seems to act throughout the entire length of small intestine, gastrin, VIP, secretin and CCK are primarily involved in jejunal postprandial hyperaemia, while NT and SP are involved in the ileal response (Granger *et al.* 1989).

7. Transport processes

In the process of digestion and absorption large volumes of fluids (10-20 l/day in humans) and nutrients (about 500 g carbohydrate and 70-100 g protein per day) are transported across the intestinal mucosa and the vascular endothelium (Lundgren 1984, Gannon and Perry 1989). The mucosa requires a relatively high rate of blood flow in order to fuel the enormous metabolic activity of the enterocytes required to exchange fluids and solutes (Granger *et al.* 1989). The resulting increase in postprandial mucosal blood flow is almost exclusively limited to the villi in order to accommodate the energy requirements of the

absorptive process (Lundgren 1984, Gannon and Perry 1989). In accordance with the Starling principles, mucosal blood flow, lymph flow, capillary permeability, interstitial fluid volume, hydrostatic conductance and colloid osmotic pressure all act to determine the rate of transport (Granger *et al.* 1989). During periods of net absorption, there is an increase in intestinal fluid, interstitial pressure, and lymph flow, together with a concomitant reduction in capillary filtration. Periods of net secretion, on the other hand, are marked by a decrease in intestinal fluid, interstitial pressure, and lymph flow, but enhanced capillary filtration (Ooms and Degryse 1986, Granger *et al.* 1989). The earliest manifestation of intestinal ischaemia is an increase in capillary permeability followed by disruption of the mucosal epithelial layer. While all transport processes are affected both during and following ischaemia, some are more sensitive to hypoxia than others (Lundgren 1984).

Absorption

There is a correlation between the rate of intestinal mucosal absorption and blood flow (Donowitz *et al.* 1979, Granger *et al.* 1989, Beubler 1995). Absorption of substances from the gut lumen into the circulation involves first, the diffusion or transport of solutes across the mucosal barrier into the interstitium, and second, the diffusion or transport of the absorbed substances into the lymph or blood. The capillaries play a more important role than lymphatics in removing absorbed fluid from the mucosal interstitium. Mucosal blood flow is regulated by local metabolic factors (e.g. CO_2, NO and adenosine), which diffuse into the arterioles and cause relaxation. While these metabolic mechanisms maintain adequate blood flow and oxygen delivery, myogenic factors keep the intestinal capillary pressure and transcapillary fluid exchange constant (Ooms and Degryse 1986). Absorbed solutes are transported passively. This process is, however, enhanced by countercurrent exchange and multiplier mechanisms. The intestinal countercurrent exchange mechanism is suggested in anatomical studies of animals by the presence of a subepithelial capillary network, in which the flow is opposite to that of the central arteriole. Because of this countercurrent arrangement of vessels, the circulating blood vessels form an exchange multiplier which enhances the ability of the circulation to clear the absorbed material (Lundgren 1984). Absorption rates are also proportional to the arterial perfusion pressure, the solute concentration difference between the intestinal lumen and the capillaries, capillary permeability and surface area as well as the blood flow within the villi. Although there is a positive correlation between blood flow and the active transport of solutes, the relationship is quite complex. As such, absorption rates of amino acids, monosaccharides, water and electrolytes by the enterocyte are highly sensitive to hypoxia and ischaemia (Lundgren 1984, Granger *et al.* 1989).

Secretion

In general, the intestinal circulation serves a permissive role in secretory processes, and thus the most active secretagogues are potent vasodilators. Convincing evidence indicates that some endotoxins and enterotoxins (e.g. cholera toxin) and endogenous secretagogues (e.g. 5-HT in patients with carcinoid syndrome) induce fluid secretion *via* nervous reflexes which cause an increase in blood flow to the mucosa and submucosa (Cedgaard *et al.* 1978, Ahlman 1985a,b). The circulation provides the enterocytes with the necessary nutrients, oxygen, and fluid to support active secretory processes However, simple increases in blood flow do not automatically lead to hypersecretion (Gallavan *et al.* 1989), and active hyperaemia is not always present in hypersecretory states (Donowitz *et al.* 1979, Vanner *et al.* 1993, Beubler 1995). During active fluid secretion, protein-free fluid moves out of the interstitium and into the epithelial cells, thereby decreasing intestinal fluid volume and pressure, which causes blood flow to increase (Granger *et al.* 1989). Increased blood flow in hypersecretory disorders can also result from increased local metabolism, which causes the cells to rapidly utilize the tissue fluid nutrients and also to release large quantities of vasodilator substances (Ooms and Degryse 1986, Vanner *et al.* 1993). The reverse relationship, i.e., alteration of secretion by a change in blood flow, has been documented, but only when blood flow is reduced to a limiting value (Lundgren 1984, Shepherd and Kiel 1989). Blood flow-independent hypersecretion can also occur. This usually results from increases in venous pressure and altered plasma colloid osmotic pressure (Lundgren 1984). Finally, it has been hypothesized that segmental intestinal blood flow may be influenced by migrating action potentials originating in adjacent segments of bowel. These migrating action potentials travel in an aboral fashion, having a maximal transit distance of approximately 1.5 cm. Experiments testing this hypothesis, however, have not been able to demonstrate altered transport processes or blood flow in segments of small intestine adjacent and in continuity with secretagogue-exposed segments (Hubel *et al.* 1991, Jodal and Lundgren 1995).

Motility

Experimental evidence suggests that mean intestinal blood flow is inversely correlated with the intestinal motility index. However, spontaneous motility does not seem to affect blood flow significantly. On the other hand, reduction of blood flow increases motility initially and later inhibits motor activity (Ooms and Degryse 1986). Muscle contractions affect blood flow by at least two mechanisms: mechanical (muscle contractions), and metabolic (mucosal absorption and secretion). The two mechanisms usually produce opposite effects. The compression effect of muscle contractions decreases the blood flow to all layers of the intestine, while the metabolic hyperaemia caused by muscle contractions occurs in the mucosal layer only. Furthermore, the vascular smooth muscle of the blood vessels in the mucosal and muscularis layers may respond differently to a given stimulus. For example, adenosine decreases mucosal and increases muscularis blood flow (Lundgren 1984, Chou 1989, Granger *et al.* 1989).

