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Neuronal and endothelium-derived mediators in the modulation of the gastric microcirculation: integrity in the balance

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Introduction

J.H. Gaddum began his long and most productive career in pharmacology in 1925, when he joined the Wellcome Research Laboratories in Beckenham, under the guidance of J.W. Trevan. It was here that he was exposed to problems in bioassay, the generation of quantitative data and its subsequent mathematical and statistical evaluation. Three years later, when he joined Sir Henry Dale at the National Institute of Medical Research in Hampstead, he retained his enthusiasm for such analytical pharmacology, while developing interests in many other areas including peripheral neuronal regulatory mechanisms and the production and actions of novel local mediators.

Of particular relevance to the theme of this review was his detection in 1931, along with U.S. von Euler, of an unknown depressor substance in extracts of various tissues, particularly the brain and intestine (Euler & Gaddum, 1931). This factor, which they called substance P, for no better reason than this was the letter on the label used on the original standard preparations of the extracts, was more fully characterized in collaboration with H.O. Schild (Gaddum & Schild, 1934). These studies by Gaddum thus initiated the field of pharmacological study on sensory neuropeptides. Only more recently, however, has there been appropriately detailed research on the actions and role of this diverse group of peptides with potent biological properties. As will be discussed later, the sensory neuropeptide, calcitonin gene-related peptide (CGRP), plays an important role in the regulation of gastric mucosal integrity, interacting with other local mediators.

During his time at the College of the Pharmaceutical Society in Bloomsbury, Gaddum also developed and refined techniques to determine the release of neurotransmitters into the perfused vasculature of isolated tissues (Gaddum *et al.*, 1939). It is therefore fitting, in view of his interest in neuronal modulatory processes and the release of local mediators, to describe in this Thirteenth Gaddum Memorial Lecture, our current understanding of the mechanisms that regulate microvascular blood flow in the gastric mucosa. It will become apparent that local neuronal and endothelium-derived factors that affect the microcirculation have a crucial influence on the processes that allow the gastric mucosa to

fulfil its secretory function and to resist the continual onslaught of aggressive agents including physiological concentrations of luminal acid, pepsin and bile, and the ingestion of irritants such as ethanol and spicy foods.

Importance of the gastric microcirculation

The stomach, like all organs in the body, requires an adequate blood flow to maintain its structural and cellular integrity and to conduct its physiological functions. Indeed, because of the extensive metabolic requirements for the elaboration and secretion of hydrogen ions from the parietal cell, gastric mucosal blood flow can be a prime factor in the regulation of gastric secretion. Early studies using aminopyrine or aniline clearance techniques in the dog, cat and rat suggested a close correlation between acid output and mucosal blood flow during stimulation with histamine or pentagastrin (Jacobson *et al.*, 1966; Harper *et al.*, 1968; Main & Whittle, 1973; Kauffman, 1982; Guth & Leung 1987).

It is now known, however, that the clearance of weak bases is not an accurate index of mucosal blood flow under secretory conditions. Using the well-validated hydrogen gas clearance technique in more recent studies, increases in mucosal blood flow were also observed in the rat during stimulation of acid secretion by pentagastrin infusion (Murakami *et al.*, 1982; Leung *et al.*, 1984; Pique *et al.*, 1992a). Furthermore, using both direct visualization of rat submucosal vessels and laser Doppler flowmetry, pentagastrin-stimulated acid secretion was associated with an increase in microvascular perfusion, although a direct correlation between these parameters was not observed (Holm-Ruttili & Berglinde, 1986). However, when hydrogen gas clearance was used to determine rat mucosal blood flow, a significant correlation with acid output during pentagastrin stimulation was obtained by others (Pique *et al.*, 1988).

These discrepancies in the degree of mucosal hyperaemia may reflect not only differences in the techniques used to determine blood flow, but also the possibility that different rates of oxygen extraction from the mucosal blood supply may operate under different experimental conditions (Perry *et al.*, 1983). Hence the need for an equivalent degree of local vasodilatation to support active acid secretion under different conditions may not be essential. Apart from the possible local generation of vasoactive waste products of parietal cell stimulation, these local vasodilator responses following

Figure 1 Role of the gastric mucosal microcirculation in removing hydrogen ions that have back-diffused into the mucosal tissue. (A) Under physiological conditions, blood flow will wash out any such hydrogen ions. (B) Following an acid load or topical damage to the, apical mucosa by irritants, the excess intra-mucosal hydrogen ions are removed by an augmented blood flow. (C) Limitation of this blood flow response by vasoconstrictors and ischaemia leads to intramucosal accumulation of acid, provoking haemorrhage and tissue necrosis.

secretory stimulation may reflect the release of vasoactive substances into the microcirculation. Early studies had suggested the involvement of local prostaglandins (Main & Whittle, 1973; 1975) and more recently, adenosine (Gerber & Guth, 1989), but the pharmacological evidence for these substances in secretory vasodilatation cannot now be considered compelling. Thus, the identity of this local vasodilator mediator has remained under investigation up to the present time (Walder *et al.*, 1990; Pique *et al.*, 1992a).

Protective role of mucosal blood flow

An adequate blood flow is also essential for gastric mucosal tissue to withstand the challenge of both endogenous and exogenous aggressors. Thus, blood flow, by providing tissue oxygenation and nutrient delivery, will act to enhance the mucosal defence mechanisms, while an inappropriate reduction in blood flow will lead to mucosal injury or make the tissue more susceptible to damage (Figure 1). Indeed, local ischaemia has consistently been proposed over the past 100 years as a mechanism underlying various forms of gastric damage, ranging from acute gastric erosions to chronic peptic ulceration. The mechanisms by which mucosal blood flow is regulated thus assume significant importance in considering most aspects of the physiology and pathophysiology of the stomach.

An important factor in the relationship between mucosal blood flow, tissue integrity and mucosal defence is the maintenance of intramucosal acid-base neutrality (Kivilaakso *et al.*, 1978). When the mucosal epithelial cell layer is undamaged, only minimal levels of the hydrogen ions that have been actively secreted into the lumen, will diffuse back into the gastric tissue. Under conditions where acidity in the lumen is pH 2 or above, the secretion of bicarbonate and mucus from surface epithelial cells will form an unstirred layer capable of adequately buffering such acid back-diffusion (Allen & Garner, 1980; Flemstrom, 1987). Any remaining hydrogen ions that gain entry into the tissue will be buffered by the bicarbonate derived from the blood or from the alkaline tide generated by parietal cells activity, as well as being removed by microcirculation (Figure 1).

Limitation of mucosal blood flow will, therefore, allow the intramucosal accumulation of hydrogen ions by reducing both their neutralisation and their washout. Should the intramucosal pH drop significantly, then cellular damage will rapidly ensue, either as a direct consequence of the acidic environment or by the subsequent release of tissue-damaging mediators (Figure 1). This situation is exacerbated by high intragastric acid concentrations (an acid load) or following luminal application of weak irritants such as bile salts, ethanol or salicylates, the so-called 'barrier breakers', which disrupt the continuity of surface epithelial cells and enhance back diffusion of acid into mucosal tissue. These latter events may not necessarily lead to deep necrotic damage in the mucosa if there is adequate blood flow to allow buffering and washout of the hydrogen ions (Figure 1). Indeed, many studies show that a hyperaemic response can be provoked by such irritation, perhaps a reflection of a pathophysiological defence mechanism that involves the release of local vasodilator mediators and neuronal pathways (Ritchie, 1975; Whittle, 1977; Druggeman *et al.*, 1979; Holzer *et al.*, 1991a,b; Lippe & Holzer, 1992).