8. Platelets

Platelets contain vasoactive substances such as 5-HT, adenosine diphosphate (ADP), ATP, and Ca^{2+}.

However, there are marked species differences in both content and release mechanisms (Meyers *et al.* 1982). Physiological stimuli (e.g. platelet adhesion and several neurohumoral agents including ADP, thrombin, 5-HT, vasopressin and epinephrine), and pathologically induced stimuli (e.g. antigen-antibody complexes, foreign substances, endothelial damage, abnormal platelet function in diabetes or arteriosclerosis) can activate platelets. Under either physiological or pathological conditions, the pattern by which platelets respond to stimuli is identical. Platelets first undergo a change in their shape. The cell membrane then become sticky, resulting in platelet aggregation. Finally, the platelets release (secrete) the contents of their granules. Platelets contain several types of granules. These include dense granules containing 5-HT, ADP, ATP and Ca^{2+}, alpha granules containing fibrinogen, albumin and other proteins, and lysosomes containing hydrolytic enzymes. In addition to platelet aggregation itself, prostanoids and thrombin are also stimuli for platelet degranulation (Holmsen 1985, Zucker and Nachmias 1985).

The functional effects of platelet degranulation on vasoactivity was discovered as a result of the observation that coagulation and platelet lysis caused local vasoconstriction. In 1947 Rapport and coworkers identified 5-HT as the substance responsible for this platelet-induced vasoconstriction (Rapport 1997, Stoltz 1985). Circulating 5-HT is bound to platelets by serotonectin, a protein located on the surface of platelets. 5-HT is taken up by platelets and stored in a complex formation with ATP and divalent ions (Stoltz 1985). When platelets release their dense granules, the liberated 5-HT feeds back on the platelets to activate further platelet aggregation in a ketanserin-sensitive manner (Holmsen 1985). NO, derived from the vascular endothelium and platelets, counteracts platelet aggregation (Barry *et al.* 1994).

The clinical significance of abnormal platelet function is exemplified in diabetes mellitus, in which there is an increase in platelet activation, aggregation, and adherence to the vascular endothelium. This increases the local release of vasoactive substances, such as 5-HT. Thus alterations in platelet 5-HT transport could, in part, explain the higher incidence of vascular complications in diabetic patients. Insulin therapy appears to reverse the abnormal platelet function (Forster 1994, Martin *et al.* 1995).

9. Disease states

Vascular autoregulation is significantly altered in chronic vascular disease states, such as arterial hypertension, due to altered vascular morphology and physiology (Granger *et al.* 1989). In portal hypertension and early stages of inflammatory bowel disease, there is an increase in intestinal blood flow due to accumulation and release of vasodilators and a reduction in the sensitivity of the vasculature to vasoconstrictors such as NE and vasopressin (Gallavan *et al.* 1989, Crissinger and Granger 1995). On the other hand, in states of non-occlusive ischaemia (e.g. atherosclerosis, diabetes mellitus, myocardial infarction, hypovolaemia, haemorrhagic and traumatic shock, septic and anaphylactic shock, cardiac arrhythmias, surgery, burns and congestive failure), mesenteric and intestinal vascular insufficiency often results from microvascular vasospasms (Corday *et al.* 1962, Gallavan *et al.* 1989, Crissinger and Granger 1995). Failure of the autoregulatory mechanisms and selective hypersensitivity to endogenous neurohumoral agents seems to contribute to the occurrence of mesenteric and intestinal ischaemia (Rogers and David 1995). Two major pathophysiological mechanisms have been suggested for mucosal injury associated with ischaemia and reperfusion: tissue hypoxia and oxygen free radical-induced damage (Rogers and David 1995, Bastidas *et al.* 1995). Arterial hypertension, diabetes mellitus, carcinoid tumors, and non-occlusive ischaemia, are examples of disease states which may cause profound disturbances in the intestinal microvascular morphology and function.

Arterial hypertension

Chronic hypertension results in smooth muscle hypertrophy and hyperplasia in the wall of the small resistance arteries of the mesentery and intestines (Pourageaud and Freslon 1995). Since the mesenteric arteries contribute significantly to blood pressure control, the structural changes induced by prolonged hypertension may be responsible for various selective functional alterations, such as increased contractility and decreased distensibility of the microvasculature. For example, in rats with angiotensin II-induced hypertension, mesenteric arterial microvascular responses to angiotensin II are increased (Abraham and Simon 1994). In contrast, the contractile responses to 5-HT and adrenergic agonists are either augmented (without change in receptor affinity) (Agrawal and McNeil 1987) or unchanged with inhibited Ach relaxation (Pourageaud and Freslon 1995). Finally, in portal hypertension, vascular responses to agents such as glucagon and epinephrine are altered, leading to intestinal hyperaemia (Agrawal and McNeil 1987, Granger *et al.* 1989). Significant evidence points to up-regulation in the production of PGI_2 (prostacyclin) as a cause for this phenomenon.

There is a growing body of evidence to support the hypothesis that chronic hypertension may lead to altered endothelial cell function and subsequent abnormal (reduced) vasorelaxation. Although vessels are hyperactive, the intestinal blood flow is usually unchanged in conditions of hypertension (Gallavan *et al.* 1989). Normal intestinal blood flow in these conditions may reflect compensatory responses of the intestinal microvasculature, such as changes in receptor binding affinity or changes in receptor density on the vascular endothelial cells.

Diabetes mellitus

Intestinal blood flow, motility, secretion and absorption are all affected in diabetics (Rogers and David 1995, Batra and Whitehouse 1995). Diabetes is one of the common predisposing factors for mesenteric arterial insufficiency (Camilleri *et al.* 1983, Forster 1994, Rogers and David 1995). Alterations in these functions lead to significant clinical manifestations including diarrhoea, gastroparesis, steatorrhoea and constipation. The causes of impaired intestinal function resulting in abnormal motility and transport processes in chronic diabetes. Increased peristaltic activity, rapid transit and decreased intestinal tone appear to be

multifactorial (Forster 1994). These include autonomic neuropathy and microvasculopathy, intravascular hyperviscosity, and increased platelet adhesiveness. These events make diabetes one of the common predisposing factors for chronic mesenteric arterial insufficiency (Rogers and David 1995). Focal ischaemia is usually due to limited thromboembolism or non-occlusive vasospasm in the setting of diabetes (Rogers and David 1995).

In diabetes, intestinal blood flow is increased and the arterioles are abnormally dilated (Gallavan *et al.* 1989). The intestinal vasculature is characterized by atrophy of the smooth muscle layer of mesenteric vessels and decreased capillary density (Gallavan *et al.* 1989), although there are usually no characteristic pathological lesions in the intestinal mucosa (Batra and Whitehouse 1995). However, Belai *et al.* (1988), have also reported that in diabetes, there is an increase in the number of enterochromaffin cells accompanied by a decrease in the content of 5-HT. Intestinal neuropathies associated with diabetes include primarily functional abnormalities in the sympathetic and parasympathetic autonomic nervous system, associated with few pathological alterations in the myenteric or submucosal plexuses in experimental diabetes (Lincoln *et al.* 1984, Belai *et al.* 1988). Conversely, others have reported significant pathological changes in mesenteric sympathetic neurons, including both axonal dystrophy and reduced axonal transport (Gallavan *et al.* 1989, Batra and Whitehouse 1995).

Changes in the content of neurohumoral substances is dependent on the intestinal segment and the stage of diabetes. For example, methionine-enkephalin levels have been reported to be reduced, VIP levels increased, and SP levels are unchanged in early diabetes. However, these all are reduced at later stages of the disease (Belai *et al.* 1988). 5-HT levels have been shown to be increased in the duodenum but unchanged throughout the rest of the intestine in similar disease states (Belai *et al.* 1988). Thus, the synthesis of specific neurohormones are likely altered in diabetes, and these changes seem to be localized to specific segments of the intestine. In fact, these functional changes begin before any structural changes can be detected (Takiguchi *et al.* 1988).

Vasoconstrictor responses to most stimuli, such as catecholamines and sympathetic nerves have been reported to be potentiated in early stages of diabetes (Poston and Taylor 1995) and decreased in later stages (Takiguchi *et al.* 1988). However, the data are far from clear in this regard. Streptozotocin (STZ)-induced diabetes in rats, a commonly used model for human diabetes, produces increased vasoconstrictor responses of the 3A mesenteric arterioles to angiotensin II, but a decrease in responsiveness to vasodilators, such as PGI_2 and PGE_2 (Hill and Larkins 1989), isoproterenol, histamine and Ach (Takiguchi *et al.* 1988). These data suggest that there may be an endothelium-dependent defect in the relaxation mechanisms of the mesenteric vasculature. Recent experiments in our laboratories suggest that prostaglandins are not involved in the blunted vasodilator responses of the mesenteric microvasculature in diabetic animals, and that it is unlikely to be caused by alterations in NO synthetic mechanisms. Since the smooth muscle responses to nitroprusside (endothelium-independent NO donor) remain normal, as do the vasodilator responses to calcium ionophore A23187, which increases endogenous endothelial NO production *via* NO synthase, vasodilator abnormalities more likely reside in the receptor pathways leading to the stimulation of NO synthesis, rather than in the NO synthetic mechanism itself (unpublished data). In support of hyperglycaemia alone being a pathophysiological key factor (Taylor and Poston 1995), treatment of diabetic animals with insulin seems to restore the vasoreactivity in cremaster muscle arterioles (Hill and Larkins 1989) and mesenteric arterioles (Taylor *et al.* 1994).

Carcinoid tumors

Carcinoid tumors are neuroendocrine tumors which are the most common small intestinal tumors, accounting for 15 % of all neuroendocrine gastroenteropancreatic neoplastic lesions. These tumors arise from Kultschitzky or enterochromaffin (EC) cells. Many biologically active peptides and amines (e.g. motilin, enkephalin, 5-HT, neurokinin A, neuropeptide K, gastrin, gastrin releasing peptide, SOM, pancreatic polypeptide, glucagon, SP, kallikrein, histamine, dopamine, NT and eledoisin), may be synthesized and contained within secretory granules in the cells of these tumors (Ahlman 1985a,b). Close contacts (neuroendocrine complexes) exist between EC cells and neighboring nerve elements of both afferent and efferent terminals adjacent to the basal lamina of the mucosa. The neuroendocrine substances contained within the secretory granules are released locally into the lumen and into the portal blood flow by a number of chemical, mechanical, bacteriological and nervous stimuli. Release of these substances may elicit functional changes within the intestines, including but not limited to, postprandial hyperaemia (vasodilation), as well as changes in absorption, secretion and motility of the small intestine (Ahlman 1985a,b).

Approximately 10 % of carcinoid tumors are associated with the carcinoid syndrome, a symptom constellation which usually includes diarrhea, hepatomegaly, facial flush, right-sided cardiac valvular disease, retroperitoneal fibrosis, and asthma. Symptoms usually arise when the liver's capacity to metabolize the released vasoactive substances (5-HT, SP, tachykinins, bradykinins) is exceeded. Carcinoid tumors differ widely in their ability to produce or store these substances, but about 25 % of patients with carcinoid tumors have elevated SP and NT levels in peripheral blood and about 75 % have elevated levels of 5-hydroxyindoleacetic acid (5-HIAA) in the urine, making these measurements useful as tools in diagnosing and managing the carcinoid syndrome (Feldman and O'Dorisio 1986). In fact, platelet 5-HT is the most sensitive parameter for the diagnosis and follow-up of carcinoids, since platelet 5-HT, in contrast to 5-HIAA, is not influenced by short-term dietary intake (Kema *et al.* 1993). Carcinoid abdominal crisis (pain, hypermotility, diarrhea) are attributed to effects of 5-HT and possibly SP, while catecholamines, prostaglandins, bradykinin and SP are believed to be responsible for the flushing and pulmonary symptoms (Ahlman 1985a,b, Norheim *et al.* 1986).