In support of this concept of a protective hyperaemia, are the findings that when the mucosa of rat or dog is exposed to an acidified solution of the mild irritant, taurocholate, limited macroscopic damage can be observed. However, following a reduction in blood flow or concurrent administration of a vasoconstrictor agent such as vasopressin or noradrenaline, deep necrotic damage rapidly occurs (Richie, 1975; Whittle, 1977; 1983). The vasoconstrictor metabolite of arachidonic acid, thromboxane A_2 or its stable epoxy-methano mimetic also potentiates the mucosal injury by acidified bile salts or ethanol in the dog and rat gastric mucosa (Whittle *et al.*,

1981; Esplugues & Whittle, 1988a; Whittle & Esplugues, 1989). In addition, local or systemic infusion of the low molecular weight phospholipid, platelet activating factor (PAF), which reduces mucosal blood flow and causes haemorrhagic erosions in the gastric mucosa (Rosam *et al.*, 1986; Whittle *et al.*, 1986), greatly potentiates mucosal damage brought about by low intraluminal concentrations of ethanol or bile salts (Wallace & Whittle, 1986; Esplugues & Whittle, 1988b). Thus, any limitation of microvascular blood flow can greatly enhance the susceptibility of the mucosa to disruption by topical irritants, as well as itself provoking mucosal injury.

Microvascular ischaemia and injury

Gastric damage which results from inadequate vascular perfusion may reflect the period of relative anoxia and hence disturbances in cell metabolism and integrity. However, such damage may also involve the subsequent local release of tissue-damaging mediators, among which oxygen-derived free radicals are likely candidates (Parks *et al.*, 1982). These highly-labile and reactive moieties have the capacity to disrupt cell membranes by lipid peroxidation of membrane constituents and destroying the interstitial matrix following degradation of collagen and hyaluronic acid (Del Maestro *et al.*, 1980). This cellular injury can induce the further release of endogenous cytotoxic agents, including lysosomal enzymes. Thus, tissue damage may not only occur during the anoxic episode, but appear as subsequent reperfusion injury. Such damage in the stomach following hypovolemic shock is reduced by agents that interfere with free-radical generation, the xanthine oxidase inhibitor, allopurinol, or by free-radical scavengers (Itoh & Guth, 1985).

More direct evidence for the tissue-damaging actions of such oxygen metabolites in gastric tissue has come from studies where local infusion of a superoxide generating system into the rat coeliac artery caused gastric mucosal bleeding, as assessed by the leakage of radiolabelled erythrocytes (Wadhwa & Perry, 1987). Furthermore, local intra-arterial infusion of either hydrogen peroxide, which can form hydroxyl radicals, or a superoxide generating system, induced macroscopically apparent damage in the mucosa, that was attenuated by concurrent administration of catalase or superoxide dismutase, respectively (Esplugues & Whittle, 1989a). In these studies, the initial site of injury was considered to be the microvascular endothelium.

Endothelial cells can regulate mucosal integrity by providing a local diffusion barrier and by the release of factors that interact to modulate tone of the microvascular smooth muscle or affect the processes of cellular adhesion. The role of endothelium-derived mediators in the physiological modulation of mucosal blood flow and integrity in the stomach will therefore be considered further. The influence of neuronally derived vasoactive mediators in the process underlying damage and protection of the gastric mucosa will, however, be initially described.

Neuronal mediators in the gastric mucosa

Noradrenaline and neuropeptide Y

Effects of noradrenaline on gastric blood flow In early studies in cat, intravenous infusion of adrenaline or splanchnic sympathetic stimulation was shown to reduce gastric blood flow (Thompson & Vane, 1953; Reed *et al.*, 1971). Other early studies in the dog demonstrated a reduction in gastric blood flow following parenteral administration of adrenaline or noradrenaline, as determined both by a clearance technique and by electromagnetic flowprobes (Cowley & Code, 1970; Zinner *et al.*, 1975). Likewise, noradrenaline infusion reduced both acid secretion and blood flow in the dog during stimulation with histamine, using venous outflow determinations

(Cumming *et al.*, 1963), electromagnetic flowmeters (Nicoloff *et al.*, 1964) and a clearance technique (Jacobson, 1970). This reduction in blood flow by noradrenaline appeared to limit the secretory process rather than to be secondary to the reduction in functional vasodilator drive. Both total gastric and mucosal blood flow were reduced by intravenous administration of noradrenaline in the dog stomach (Varro *et al.*, 1978), while in the gastric chamber preparation of dog stomach *in situ*, noradrenaline increased vascular perfusion pressure, indicating vasoconstriction (Kauffman & Whittle, 1982). Similarly, in the isolated perfused vasculature of the rat and rabbit stomach, vasoconstrictor actions of noradrenaline were also demonstrated (Salvati & Whittle, 1981).

The vasoconstrictor activity of noradrenaline has been observed directly using *in vivo* microscopy techniques in the rat following topical application to the exposed gastric submucosal microvessels (Guth & Smith, 1975a,b; Oren-Wolman & Guth, 1984). More recently, locally infused noradrenaline was shown to induce a dose-dependent reduction in rat gastric mucosal blood flow determined by laser Doppler flowmetry (Tepperman & Whittle, 1991). Submucosal injection of adrenaline, or its application directly to the exposed rat gastric submucosal vessels also reduced laser Doppler flow (Chung *et al.*, 1990; Leung, 1992).

Effects of neuropeptide Y and related peptides In addition to the classical neurotransmitters, sympathetic neurones can synthesize biologically active peptides including the 36 residue vasoconstrictor, neuropeptide Y (NPY; Lundberg & Tatemoto, 1982). NPY has been identified in postganglionic nerves supplying arteries and veins, co-existing with noradrenaline, both being released by sympathetic stimulation (Sundler *et al.*, 1983; Lundberg *et al.*, 1984). In the gastrointestinal tract, NPY-like immunoreactivity has been found in sympathetic nerves associated with blood vessels (Lee *et al.*, 1985; Ekblad *et al.*, 1985) and in enteric neurones originating from the myenteric and submucosal plexus (Furness *et al.*, 1983; Lee *et al.*, 1985; Su *et al.*, 1987). In addition, peptides that are structurally similar to NPY, including pancreatic polypeptide (PP) and polypeptide YY (PYY), are also found in the gut, although primarily in endocrine cells (Lundberg *et al.*, 1982; El-Salhy *et al.*, 1983).

Investigations on the possible physiological role of NPY in the gastro-intestinal tract have included studies on its effects on non-vascular smooth muscle tone (Allen *et al.*, 1987; Hellstrom, 1987; Holzer *et al.*, 1987) and intestinal ion and water transport (Saria & Beubler, 1985). Systemic administration of NPY stimulates duodenal alkaline secretion, through vagal non-cholinergic neuronal mechanisms (Pascual *et al.*, 1993). The release of immunoreactive-NPY from the rat isolated stomach can be provoked by acetylcholine, probably through nicotinic-receptor stimulation of intrinsic ganglia (McIntosh *et al.*, 1992), while intracerebroventricular administration of NPY stimulated both gastric acid and pepsin secretion through a vagally mediated process (Matsuda *et al.*, 1991). By contrast, injection of NPY into the rat hypothalamic paraventricular nucleus inhibited acid secretion through a mechanism involving the suppression of vagal cholinergic tone following activation of α_2 -adrenoceptors, but did not affect gastric mucosal blood flow (Humphreys *et al.*, 1992).