Carcinoid tumors have also been found to be associated with ischaemic bowel necrosis due to

sclerosis and narrowing of the mesenteric and intestinal blood vessels (Anthony and Drury 1970). 5-HT in high concentrations causes constriction of mesenteric vessels. This response may be exaggerated in patients with carcinoid syndrome because the arteries in these patients may be narrowed by severe fibroelastosis (Anthony and Drury 1970), causing vasospastic episodes, ischaemia and necrosis of the intestine and symptoms as diarrhea and pain (Ahlman 1985a,b, Ahlman *et al.* 1992).

10. 5-Hydroxytryptamine

In 1947 Rapport and colleagues identified an endogenous vasoconstrictor, which has become known as serotonin. Serotonin was identified as 5-hydroxytryptamine and as an enteromine in the EC cells by Erspamer and others in the 1950's (Saxena 1995). Since then it has become clear that 5-HT has many intestinal functions. It is a regulator of intestinal blood flow and transmural transport processes in both health and disease (Martin 1994, Hansen and Skadhauge 1997). Studies demonstrated that 5-HT acts as a transmitter in sensory and reflex responses to mucosal irritation from physiological (food) and pathophysiological (antigens, toxins) stimuli. Thus, 5-HT is closely implicated in such phenomena as emesis, diarrhea, and ischaemic abdominal pain (Martin 1994, Hansen and Skadhauge 1997).

Synthesis, location and release

Serotonin is primarily synthesized and released from the EC cells of the intestinal epithelium, and is actively taken up by platelets and vascular adrenergic nerves in addition to the endothelial cells in the liver and lungs (Ravelic *et al.* 1992, Martin 1994, Read and Gwee 1994). The intestinal mucosa has been reported to contain 5-HT in concentrations as high as 10 μg per gram of tissue (Ooms and Degryse 1986), whereas 5-HT content in longitudinal muscle preparations average approximately 100 ng/g. The concentration of circulating 5-HT is approximately 3 ng/ml in plasma (Saito and Yoshioka 1994) and about 1 μg/ml intraluminally when rat jejunum is perfused with saline at a rate of 1 ml/min (Gronstad *et al.* 1986). Such low 5-HT concentrations are not expected to have any physiological or pharmacological importance. Thus, the actions of 5-HT are probably dependent upon transient local increases of its release and concentration. 5-HT is co-located with several other neurohumoral agents (e.g. SP, motilin and enkephalin) in the EC cells, neurons and mast cells (Ahlman 1985a,b). Similar to histamine, 5-HT is tonically released at low rates from the intestinal mucosa (Jodal and Lundgren 1989, Vanner *et al.* 1993). It is released into the portal blood and into the intestinal lumen following a number of stimuli, including chemical stimulation (e.g. lowering of lumen pH), mechanical stimulation (e.g. feeding and mucosal irritation), enhanced nervous input (e.g. increased vagal tone) and pharmacological modulation (e.g. catecholamines, morphine, nicotine and reserpine) (Hansen 1995). The control of the release of 5-HT into the lumen seems mainly to be cholinergic, while release into the portal circulation seems mainly to be under adrenergic control (Ahlman 1985a,b).

Functions in health

5-HT affects almost all of the components of the intestinal wall, including the longitudinal and circular layers of smooth muscle, enteric nerves, vascular smooth muscle, and the enterocytes (Hansen 1995). The physiological role of 5-HT in the cardiovascular system and its importance in pathological conditions have clearly been demonstrated. This amine stimulates or inhibits the function of a variety of smooth muscles and autonomic nerves, although the responses are heterogeneous (Martin 1994). Released 5-HT is involved in normal digestive processes (postprandial hyperaemia, secretion and motility), as well as excretory function (hypermotility and diarrhoea). All of these are mediated principally by intrinsic reflexes activated within the mucosa (Ahlman 1985a,b, Jodal and Lundgren 1989). For example, exogenous 5-HT or mucosal stroking result in dilatation of submucosal arterioles in a tetrodotoxin-sensitive and 5-HT receptor-specific manner, suggesting that mechanosensory afferent fibers initiate this neuronally mediated reflex pathway, resulting in vasodilatation of submucosal arterioles (Lundgren 1984, Vanner *et al.* 1993). This phenomenon occurs only in the affected intestinal segment, but not in adjacent segments (Gronstad *et al.* 1986).

Motility

The effect of 5-HT on motility and blood flow has been shown to be dependent on the amount of 5-HT released. Low doses of 5-HT produce vasodilatation without altering luminal pressure. Medium dosage of 5-HT increase motility without altering blood flow, while high dosage produces vigorous intestinal contractions and vasoconstriction (Chou 1989). 5-HT induced contractions are mediated by 5-HT_2, 5-HT_3, and 5-HT_4 receptors, while relaxation is modulated by 5-HT_1 receptors that triggers the release of inhibitory neurotransmitters such as ATP, VIP and NO (Briejer *et al.* 1992).

Blood flow

The influence of 5-HT on the intestinal blood flow remains controversial. 5-HT appears to have variable effects depending on the dose employed, species used, experimental preparation, and is also dependent upon the state of intestinal motor activity. No changes in blood flow are detected in continuously hyperserotonergic experimental animals (Donowitz *et al.* 1979, Zinner *et al.* 1983, Yeo *et al.* 1989), but the initial response to applied 5-HT is usually vasodilatation in low doses and vasoconstriction in high doses (Granger *et al.* 1989, Jodal and Lundgren 1989). *In vitro,* 5-HT is usually a constrictor, but it appears to be a vasodilator *in vivo* (Edvinsson *et al.* 1976, Van Nueten *et al.* 1981). These data support the theory that 5-HT acts by transient local increases of its release and concentration.

Mechanisms

5-HT modulates intestinal vascular smooth muscle cell activity through activation of 5-HT receptors located directly on smooth muscle and endothelial cells (Martin 1994). The constrictor or vasodilating effects of 5-HT depend upon which of the

mechanisms predominates (Coughlin *et al.* 1984). Similar to other neurohumoral substances, 5-HT regulates vascular smooth muscle tone, by postsynaptic actions on the smooth muscle cells themselves, and by direct action upon presynaptic noradrenergic nerves which inhibits the release of NE in response to nerve stimulation (Su and Uruno 1985). Consequently, vascular responses to 5-HT are dependent on the state of sympathetic vascular tone because 5-HT causes constriction when sympathetic tone is low and vasodilatation when tone is high (Martin 1994). 5-HT is predominantly a vasoconstrictor (Moreland *et al.* 1985, Su and Uruno 1985, Tornebrandt *et al.* 1987, Martin 1994) and venoconstrictor (Cummings *et al.* 1986), however it acts as an attenuator of the actions of other vasoconstrictor influences such as NE, angiotensin II (Ang II), degradation products of fibrin and fibrinogen, $PGF_{2\alpha}$, thromboxane A_2 (TXA_2), hypoxia, and cooling (Van Nueten *et al.* 1985, Moreland *et al.* 1985). These actions could result from a receptor cross-talk mechanism, which has been demonstrated to exist among 5-HT_1-like, 5-HT_{2A}, α_2 adrenergic, NPY, TXA_2, histamine H_1 and Ang II receptors (Martin 1994). Furthermore, it appears that the effect of other neurohumoral substances are partially mediated through 5-HT receptor mechanisms, exemplified by CCK-mediated vasodilatation (Granger *et al.* 1989). Conversely, 5-HT seems to require the release of VIP in order to achieve its effects on intestinal motility and blood flow (dilatation) (Chou 1989). The dilatation caused by 5-HT is primarily due to direct activation of endothelial cells, stimulation of prostacyclin synthesis by vascular smooth muscle cells, and due to the increase in VIP release (Van Nueten *et al.* 1985). 5-HT causes vasoconstriction through vascular smooth muscle K^+ efflux and Ca^{2+} and Na^+ influx (Moreland *et al.* 1985) in a protein kinase C-dependent manner (Northover and Northover 1994). 5-HT increases sarcoplasmic Ca^{2+} levels, through activation of 5-HT_2 receptors, and increases Ca^{2+} from internal stores *via* phosphoinositol hydrolysis and through the voltage-dependent Ca^{2+} channels by a mechanism independent of phosphoinositol hydrolysis (Van Nueten *et al.* 1985, Feniuk and Humphrey 1989). Therefore it is surprising, that in isolated dog mesenteric arteries, 5-HT produces concentration-dependent contractions, which are attenuated by long-acting Ca^{2+} antagonists (Suzuki *et al.* 1995).

Receptors

5-HT receptors can be present or absent, functionally active or silent (latent). This has been demonstrated for the 5-HT_1-like receptor in isolated rabbit mesenteric arteries. These receptors are predominantly silent, unless tissues are subjected to a modest precontraction (Choppin and O'Connor 1995). There is also substantial species and distribution heterogeneity for 5-HT receptors (Humphrey *et al.* 1988, Okoro *et al.* 1995, Cambridge *et al.* 1995). Undoubtedly, the constrictor action of 5-HT at the arteriolar level as well as within large conduit vessels is mediated by several receptors, including the methiothepin-sensitive and postjunctional stimulatory 5-HT_1-like receptors located on sympathetic neurons and smooth muscle cells (Yildiz and Tuncer 1994, Martin 1994) and the ketanserin-sensitive 5-HT_2 receptors on smooth muscle cells (Su and Uruno 1985, Tornebrandt *et al.* 1987, Yildiz and Tuncer 1995, Wallerstedt *et al.* 1996). Several studies suggest that additional mechanisms are involved in 5-HT-induced arteriolar constriction. 5-HT could activate adrenoreceptors directly, and indirectly through the release of NE from sympathetic nerve endings by activation of ondansetron-sensitive 5-HT_3 receptors (Feniuk and Humphrey 1989).

5-HT also induces arterial relaxation through several mechanisms. 5-HT activates 5-HT_1-like receptors located on the endothelium and prejunctional inhibitory receptors of sympathetic neurons (Martin 1994, Hindle 1994). Conversely, 5-HT activates 5-HT_3 receptors on cholinergic vasomotor neurons in the submucosal plexuses to cause arteriolar dilatation (Vanner *et al.* 1993). Furthermore, in rat mesenteric arteries, 5-HT induces relaxation indirectly by causing the release of endothelium-dependent relaxing factor (NO) and PGI_2 through activation of vascular 5-HT_{2A} and atypical 5-HT receptors (Cummings *et al.* 1986, Martin 1994). The 5-HT_4 receptors have been identified on peripheral autonomic neurons and vascular smooth muscle cells (Martin 1994) and have been shown to be involved in 5-HT actions in sheep pulmonary veins (Cocks and Arnold 1992). They may also be involved in intestinal vasoactivity. Finally, the 5-HT_7 subtype has also been detected in vascular smooth muscle cells (Ullmer *et al.* 1995) and as such may also play a role in the vascular response to 5-HT.