NPY induces vasoconstriction in the vascular beds of the spleen and large and small intestine (Lundberg *et al.*, 1982; Hellstrom, 1987; Westfall *et al.*, 1987; MacLean & Hiley, 1990; Sheikh, 1991). In addition, local intra-arterial infusion of NPY has been shown to reduce rat gastric mucosal blood flow, as determined by laser Doppler flowmetry (Tepperman & Whittle, 1991). Although the structurally related peptide, PP and PYY were effective vasoconstrictors in the cat submandibular gland (Lundberg & Tatemoto, 1982), PYY produced responses as potent but more variable than those to NPY, while PP was minimally active in the rat gastric microcirculation (Tepperman & Whittle, 1991). By contrast, in the

pithed rat, intravenous infusion of NPY elevated blood pressure and induced vasoconstriction in the mesenteric bed, but did not increase vascular resistance in the stomach, as estimated by radiolabelled microspheres (MacLean & Hiley, 1990). The inability of the α_1 -adrenoceptor antagonist, prazosin, to overcome the effect of NPY in the gastric circulation (Tepperman & Whittle, 1991) is consistent with other findings in the mesenteric circulation of the rat (Westfall *et al.*, 1987), indicating that NPY, like noradrenaline, affects the gastric microcirculation by actions directly on gastric vascular smooth muscle, but through activation of a distinct receptor type.

There is a close anatomical relationship between NPY and noradrenergic neurones in the rat stomach and after treatment with 6-hydroxydopamine to deplete noradrenergic nerves, NPY-containing fibres around blood vessels also disappear (Wang *et al.*, 1987). Although NPY may modulate the actions of noradrenaline by acting either prejunctionally to suppress noradrenaline release or postjunctionally to potentiate the noradrenaline response (Hakanson *et al.*, 1986), this did not occur in the rat gastric microcirculation (Tepperman & Whittle, 1991). Furthermore, unlike its actions on the gastro-epiploic artery, NPY did not potentiate the vasoconstrictor effects of noradrenaline in segments of gastro-epiploic vein (Wahlestedt *et al.*, 1985), nor suppress noradrenaline release (Ekblad *et al.*, 1984). Thus, the nature of the interactions between NPY and noradrenaline in the gut appear to be organ or tissue specific.

Effects of noradrenaline and neuropeptide Y on gastric mucosal integrity Intravenous infusion of noradrenaline for 3 h in the rat potentiated mucosal damage induced by intragastric perfusion of bile salts (Whittle, 1983). In other studies, close-arterial infusion of NPY or noradrenaline for only 10 min, resulted in dose-dependent haemorrhagic damage to the gastric mucosa in the presence of physiological concentrations of luminal acid (Tepperman & Whittle, 1991). As with the microcirculatory actions, the injurious effects of noradrenaline, but not NPY, were inhibited by α_1 -adrenoceptor blockade. The substantial vasoconstriction induced by both NPY and noradrenaline is likely to underlie these mucosal injurious actions, which re-inforces the importance of adequate microvascular perfusion even in the absence of local irritants.

It is feasible that elevated levels of noradrenaline following sympathetic stimulation, that could occur under situations of stress, may be associated with any accompanying mucosal injury. In addition, plasma NPY levels are increased after haemorrhagic shock in both rats and pigs (Morris *et al.*, 1987; Lundberg *et al.*, 1987). Since haemorrhagic shock is usually followed by gastric damage and bleeding (Guth & Leung, 1987), the release of NPY may play some role in this type of gastric injury.

Neuronal mechanisms in mucosal vasodilatation and protection

Cholinergic and non-cholinergic processes The direct effects of cholinergic stimulation on the gastric microcirculation may be obscured by concurrent induction of acid secretion and hence an accompanying vasodilatation. However, an increase in mucosal blood flow following vagal stimulation has been observed to precede the secretion of acid, indicating a direct vasodilator action (Martinson, 1965; Guth & Smith, 1975b). The gastric vasodilatation induced by vagal stimulation can be blocked by hexamethonium, and reduced but not abolished by atropine in doses sufficient to abolish the response to acetylcholine (Martinson, 1965; Yano *et al.*, 1983; Kitagawa *et al.*, 1987). This could suggest the release of a vasodilator mediator other than acetylcholine acting on non-muscarinic sites following vagal stimulation.

Local non-adrenergic, non-cholinergic (NANC) neuronal

processes within the gastric mucosa modulate its ability to withstand noxious challenge. Thus, local infusion through the left gastric artery of the neurotoxin, tetrodotoxin, which did not itself induce gastric mucosal injury, substantially potentiated the haemorrhagic damage following local administration of PAF (Esplagues *et al.*, 1989). By contrast, pretreatment with atropine or the adrenoceptor antagonists, phentolamine and propranolol did not augment such mucosal injury, suggesting the involvement of a NANC neuronal pathway (Esplagues & Whittle, 1989b). In further studies, local infusion of tetrodotoxin also potentiated the mucosal injury induced by intra-arterial administration of a vasoconstrictor thromboxane mimetic (Whittle & Esplagues, 1990), as well as that brought about by intragastric application of acidified ethanol (Holzer *et al.*, 1991 a,b), again indicating the involvement of a local neuronal mechanism in the regulation of mucosal integrity.

Sensory neurones The release of vasodilator neuropeptides from afferent sensory neurones, through a local reflex, has been suggested to be a protective mechanism in the gastric mucosa. Much of the early evidence for such a role of sensory neurones came from studies with capsaicin pretreatment (Szolcsanyi & Bartho, 1981; Holzer & Sametz, 1986), a pungent extract of red peppers, (8-methyl-N-vanillyl-6-nonenamide), which can deplete primary afferent sensory neurones of their neuropeptide content and cause their functional ablation (Sternini *et al.*, 1987; Green & Dockray, 1988; Holzer, 1991). Thus, capsaicin pretreatment, which itself did not injure the mucosa, enhanced mucosal damage following a number of pro-ulcerogenic procedures including acid distension and pylorus ligation as well as challenge with indomethacin, ethanol and PAF (Szolcsanyi & Bartho, 1981; Holzer & Sametz, 1986; Esplagues *et al.*, 1989; Esplagues & Whittle, 1990).

Another approach to the pharmacological modulation of sensory neuronal activity in the stomach has been the use of opioids. Since the early description of the pro-ulcerogenic potential of morphine (Selye, 1935), administration of opioids, including morphine, has been demonstrated to augment gastric mucosal injury in a variety of experimental models. Thus, relatively low doses of morphine augmented the gastric damage induced by pylorus ligation (Ho *et al.*, 1984) indomethacin administration (Gyires *et al.*, 1985), or restraint stress (Morley *et al.*, 1982; Till *et al.*, 1988). The mechanisms underlying this action of morphine have not been clear and may not be seen in all experimental models. Studies on vascular permeability and analgesia have however suggested that opioids can modulate the activity of primary afferent neurones (Ferreira & Nakamura, 1979; Bartho & Szolcsanyi, 1981; Smith & Buchan, 1984; Lembeck & Donnerer, 1985; Russell *et al.*, 1987). It is therefore feasible that opioids could affect the ability of the mucosa to withstand mucosal injury by exerting a peripheral action on local afferent neuronal mechanisms (Esplagues *et al.*, 1989). Indeed, morphine can augment the gastric mucosal damage and detrimental blood flow changes induced by local infusion of PAF or following challenge with ethanol to a comparable degree as capsaicin pretreatment (Esplagues *et al.*, 1989; Pique *et al.*, 1990; Esplagues & Whittle, 1990). These effects are exerted on peripheral μ -opioid receptors for they were shared by N-methyl morphine that does not penetrate the brain, and were inhibited by administration of naloxone or by the quaternary antagonist, N-methyl nalorphine (Esplagues *et al.*, 1989; Esplagues & Whittle, 1990).