Functions in disease

In recent years, much attention has been focused on the pathological role of 5-HT in vascular alterations found in a number of diseases including hypertension, diabetes mellitus, hypothyroidism, inflammatory bowel disease, angiodysplasia, tumors, radiation sequels and allergy. Thus the response of intestinal arteries to 5-HT released by platelets and other sources (e.g. the EC cells), could be implicated in the pathogenesis of non-occlusive intestinal ischaemia and other vascular complications occurring in diabetes as well as other vascular and platelets disorders. Possible mechanisms include loss of function of 5-HT receptors on the damaged endothelium (atherosclerosis), activation of silent 5-HT receptors in certain pathophysiological states (atherosclerosis and diabetes), and increased release of 5-HT from local (EC cells) and circulating stores (platelets) in patients with carcinoids or diabetes. Platelet-derived 5-HT has been implicated in several other vascular diseases, including cerebral vasospasm following subarachnoid haemorrhage, coronary vasospasm, Raynaud's phenomenon, hypertension, as well as in intestinal pathologies, including ischaemic and stress injuries of the gastric (Salim 1992) and duodenal mucosa (Tsukamoto *et al.* 1991). 5-HT may to play an important role in the pathogenesis of mesenteric and intestinal vasospasm through its release by platelets which adhere and aggregate at the site of atherosclerotic lesions (Lopez *et al.* 1989). This hypothesis is supported by the fact that 5-HT-induced decreases in mucosal blood flow play an important role in the pathogenesis of gastric and duodenal ulcers (Tsukamoto *et al.* 1991). Accordingly, vascular insufficiency following 5-HT-induced vasospasm can

cause mesenteric ischaemia and its clinical sequels, such as diarrhea (Ooms and Degryse 1986), abdominal angina and infection (Ende 1958, Siegelman *et al.* 1974), and colonic anastomotic dehiscence (Fawcett *et al.* 1995).

Transport processes

5-HT is an intestinal secretagogue and is one of the mediators responsible for the diarrheal symptoms of several toxins (Hansen and Skadhauge 1995), as well as those in patients with carcinoid syndrome (Ahlman 1985a). Stimulation of the EC cells results in the release of 5-HT, which mediates intestinal secretion through activation of at least the epithelial 5-HT_2 and neuronal 5-HT_3 and 5-HT_4 receptors in the submucosal plexus, including those in the reflex arc. Intracellular mediators include PGE_2, Ca^{2+}, inositol triphosphate (IP_3), NO, and possibly some of the cyclic nucleotides (Hansen 1995). Due to a limited amount of experimental data, the effects of secretory 5-HT on changes in intestinal blood flow can currently only be speculated upon. Observations obtained from experiments with isolated mucosa performed in flux chambers argue against an essential role of blood flow in the secretory effects of 5-HT (Beubler 1995). On the contrary, 5-HT causes a ketanserin- and methysergide-sensitive increase in vascular permeability involving endogenous NO (Maling *et al.* 1974, Fujii *et al.* 1994), suggesting the participation of vascular 5-HT_1-like and 5-HT_2 receptors in the secretory effects of 5-HT.

Disorders

In normal monkeys, 5-HT has little effect on gastric, duodenal or colonic blood flow. In monkeys with arteriosclerosis, however, 5-HT totally blocks blood perfusion to the colon and decreases both gastric and duodenal blood flow (Lopez *et al.* 1989). Likewise, arteriosclerosis and hypertension have been demonstrated to influence blood flow in the small intestine by causing the mesenteric vessels to be hyperresponsive to 5-HT as compared with normals. Such change is caused by activation of 5-HT_2 receptors (Henry and Yokoyama 1980, Cummings *et al.* 1986, Lopez *et al.* 1989, Martin 1994). These findings are disputed, however, by the observation that the response to 5-HT in spontaneously hypertensive rats are unchanged (Pourageaud and Freslon 1995). An explanation for the altered vascular reactivity found in arteriosclerosis could be that atherosclerotic disease of the vessel wall leads to damage of the endothelium and therefore reduces the number of the vasorelaxing 5-HT-like endothelial receptors, thereby favoring the vasoconstrictor activity of the 5-HT_2 receptors. This mechanism (changes in number of certain 5-HT receptors) has been observed in aortic strips from hypercholesterolaemic rabbits (Henry and Yokoyama 1980) and diabetic rats (Sikorski *et al.* 1993), as well as in coronary arteries of arteriosclerotic patients (Chester *et al.* 1990). These phenomena may also be explained by altered 5-HT receptor activity, and thereby neurotransmission, since it has been reported that 5-HT inhibits presynaptic NE neurotransmission in normal, but not in spontaneously hypertensive rats (Su and Uruno 1985, Agrawal and McNeil 1987). Thus, since an increased response to adrenoreceptor agonists and 5-HT are present in various types of atherosclerotic disease, the involvement of α-adrenoreceptors and 5-HT receptors in the development and/or maintenance of hypertension and intestinal ischaemia is possible.

Diabetes mellitus

Studies of 5-HT in experimental diabetes have not fully characterized the changes in 5-HT effects on intestinal blood flow, especially at the level of the resistance vessels of the mesentery. Conflicting results on the effects of exogenous 5-HT have been reported. This could result from the differing types of anaesthesia, the type of diabetic models used (Andersen *et al.* 1992), the age of the animals, the duration of disease (Martin *et al.* 1995), differences in the regions studied (Sarioglu *et al.* 1993, Feletou *et al.* 1994) or differences in the vessels studied (Alsip *et al.* 1992). Preliminary results from our laboratory suggest enhanced mesenteric arteriolar vasoconstrictor responses to exogenous 5-HT in STZ-induced diabetic rats. These results are consistent with the reported increase in responsiveness to 5-HT in diabetic patients. One explanation for the observed changes in vascular responsiveness could be up-regulation of receptor number, affinity, and/or density, due to a decrease in 5-HT levels (Hill and Larkins 1989). Other explanations for the observed changes in diabetic animals are equally as plausible. Platelet content, release and affinity of 5-HT have been demonstrated to be higher in diabetes (Martin *et al.* 1995). The intestinal vasospastic phenomenon responsible for catastrophic complications in some diabetics could potentially result from 5-HT release by platelets and other sources (e.g. the EC cells). Supporting evidence for diabetes-induced abnormalities in the intestinal neurohumoral system is the fact that the density of 5-HT-containing cells in the gut is altered in diabetic animals. The effects seem to be dependent upon the type of diabetic model and the intestinal segment (Portela-Gomes *et al.* 1990, Pinto *et al.* 1995). The number of cells containing 5-HT increases in the jejunum, but decreases in the large intestine of genetically diabetic mice, while the converse pattern is seen in STZ-induced diabetic mice (Pinto *et al.* 1995, Portela-Gomes *et al.* 1990). The effect of diabetes on 5-HT-induced vasoactivity could also depend on the developmental state of diabetes (Agrawal and McNeil 1987), although other studies using the same experimental model (Rosenblum *et al.* 1985, Takiguchi *et al.* 1988) have reported that 5-HT-induced constriction of intestinal vessels is unaltered after 4–12 weeks of STZ-induced diabetes. Finally, 5-HT and its receptors have also been implicated in the regulation of glucose homeostasis (Jamnicky *et al.* 1993).