Involvement of calcitonin gene-related peptide The predominant neuropeptide localized by immuno-histochemical techniques in capsaicin-sensitive neurones in the rat stomach is CGRP (Green & Dockray, 1988) and such neurones are found in close proximity to the submucosal microvasculature (Ekblad *et al.*, 1985; Su *et al.*, 1987; Sternini *et al.*, 1987). This neuropeptide occurs principally in the form of α -CGRP

in the sensory neurones innervating gastro-intestinal tissue (Mulderry *et al.*, 1988). Intragastric instillation of capsaicin to stimulate mucosal sensory neurones, induces acute gastric mucosal vasodilatation (Lippe *et al.*, 1989b; Holzer *et al.*, 1990; 1991b; Whittle *et al.*, 1992b). This acute hyperaemia involves the release of CGRP from spinal sensory neurones innervating the gastric mucosa, since it was inhibited by concurrent infusion of the CGRP-receptor antagonist CGRP₈₋₃₇, as well as by selective ablation of pericoeliac nerves by prior application of capsaicin (Li *et al.*, 1991).

Activation of sensory neurones following acid back diffusion induced by intragastric application of acid-ethanol is also considered to be involved in the associated protective increase in gastric mucosal blood flow (Holzer *et al.*, 1991 a,b). This mucosal hyperaemia was not altered by bilateral vagotomy but was abolished by removal of the coeliac-mesenteric ganglionic complex or transection of the greater splanchnic nerves (Holzer & Lippe, 1992). It was also blocked by tetrodotoxin and by morphine administration, as well as by capsaicin desensitization and by local infusion of the antagonist CGRP₈₋₃₇, indicating the involvement of CGRP (Holzer *et al.*, 1991a,b).

Acute mucosal application of capsaicin protected against mucosal damage provoked by ethanol and acid load (Holzer & Lippe, 1988; Holzer *et al.*, 1990; Li *et al.*, 1992). Furthermore, subcutaneous or intra-arterial administration of rat α -CGRP inhibited the gastric injury induced by intragastric instillation of aspirin or ethanol (Maggi *et al.*, 1987; Lippe *et al.*, 1989a). Local infusion of CGRP also prevented the vascular and haemorrhagic injury induced by close-intra-arterial infusion of endothelin-1 (Whittle & Lopez-Belmonte, 1991).

Intravenous infusion of antisecretory doses of rat α -CGRP did not alter rat mucosal blood flow (Leung *et al.*, 1987), whereas intravenous administration of higher doses of CGRP increased blood flow in the rat and rabbit stomach (Dipette *et al.*, 1987; Bauerfeind *et al.*, 1989). Moreover, close-intra-arterial infusion of rat α -CGRP increased resting mucosal blood flow, as determined by hydrogen-gas clearance (Holzer & Guth, 1991; Li *et al.*, 1991) and laser Doppler flowmetry (Whittle *et al.*, 1992b). By contrast, local infusion of the sensory neuropeptides, substance P or neurokinin A, did not elevate resting mucosal blood flow (Holzer & Guth, 1991). Thus, CGRP has the profile of actions compatible with its proposed role as an endogenous vasoactive mediator involved in the regulation of gastric blood flow and integrity.

Endothelium-derived factors – the endothelins

Vascular endothelial cells synthesize a 21-residue peptide known as endothelin-1 (ET-1) which can exert vasoconstrictor actions both *in vitro* and *in vivo* (Yanagisawa *et al.*, 1988; Inoue *et al.*, 1989). Intravenous administration of ET-1 can, however, decrease, increase or exhibit a biphasic action on rat systemic arterial blood pressure depending on the dose used, the level of anaesthesia or the existing blood pressure (Wright & Fozard, 1988; De Nucci *et al.*, 1988; Whittle *et al.*, 1989a,b; Gardiner *et al.*, 1989). Moreover, ET-1 induces the release of prostacyclin and nitric oxide from the vascular endothelium which accounts for the vasodilatation seen in the isolated mesenteric vascular bed (De Nucci *et al.*, 1988). Endothelin-1-like immunoreactivity has been demonstrated in the rat gastric mucosa in both antral and corpus regions (Matsumoto *et al.*, 1989; Takahashi *et al.*, 1990). Much lower levels of immunoreactivity to endothelin-3 (ET-3), which differs from ET-1 by alterations in six amino acids (Inoue *et al.*, 1989) have also been found in the rat stomach (Matsumoto *et al.*, 1989).

Local intra-arterial infusion of picomole quantities of ET-1 induces substantial gastric mucosal vasocongestion and haemorrhagic injury in the rat (Whittle & Esplugues, 1988;

Whittle *et al.*, 1989a,b; Whittle & Lopez-Belmonte, 1991). Furthermore, intravenous infusion of ET-1 augments mucosal damage induced by intragastric instillation of ethanol or acid (Wallace *et al.*, 1989a; MacNaughton *et al.*, 1989b; Peskar *et al.*, 1992). ET-1 and ET-3 were equipotent in inducing rat gastric haemorrhage following intravenous infusion (Wallace *et al.*, 1989b). Local intra-arterial infusion of high doses of ET-3 induced vascular lesions in the rat gastric mucosa that involved the venules and capillaries and potentiated the vascular injury induced by acid and ethanol (Morales *et al.*, 1992). Furthermore, an anti-ET-3 serum reduced the extent of mucosal damage induced by intragastric ethanol, suggesting that acute release of endogenous endothelins are involved in such mucosal injury (Morales *et al.*, 1992).

Microcirculatory actions of endothelins

The vasoconstrictor actions of ET-1 have been demonstrated in the perfused vasculature of the rat isolated stomach (Wallace *et al.*, 1989a; Peskar *et al.*, 1992). Furthermore, the vasoconstriction that follows intravascular infusion of ethanol in the rabbit isolated perfused stomach was accompanied by the release of ET-1 into the perfusate, and was inhibited by an anti-ET-1 antibody (Masuda *et al.*, 1992). ET-3 also induced small increases in canine gastric vascular resistance following close-arterial infusion (Wood *et al.*, 1992).

More recently, close intra-arterial infusion of higher doses of ET-1 to the rat stomach *in vivo* has been demonstrated to induce an initial vasodilatation followed by a sustained vasoconstrictor response (Lopez-Belmonte & Whittle, 1993). This vasoconstriction may reflect actions on the ET_A receptor, located on vascular smooth muscle (Arai *et al.*, 1990; Sakurai *et al.*, 1990). By contrast, local infusion of low doses of ET-1 induced only a sustained vasodilatation (Lopez-Belmonte & Whittle, 1993). Whether this reflects a direct vasodilator response through an uncharacterized mechanism, or is the consequence of the release of local vasoactive mediators (De Nucci *et al.*, 1988), perhaps through ET_B receptors located on the vascular endothelium (Masaki *et al.*, 1991) or following endothelial perturbation, will require further evaluation.