11. Nitric oxide

Nitric oxide (NO) is a biologically active volatile gas that plays an important role in the physiology and pathophysiology of the gastrointestinal tract. NO is produced by a number of tissues and cell types in the large and small intestine and performs a variety of physiological and pathophysiological regulatory functions, including regulation of vascular tone, modulation of the immune function, neuronal

inhibitory innervation, and mucosal protection (Rodeberg *et al.* 1995).

Endothelium

In the mesenteric vascular bed, endothelial production of NO participates in the control of vascular tone and regulation of mucosal blood flow. Endothelial cells respond to a wide variety of stimuli, including luminal flow, shear stress, neurohumoral agents, and other cellular, molecular, chemical, and physical factors (Ryan *et al.* 1992). The endothelium is regionally differentiated and regulates vascular tone in both conduit and small resistance vessels. Endothelial cells communicate in a paracrine fashion with neighboring smooth muscle cells by their synthesis and release of vasoactive compounds including endothelin, prostaglandins, NO and related nitrosothiols. Endothelial cells also respond to paracrine and hormonal substances with a full complement of membrane receptors linked to ion exchange pumps, synthetic and degradative pathways (Vanner *et al.* 1993, Rodeberg *et al.* 1995). In addition to regulation of vascular tone, endothelial cells of normal vessels prevent spontaneous thrombosis by the production of NO and prostacyclin, both of which inhibit platelet aggregation. When the endothelium is damaged, however, platelets aggregate and release vasoactive substances, including 5-HT, nucleotides, prostaglandins and catecholamines (Rodeberg *et al.* 1995) causing vasoconstriction and thrombosis.

Synthesis, location and release

NO is enzymatically synthesized from L-arginine (Lewis and Smith 1992) by variety of nitric oxide synthases (NOS). Constitutive (cNOS) and inducible (iNOS) types of NO synthases are present in the vascular endothelial cells, smooth muscle cells, platelets, peripheral nerves, enterocytes and other tissues, where they exists as Ca^{2+}-CAM-requiring enzymes (Barry *et al.* 1994, Salzman 1995). In general, the constitutive isoform of NOS (cNOS) is expressed under resting (basal) conditions, whereas inflammatory stimuli are required to induce the production of iNOS (Salzman 1995). In vascular endothelium, NO is produced continuously in small amounts by cNOS and can be stimulated to release additional amounts of NO by a wide variety of stimuli, including shear stress, stretch, and various neurohormones (ADP, thrombin, Ach, bradykinin, gastrin and CCK). These substances act by binding to specific receptors on the luminal surface of the endothelium. Receptor binding causes the opening of Ca^{2+} channels and a rise of intracellular Ca^{2+} (Salzman 1995) through a variety of mechanisms G protein-linked processes (Rodeberg *et al.* 1995). In addition, physical and chemical stimuli including change in shear stress can cause such rise in intracellular calcium. This rise in intracellular calcium activates NOS to produce additional NO from L-arginine. The L-arginine-NO-pathway can be inhibited at several levels (Briejer *et al.* 1992). Specifically, NO production by NOS can be pharmacologically impaired through competitive inhibition by L-arginine analogues.

NO and NOS are found in both endothelial and non-endothelial tissues. Non-endothelial sources of NO in the gut include: intrinsic intestinal tissue (mast cells, epithelium, smooth muscle cells, neural plexuses), and resident and/or infiltrating leukocytes (neutrophils, monocytes). Additionally, luminal sources of NO include luminal reduction of gastric nitrates and denitrification of intestinal contents by commensal anaerobes (Salzman 1995).

Mechanisms

In the intestine NO seems to have three mechanisms of action: vasorelaxant, neurotransmitter and paracrine hormone (Rodeberg *et al.* 1995). In response to agonist-induced Ca^{2+} entry, NOS is activated and NO production is increased. NO can then freely diffuse from the endothelial cells to the vascular lumen, where it is rapidly inactivated following a covalent interaction with haemoglobin (Salzman 1995). Likewise, abluminal diffusion of NO into the vascular smooth muscle occurs. In the vascular smooth muscle cell, NO activates guanylate cyclase (GC), resulting in increased amounts intracellular cGMP and thereby activation of a variety of cGMP-dependent protein kinases as well as dephosphorylation of myosin light chains. This causes relaxation of individual vascular smooth muscle cells, decreased vessel tone and increased luminal blood flow (Salzman 1995, Rodeberg *et al.* 1995). NO is also released from myenteric neurons and together with ATP and VIP act as NANC (non-adrenergic, non-cholinergic) neurotransmitters. NO readily diffuses through presynaptic membranes into the target cells, where it is thought to activate GC, and increase intracellular cGMP levels (Briejer *et al.* 1992, Barry *et al.* 1994).