Interactions of endothelins in the gastric mucosa

The injurious action of ET-1 on the rat gastric mucosa following its intra-arterial infusion was not inhibited by pretreatment with atropine nor α - and β -adrenoceptor antagonists, indicating no involvement of adrenergic or cholinergic mechanisms (Whittle & Esplugues, 1988). In addition, pretreatment with a 5-lipoxygenase inhibitor had no effect on the mucosal injury, showing that local release of vasoconstrictor leukotrienes (Whittle *et al.*, 1985) did not contribute to the mucosal damage with ET-1 (Whittle & Esplugues, 1988). An increase in PAF formation by the gastric mucosa has been reported to be stimulated by ET-1, along with activation of the fibrinolytic system, while a PAF receptor antagonist attenuated mucosal injury induced ET-1 (Kurose *et al.*, 1992). By contrast, others have found no protection by a PAF receptor antagonist against ET-1 induced mucosal damage (Wallace *et al.*, 1989a). Whether the release of PAF or fibrinolytic activation are secondary responses, perhaps as a consequence of endothelial injury by ET-1, therefore requires consideration.

Inhibition of cyclo-oxygenase by indomethacin augmented the mucosal damage induced by intravenous ET-1 in combination with an acid load or ethanol (MacNaughton *et al.*, 1989b; Wallace *et al.*, 1989a), or following its local infusion (Whittle & Lopez-Belmonte, 1991). Furthermore, capsaicin-pretreatment or morphine administration substantially elevated the mucosal damage provoked by ET-1, while close-arterial infusion of CGRP inhibited the damage (Whittle & Lopez-Belmonte, 1991). These findings suggest an interaction

between the vascular effects of ET-1 and those of sensory neuropeptides and prostaglandins (Figure 2). This certainly would be applicable under conditions where ET-1 induces mucosal damage through its vasoconstrictor actions, which would be thus offset by the vasodilator actions of endogenous CGRP or prostacyclin released under physiological conditions or in response to ET-1.

Such interactions occur in other microvascular beds, for intravenous infusion of CGRP can reverse the vasoconstrictor action of ET-1 on the rat internal carotid vasculature (Gardiner *et al.*, 1990a), and local CGRP administration reduces the vasoconstriction induced by ET-1 in rabbit skin (Brain *et al.*, 1988). Furthermore, it is of interest that ET-1 can be localized using *in situ* hybridization and immunostaining techniques in both sensory and motor neurones and dorsal root ganglia from human spinal tissue, and can co-exist with CGRP and substance P (Giaid *et al.*, 1989). In addition, ET-1 induces depolarization of rat spinal neurones *in vitro*, which can be inhibited by a substance P antagonist (Yoshizawa *et al.*, 1989). These findings may indicate an interaction between ET-1 and such neuropeptides in the modulation of sensory neuronal function.

More recently, however, it has been found that local infusion of low doses of ET-1, that only induce vasodilatation in the gastric microcirculation, can provoke substantial mucosal injury. This hyperaemia may be the consequence of initial damage to the endothelium, since concurrent local infusion of vasodilator doses of CGRP that inhibited mucosal damage paradoxically suppressed the mucosal hyperaemia (Lopez-Belmonte & Whittle, 1993). It would therefore appear that the interactions between ET-1 and CGRP in the mucosal microcirculation are not simply due to opposing vasoactive properties, but may also reflect more-complex events, perhaps involving the release of local mediators and actions on the continuity of the vascular endothelium.

Prostacyclin

The synthesis of the labile vasodilator cyclo-oxygenase product, prostacyclin (PGI_2), from the fatty acid precursor, arachidonic acid, was originally identified in endothelial cells

Figure 2 Interactions of the endothelium-derived mediators, endothelin-1 (ET-1) and prostacyclin (PGI_2) and the sensory neuropeptide, calcitonin-gene-related peptide (CGRP) in the mucosal microcirculation. Attenuation of the synthesis or release of the vasodilator mediators, prostacyclin and CGRP respectively, augments ET-1 induced mucosal injury.

(Moncada *et al.*, 1976; Moncada & Vane, 1979). Its formation can be detected in gastric mucosal tissue using bioassay or radioimmunoassay techniques and is inhibited both *in vitro* and *in vivo* by non-steroid anti-inflammatory drugs such as aspirin and indomethacin (Whittle *et al.*, 1980; Whittle & Vane, 1987). Such actions on prostanoid synthesis are likely to contribute to the complex multifactorial mechanisms that underlie the gastric mucosal injury induced by these agents (Whittle *et al.*, 1980; Whittle, 1992).

Prostacyclin can inhibit gastric acid secretion in a number of experimental preparations (Whittle *et al.*, 1978; Gerkens *et al.*, 1978; Kauffman *et al.*, 1979a; Konturek *et al.*, 1980; Whittle, 1981; Shea-Donohue *et al.*, 1982) and can stimulate the secretion of bicarbonate, a luminal protective factor (Whittle *et al.*, 1984). Prostacyclin and its more-stable analogues, like other prostanoids particularly of the E series (Robert *et al.*, 1979), exert potent protective actions against gastric mucosal damage in a number of experimental models (Whittle *et al.*, 1978; Whittle & Boughton-Smith, 1979; Kauffman *et al.*, 1979b; Konturek *et al.*, 1981; 1984).

Effects on the gastric microcirculation

Under resting conditions, intravenous infusion of prostacyclin elevated basal mucosal blood flow (estimated using clearance techniques) in the rat and dog (Whittle *et al.*, 1978; Konturek *et al.*, 1980; Walus *et al.*, 1980). Using radio-labelled microspheres in the dog, infusion of low doses of prostacyclin into the left atrium or directly into the gastric artery increased blood flow in fundic and antral mucosa, (Gerber & Nies, 1982; Einzig *et al.*, 1980), as also found following intravenous infusion in the pig (Gaskill *et al.*, 1982). During partial inhibition of gastric acid secretion induced by prostacyclin in the rat, mucosal blood flow, estimated by a clearance technique, was elevated, whereas at higher doses mucosal blood flow fell, presumably as a consequence of reduced functional vasodilator drive following the substantial secretory inhibition (Whittle *et al.*, 1978). In the dog, acid inhibition by prostacyclin was also accompanied by a fall in mucosal blood flow, but the ratio of these parameters remained unaltered indicating that the vascular changes were secondary to the secretory events (Kauffman *et al.*, 1979a).

Under basal conditions, prostacyclin was a potent vasodilator in the blood-perfused canine gastric circulation *in situ* following close-arterial infusion or bolus injection (Gerkens *et al.*, 1978; Walus *et al.*, 1980; Kauffman & Whittle, 1982) and in the gastric circulation of the isolated stomach of rat and rabbit (Salvati & Whittle, 1981). The stable analogues, $6\beta\text{-PGI}_1$ and the 16-phenoxy derivative of prostacyclin, increased the ratio of blood flow to acid output during intravenous infusion of antisecretory doses in the conscious dog (Kauffman *et al.*, 1979b). Both $6\beta\text{-PGI}_1$ and carbacyclin also reduced gastric vascular resistance in the dog stomach following intra-arterial injection (Kauffman & Whittle, 1982). Whereas the breakdown product 6-oxo-PGF $_{1\alpha}$ had little effect on gastric mucosal blood flow or vascular resistance, the putative prostacyclin metabolite 6-oxo-PGE $_1$ was some three to four times less active than prostacyclin as a vasodilator in the dog gastric circulation *in situ* and in the perfused gastric vasculature of rat and rabbit *in vitro* (Kauffman & Whittle, 1982; Salvati & Whittle, 1981).