In health

NO appears to be a major determinant of vascular tone in mesenteric resistance vessels and submucosal arterioles (Salzman 1995). In the intestine, the endothelium regulates blood flow in response to endocrine, neural, metabolic, and physiological stimuli, matching the demands required to accomplish digestion, tissue oxygenation, and to maintain mucosal integrity (Salzman 1995). Not all of the protective actions of NO, however, can be accounted for by increased mucosal perfusion alone. NO may protect the mucosal blood flow by inhibiting neutrophil adhesion to endothelium, blocking platelet adhesion, and preventing mast cell activation (Salzman 1995). However, experimentally engineered mice without functioning cNOS (cNOS knock out mice) have normal tissue perfusion and normal life expectancy (Shesely *et al.* 1996). Various neurohumoral substances (5-HT, eicosanoids, ADP and ATP, bradykinin, histamine, SP, pentagastrin, cGRP, thrombin, vasopressin, and angiotensin II), derived from platelets and other sources, have also been shown to mediate endothelium-dependent arterial relaxation (Ravelic *et al.* 1992, Himmel *et al.* 1993, Salzman 1995, Rodeberg *et al.* 1995). These data suggest that multiple factors working in concert and in opposition affect mucosal blood flow.

NO released from the non-adrenergic, non-cholinergic neurons plays an important role in the gut motility and sphincter relaxation. NO acts as the principal neurotransmitter of the component of the enteric nervous system and as such participates in the coordinated propagation of gut contents and sphincter relaxation. The production of NO depends on nerves

with N-type Ca^{2+} channels. NO acts to inhibit muscle contraction directly by inhibiting the release of excitatory mediators. NO and NO-releasing neurons act as the primary inhibitory determinant of small intestine smooth muscle contraction. NO, released onto small intestine wall muscle inhibits the release of excitatory mediators (Daniel *et al.* 1994). Excessive NO, produced by type 2 NOS (iNOS) in response to a variety of inflammatory conditions, results in an inhibition of smooth muscle activity and result in alteration in sphincter tone and decreased gut motility (Salzman 1995).

NO has been shown to protect the gastrointestinal mucosa through a variety of mechanisms (Salzman 1995). First, as described above, NO causes increase in mucosal blood flow, providing injured or stressed tissues with oxygen and nutrients. NO also may protect the gastrointestinal mucosa by preventing the accumulation of inflammatory neutrophils (Payne and Kubes 1993). Thirdly, NO has been shown to prevent degranulation of intestinal mast cells and the release of inflammatory mediators.

Transport processes

The intestinal epithelium is exposed to NO released from macrophages and mast cells of the lamina propria, enteric nerves, smooth muscle and the endothelium (Wilson *et al.* 1993). There is increasing evidence that NO plays a role in the regulation of salt and water transport (Barry *et al.* 1994, Salzman 1995). In rat distal colon, the NO-donor nitroprusside stimulates anion secretion and inhibits Na^+ and Cl^- absorption. NO may be acting as a neurotransmitter, because this response is TTX-sensitive (Wilson *et al.* 1993). NO could diffuse into the enteric neurons and cause electrolyte secretion directly or indirectly through the release of other secretagogues from various sources including the ENS, macrophages, mast cells and endocrine cells (Wilson *et al.* 1993, Hansen and Skadhauge 1995). Excessive NO production then may be responsible at least in part for abnormal transport processes seen in diarrhea and malabsorption.

In disease

Diminished and excessive amounts of NO plays an important role in the pathogenesis of gastrointestinal disease. Alterations in the production of NO and other vasoactive substances play a role in the changes in blood flow found in gastrointestinal disease. Although barrier function of the mucosa is protected by NO early in the inflammatory response, mucosal hyperaemia occurs as a result of local cytokine release and subsequent induction of iNOS (Salzman 1995). During more advanced stages of intestinal inflammation, greater and more toxic concentrations of NO may be produced. NO in large concentrations can cause disruption of the microorganization of the epithelium, loss of barrier function (Salzman 1995), and with endothelial injury, increase in vascular permeability and platelet deposition. Endothelial cell injury with loss of NO production may also play a role in the changes of vessel function and structure seen in atherosclerotic disease (Rodeberg *et al.* 1995). Impaired NO release may also have important implications for the pathogenesis of mesenteric ischaemia. Inhibition or loss of the ability to release NO by an artery or vein results in inappropriate vasoconstriction that is exacerbated by platelet aggregation and vasoconstrictor release (Connor *et al.* 1989). This vasospasm contributes to mesenteric ischaemic changes found in clinical intestinal angina and bowel necrosis.

Undoubtedly, other vasoactive agents are also involved in the local control of blood flow in these pathological conditions. Altered endothelial levels of vasoactive substances are implicated in the pathophysiology of hypertension. Diabetes seems to induce changes which impair NO-dependent arteriolar relaxation without altering smooth muscle responses (Salzman 1995, Umans and Levi 1995). An altered NO production may explain these findings. Diabetes impairs the vasodilator response to Ach (Wang *et al.* 1996) and augments the response to 5-HT in mesenteric arteries (Taylor *et al.* 1994). Experimental evidence suggests that this impairment is prevented or at least partly reversed by treatment with insulin (Taylor *et al.* 1994, Ponomarenko *et al.* 1997)

12. Conclusions

Over the past 40 years, we have gained a much better understanding of the physiology and pharmacology of mesenteric and intestinal blood flow. Over the past decade we have been able to truly appreciate the intricate interactions of the neurohumoral systems with the basic absorptive, secretory, motor, immunological and humoral properties of the intestine itself. As we gain an understanding of these interactions in the normal environment, we also gain insight into the possible causes of intestinal dysfunction associated with both local and systemic disease. New treatment protocols for intestinal complications of systemic disease are constantly being proposed and tested, and each of them is based on our new understanding of the involved systems. Because much of intestinal function is related to blood flow, new therapies are likely to be targeted at the modulators of mesenteric and intestinal blood flow. In this regard, 5-HT_1-like agonists and NO inhibitors as well as modulators of receptor activity are likely to influence the future therapeutic options available for the treatment of intestinal dysfunction, and may form the platform upon which methods of prevention of intestinal dysfunction and pathology complicating systemic diseases are designed.

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