Increases in local blood flow by prostacyclin would be beneficial in maintaining the functional integrity of gastric tissue and defence mechanisms, especially under conditions of relative ischaemia in the mucosa (Whittle & Vane, 1987). Although net vasodilatation would contribute to the protective actions of the prostaglandins, more local changes in microcirculation may be of greater significance. Studies on mucosal injury and ulceration have pointed to the microvascular endothelium as an initial site of damage resulting from application of the irritant, ethanol (Guth *et al.*, 1984; Szabo *et al.*, 1985; Oates & Hakkinen, 1988). Following challenge

with irritant agents, vascular engorgement can be observed, which is correlated with the stasis of blood flow within the microcirculation. Such microvascular stasis can lead to areas of deep necrosis, a predominant histological characteristic of this damage. Pretreatment with anti-ulcer prostaglandins prevents this microcirculatory stasis and the subsequent development of necrotic lesions (Guth *et al.*, 1984; Pihan *et al.*, 1986). Protection of the gastric microvasculature, either by direct actions on endothelial cell integrity or by the prevention of the release of vasoconstrictor or cytotoxic mediators (Whittle *et al.*, 1985; Boughton-Smith & Whittle, 1988) may, therefore, be an important mechanism that underlies the so-called cytoprotective actions of prostacyclin and other prostanoids.

Nitric oxide

Endothelial cells also release another highly labile humoral vasodilator substance, originally known as endothelium-derived relaxing factor (EDRF), that mediates the vascular relaxation induced by agents such as acetylcholine and bradykinin (Furchgott & Zawadzki 1980; Furchgott, 1984). The release of an endothelium-derived factor in the gastric microcirculation was considered to underlie the vasodilatation induced by local administration of acetylcholine and by vagal stimulation (Kitagawa *et al.*, 1987). It is now known that nitric oxide (NO), accounts for the biological properties of EDRF (Palmer *et al.*, 1987; 1988a; Khan & Furchgott, 1987; Ignarro *et al.*, 1987; Kelm *et al.*, 1988).

NO synthase generates NO from the terminal guanidino nitrogen atoms of L-arginine, through a process where molecular oxygen is also incorporated (Palmer *et al.*, 1988b; Palmer & Moncada, 1989; Leone *et al.*, 1991). The constitutively expressed NO synthase enzyme is calcium-, calmodulin- and NADPH-dependent (Moncada *et al.*, 1991), and has been demonstrated in rat whole stomach and gastric mucosa (Salter *et al.*, 1991; Whittle *et al.*, 1992a). In a study on the cellular distribution of NO synthase in the rat gastric mucosa, epithelial cells separated by elutriation exhibited high levels of constitutive enzyme activity (Brown *et al.*, 1992b). This localization of NO synthase may reflect a non-vascular role for NO in the modulation of mucus or bicarbonate secretion from these cells, which, as in vascular tissue (Gruetter *et al.*, 1979; Moncada *et al.*, 1991) may involve activation of guanylate cyclase and elevation of cyclic GMP. Indeed, application of NO donors or dibutyl cyclic GMP to the rat gastric mucosa *in vivo* can increase mucus thickness, and these agents can stimulate mucus output from gastric epithelial cells *in vitro* (Brown *et al.*, 1992a; 1993).

The formation of NO is inhibited by L-arginine analogues such as N^G-monomethyl-L-arginine (L-NMMA), as demonstrated in studies *in vitro* on vascular tissue (Palmer *et al.*, 1988b; Rees *et al.*, 1989b). In the rabbit, rat and guinea-pig, L-NMMA increased systemic arterial blood pressure, an effect reversed by L-arginine but not the enantiomer, D-arginine, suggesting that endogenous NO biosynthesis from L-arginine can modulate resting vascular tone *in vivo* (Rees *et al.*, 1989a; Whittle *et al.*, 1989a; Aisaka *et al.*, 1989; Gardiner *et al.*, 1990b). Local infusion of L-NMMA also increased peripheral vascular tone in man (Vallance *et al.*, 1989), while studies with other L-arginine analogues that inhibit NO biosynthesis, such as the more potent N^G-nitro-L-arginine methyl ester (L-NAME) have confirmed the importance of NO in the regulation of systemic arterial blood pressure (Rees *et al.*, 1990).

Effect on the gastric microcirculation

Intravenous administration of L-NMMA dose-dependently reduced resting gastric mucosal blood flow, determined by hydrogen gas clearance (Pique *et al.*, 1989). These effects were not shared by D-NMMA, while L-arginine but not

D-arginine reversed these actions. Subsequently, using laser Doppler flowmetry and hydrogen gas clearance, both L-NMMA and L-NAME have been shown to reduce resting mucosal blood flow, confirming the involvement of NO in the regulation of microvascular blood flow (Tepperman & Whittle, 1992; Lippe & Holzer, 1992).

The mucosal hyperaemia induced by intravenous infusion of pentagastrin was attenuated by concurrent infusion of L-NMMA or L-NAME (Walder *et al.*, 1990; Pique *et al.*, 1992a). Pretreatment with low doses of L-NMMA reduced the elevation of mucosal blood flow but had no significant effect on the plateau rates of acid secretion induced by pentagastrin, thus indicating an effect on the microcirculation independent of secretory modulation (Pique *et al.*, 1992a). These findings indicate that NO is a prime mediator of the blood flow changes associated with acid secretion, whereas inhibition of NO biosynthesis appeared to have no direct acute effect on the stimulation of acid secretion. Recent studies, have, however demonstrated the involvement of NO in the process by which acute administration of endotoxin or the cytokine, interleukin 1 α can inhibit acid secretion, perhaps through modulation of a neuronal pathway (Martinez-Cuesta *et al.*, 1992; Esplugues *et al.*, 1993a,b).

NO is involved in the gastric mucosal hyperaemia that follows acute normovolemic anaemia induced by haemodilution (Panes *et al.*, 1992). Furthermore, it is possible that excessive NO synthesis contributes to the hyperdynamic splanchnic circulation, including the gastric vasodilatation, observed in portal hypertensive and cirrhotic rats (Pizcueta *et al.*, 1992a,b; Pique *et al.*, 1992b). NO also mediates the gastric mucosal hyperaemia that is associated with renal failure (Quintero & Guth, 1992a,b).

Interactions of NO in the gastric mucosa

Interactions in the microcirculation

Interactions between NO and CGRP in the microvasculature are evident, since chronic capsaicin treatment greatly augments the fall in mucosal blood flow induced by L-NMMA and L-NAME (Tepperman & Whittle, 1992). Furthermore, the acute increase in mucosal blood flow following instillation of capsaicin directly into the gastric lumen, is abolished by concurrent administration of L-NAME (Whittle *et al.*, 1992b). Both NO and CGRP also appear to be involved in the processes underlying the mucosal vasodilatation following sensory nerve activation through acid back-diffusion into the mucosal tissue (Lippe & Holzer, 1992).

These observations may reflect physiological interactions, perhaps of a synergistic nature, between NO and sensory neuropeptides in the modulation of microvascular tone, both mediators acting directly to relax the vascular smooth muscle (Figure 3). There could also be a partial dependence upon endothelial NO for the vascular relaxation induced by CGRP, since CGRP is an endothelium-dependent vasodilator in some vascular beds (Brain *et al.*, 1985). For this to occur in the microcirculation, it would be necessary for CGRP to diffuse from neurones surrounding the vascular smooth muscle to reach the endothelium. However, the mucosal vasodilatation induced by CGRP is only partially attenuated by NO synthase inhibitors (Whittle *et al.*, 1992). Alternatively, like its involvement in NANC neurally-evoked relaxation of the stomach musculature (Li & Rand, 1990; Boeckxstaens *et al.*, 1991; Desai *et al.*, 1991), NO may be involved in local vascular neuromodulator processes. NO synthase has been detected in neuronal cell bodies and nerve fibres in the myenteric plexus using immunohistochemical techniques (Bredt *et al.*, 1990) and it is possible that NO released from sensory neurones in the stomach could directly relax vascular smooth muscle. One stage in the events leading to neuronal NO biosynthesis and release could be the influx or intracellular mobilization of calcium, as it has been shown

that brain NO synthase can be activated by submicromolar concentrations of calcium (Knowles *et al.*, 1989). NO could also be involved in modulating the activity of sensory neurones or the release of neuropeptides (Figure 3).

Interactions modulating mucosal integrity

In studies on the interactions of local mediators with endogenous NO, administration of L-NMMA induced acute gastric mucosal injury in rats pretreated with indomethacin, using doses of either agent that themselves did not provoke acute mucosal injury. L-NMMA also induced extensive haemorrhagic mucosal injury in rats chronically pretreated with capsaicin. Furthermore, L-NMMA induced deep haemorrhagic necrosis involving virtually all of the mucosal area in rats pretreated concurrently with both indomethacin and capsaicin (Whittle *et al.*, 1990). Such findings indicate a critical interaction between endogenous NO, sensory neuropeptides and prostanoids, all of which appear to subserve a modulator function in the regulation of gastric mucosal integrity (Figure 4). These mediators, which have distinct biochemical origins, may not only exert local vasodilator actions on the microcirculation essential for adequate microvascular blood flow under physiological conditions, but may act to enhance or preserve endothelial cells function and continuity, especially under conditions of challenge.

Capsaicin pretreatment or morphine administration attenuates the protective properties of PGE₂ and its 16, 16-dimethyl analogue against acute gastric challenge, suggesting a permissive role for sensory neuropeptides in the mechanisms of protection by prostanoids (Esplugues & Whittle, 1991; Esplugues *et al.*, 1992). Furthermore, the mucosal injury induced by indomethacin is augmented in capsaicin-pretreated rats, again reflecting interactions between endogenous protective sensory neuropeptides and prostanoids (Holzer & Sametz, 1986; Whittle *et al.*, 1990). However, the mucosal protective actions of a PGE₂ analogue against ethanol-induced injury do not appear to depend on endogenous NO, since they were not inhibited by N^G-nitro-L-arginine (Konturek *et al.*, 1992). By contrast, the protection against ethanol-induced injury by

Figure 3 Possible neuronal and vascular interactions between nitric oxide (NO) and calcitonin-gene-related peptide (CGRP) in the modulation of the gastric microcirculation. These mediators may interact not only to affect directly the tone of vascular smooth muscle, but NO may be involved in neuromodulation and the release of the neuropeptide.

Figure 4 Local mediators, nitric oxide (NO), prostacyclin (PGI₂) and calcitonin gene-related peptide (CGRP) within the gastric mucosal microcirculation contribute through local vasoactive effects and actions on the endothelium, to the regulation of mucosal integrity.

acute intraluminal instillation of capsaicin, is attenuated by N^G-nitro-L-arginine (Peskar *et al.*, 1991), again implying interactions between endogenous neuropeptides and NO in the mechanisms subserving mucosal protection.

NO and the enzymes that form prostacyclin are highly susceptible to attack by free radicals (Moncada & Vane, 1979; Gryglewski *et al.*, 1986; Rubanyi & Vanhoutte, 1986) and interference with the actions of these mediators may be involved in the microvascular injury seen following local release of free radicals. It is pertinent that neutrophils play a role in the genesis of gastric injury, such as that induced by PAF, ischaemia, ethanol or non-steroid anti-inflammatory agents (Smith *et al.*, 1987; Kviety *et al.*, 1990; Kubes *et al.*, 1990; Wallace *et al.*, 1991). Thus, free radical release from such leukocytes following adhesion to the endothelium may contribute to the microvascular injury by affecting the formation and stability of these endothelial cell mediators. Furthermore, inhibition of NO biosynthesis can promote neutrophil adherence and permeability changes in microvascular endothelium, as demonstrated in the cat mesentery (Kubes *et al.*, 1991; Kubes & Granger, 1992). A reduction in NO formation or its inactivation by free radicals in both endothelial cells and neutrophils (McCall *et al.*, 1989) may therefore serve to initiate or to amplify endothelial injury. Such effects may contribute to the mechanism by which L-NMMA can elevate the intestinal vascular permeability changes seen in acute endotoxin shock that follows the rapid release of PAF and thromboxane (Hutcheson *et al.*, 1990; Boughton-Smith *et al.*, 1990) and indicates a role for constitutive NO in modulating this acute inflammatory response.

Protection and damage by NO in the microvasculature

The release of NO from nitrovasodilator agents either following metabolic transformation as with glyceryl trinitrate and isoamyl nitrite, or spontaneously as with nitroprusside, is responsible for their ability to activate guanylate cyclase, elevate cyclic GMP and relax vascular smooth muscle (Gruetter *et al.*, 1979; Ignarro *et al.*, 1981; Feelisch & Noack, 1987; Feelisch, 1991). Following intragastric application, these nitrovasodilators protect against acute haemorrhagic mucosal injury induced by topical irritants and by intravenous infusion of ET-1 (Kitagawa *et al.*, 1990; MacNaughton *et al.*, 1989a). The nitrosothiol, S-nitroso-N-acetyl-penicillamine (SNAP), which spontaneously liberates NO (Ignarro *et al.*, 1981) also protects against acute microvascular injury in the stomach and small intestine induced by endotoxin or PAF (Boughton-Smith *et al.*, 1990; 1992a).

Local administration of glyceryl trinitrate or SNAP in low doses protects the gastric mucosa from damage induced by intra-arterial infusion of ET-1 (Lopez-Belmonte *et al.*, 1993). Such actions of the NO donors could reflect vascular interactions between ET-1 and the locally generated NO in the mucosal microcirculation, on both vascular tone and endothelial integrity. By contrast, mucosal injury was observed after infusion of nitroprusside or higher doses of SNAP, which may indicate cytotoxic actions of high levels of NO on the microvascular endothelium (Lopez-Belmonte *et al.*, 1993). Indeed, the excessive production of NO by an inducible NO synthase in endothelial cells is considered to underlie the reduction in viability of these cells in culture following exposure over a 48 h period to endotoxin and the cytokine, interferon- α (Palmer *et al.*, 1992), while induction of NO synthesis is also considered to be involved in damage to adenocarcinoma cells (O'Connor & Moncada, 1991). High concentrations of exogenous NO can suppress prostacyclin synthesis by endothelial cells, which may also reflect cytotoxicity (Doni *et al.*, 1988). In addition, the substantial synthesis of NO by the immunologically-induced NO synthase in macrophages accounts for the cytotoxic actions against tumour cells (Hibbs *et al.*, 1987; 1988; Marletta *et al.*, 1988; Drapier *et al.*, 1988).

Excessive NO production and the induction of NO synthase has been implicated in the cardiovascular crisis and collapse following endotoxaemia in animals and patients (Kilbourn *et al.*, 1990; Thiemermann & Vane, 1990; Fleming *et al.*, 1991; Nava *et al.*, 1991; Petros *et al.*, 1991; Wright *et al.*, 1992). Furthermore, the increase in microvascular permeability, an index of endothelial injury, seen in the rat small and large intestine four to six hours after endotoxin administration is correlated with the corticosteroid-sensitive induction of a calcium-independent NO synthase over this period (Boughton-Smith *et al.*, 1992b). Induction of NO synthase is also associated with inflammation and injury observed in a model of colitis (Boughton-Smith *et al.*, 1992c) and is seen in the inflamed colonic mucosa of patients with ulcerative colitis (Boughton-Smith *et al.*, 1992d). It is feasible that local high concentrations of NO may form tissue destructive species such as hydroxyl moieties derived from the peroxynitrite radical (Beckman *et al.*, 1990) which contribute to the endothelial injury in the microvasculature, leading to mucosal necrosis and ulceration.

A balance of vasoactive factors for mucosal integrity?

The regulation of microvascular tone and integrity is thus of critical importance for the conduct of the physiological responses of the stomach and in the prevention of mucosal injury by both endogenous and exogenous aggressors. Local neuronal activity could provoke the inappropriate release of vasoconstrictor mediators such as noradrenaline or NPY, while stimulation of sensory neurones release the neuropeptide CGRP, involved in protective vasodilatation. Pathological events that enhance the neuronal release of these vasoconstrictor agents, or that depress the release of CGRP and possibly other protective sensory neuropeptides would thus be expected to lead to mucosal injury.

As described, the endothelium is a rich source of local vasoactive mediators capable of modulating tone and integrity in the microcirculation. Thus, the local release of the vasoconstrictor ET-1 may be an initial event in some forms of mucosal injury, or be released as a consequence of endothelial injury, thus augmenting and perpetuating the original insult. Knowledge of the regulatory processes underlying endothelin biosynthesis, and the mechanism of endothelin release in the microcirculation which may only occur under

non-physiological conditions, may thus give some indication to its possible involvement in ulcer disease.

Prostacyclin and NO appear to play protective roles within the gastric microcirculation. However, it is apparent that NO may be involved in both physiological and pathological events in the gastric mucosa. Thus, endogenous NO plays an important role in the modulation of mucosal blood flow under resting and stimulated conditions, and has a key interactive role in the regulation of mucosal integrity. However, an excess, unregulated, liberation of NO has also ulcerogenic potential. The factors that regulate the synthesis and release of endogenous NO by the neuronal and endothelial constitutive enzymes, likely to be involved in physiological processes, and by inducible enzymes that may underlie certain pathological events, will thus be of importance to the understanding of tissue integrity. Indeed, it is possible that local toxins produced by the bacterial organism, *Helicobacter pylori* that is implicated in peptic ulceration, could induce an NO synthase in epithelial or other mucosal cells and hence local excess NO or its cytotoxic metabolites may contribute to the mechanisms underlying the associated cellular injury.

A balance between the release or actions of these vasoconstrictor and vasodilator mediators in the microcirculation could be involved in the physiological control of mucosal blood flow, providing a mechanism for the rapid vascular response to the functional needs of the mucosa. The systemic release of vasoactive factors may also influence the tone of the microvasculature. However, it is feasible that the complex interactions between these opposing mediators on vascular tone may only operate to modulate blood flow under conditions of challenge. Furthermore, under physiological conditions, blood flow may be regulated predominantly by only one local vasodilator mediator, with the pharmacological evidence from the use of selective inhibitors being strongest for NO. Interference with NO biosynthesis alone under acute pathological conditions may not, however, be detrimental, since prostacyclin or CGRP can subserve this vasodilator role when necessary. Indeed, inhibition of constitutively formed NO only leads to extensive acute mucosal injury when the synthesis or release of prostanoids and CGRP are concurrently depressed. Alterations in the balance between these vasodilator mediators may thus be implicated in the pathogenesis of peptic ulceration.

A further important physiological role of these mediators may be to preserve endothelial integrity, which in turn could help provide for adequate microvascular blood flow. Indeed, earlier studies had implicated the prevention of microcirculatory injury and stasis as an underlying mechanism of mucosal protection by prostanoids (Guth *et al.*, 1984; Pihan *et al.*, 1986). Moreover, interactions of these diverse local mediators at the level of the endothelial barrier, rather than solely on vascular smooth muscle tone are suggested by analysis of their microcirculatory changes and current actions on mucosal integrity (Whittle & Tepperman, 1991; Tepperman & Whittle, 1992; Lopez-Belmonte & Whittle, 1993). Under conditions of challenge, these endogenous endothelial processes may assume strategic importance in the mechanisms of resistance to mucosal injury. Whereas selective inhibition of either mediator alone does not produce acute mucosal injury, concurrent pharmacological interference with more than one such mediator induces widespread tissue damage (Whittle *et al.*, 1990). An understanding of the properties of these agents that confer endothelial resilience would therefore offer novel approaches to the attenuation of cell injury in the stomach, which could be relevant for other tissues and organs. The interplay between endogenous mediators in the microvasculature in the control of tissue integrity is thus likely to extend beyond influences on local blood flow to encompass even more fundamental regulatory processes at the cellular level.

References

- AISAKA, K., GROSS, S.S., GRIFFITH, O.W. & LEVI, R. (1989). N^G -methylarginine, an inhibitor of endothelium-derived nitric oxide synthesis is a potent pressor agent in the guinea-pig: does nitric oxide regulate blood pressure *in vivo*? *Biochem. Biophys. Res. Commun.*, **160**, 881–886.
- ALLEN, A. & GARNER, A. (1980). Mucus and bicarbonate secretion in the stomach and their possible role in mucosal protection. *Gut*, **21**, 249–262.
- ALLEN, J.M., HUGHES, J. & BLOOM, S. (1987). Presence, distribution and pharmacological effects of neuropeptide Y in mammalian gastrointestinal tract. *Dig. Dis. Sci.*, **32**, 506–512.
- ARAI, H., HORI, S., ARAMORI, I., OHKUBO, H. & NAKANISHI, S. (1990). Cloning and expression of a cDNA encoding an endothelin receptor. *Nature*, **348**, 730–732.
- BARTHO, L. & SZOLCANYI, J. (1981). Opiate agonists inhibit neurogenic plasma extravasation in the rat. *Eur. J. Pharmacol.*, **73**, 101–104.
- BAUERFEIND, P., HOF, R., HOF, A., CUCALA, M., SIEFRIST, S., VON RITTER, C., FISCHER, J.A. & BLUM, A.L. (1989). Effects of hCGRP I and II on gastric blood flow and acid secretion in anesthetized rabbits. *Am. J. Physiol.*, **256**, G145–G149.
- BECKMAN, J.S., BECKMAN, T.W., CHEN, J., MARSHALL, P.A. & FREEMAN, B.A. (1990). Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA*, **87**, 1620–1624.
- BOECKXSTAENS, G.E., PELCKMANS, P.A., BOGERS, J.J., BULT, H., DE MAN, J.G., OSSTERBOSCH, L., HERMAN, A.G. & VAN MAERCKE, Y.M. (1991). Release of nitric oxide upon stimulation of non-adrenergic non-cholinergic nerves in the rat gastric fundus. *J. Pharmacol. Exp. Ther.*, **256**, 441–447.
- BOUGHTON-SMITH, N.K. & WHITTLE, B.J.R. (1988). Inhibition by 16,16-dimethyl PGE_2 of ethanol-induced gastric mucosal damage and leukotriene B_4 and C_4 formation. *Prostaglandins*, **35**, 945–957.
- BOUGHTON-SMITH, N.K., DEAKIN, A.M. & WHITTLE, B.J.R. (1992a). Actions of nitric oxide on the acute gastrointestinal damage induced by PAF in the rat. *Agents and Actions*, (Special Conference Issue) ed. Velo, G.P., Whittle, B.J.R., Bray, M.A. C3–9.
- BOUGHTON-SMITH, N.K., EVANS, S.M., WHITTLE, B.J.R. & MONCADA, S. (1992b). Dexamethasone inhibits endotoxin-induced vascular permeability and nitric oxide synthase in the rat intestine. *Br. J. Pharmacol.*, **107**, 79P.
- BOUGHTON-SMITH, N.K., EVANS, S.M., WHITTLE, B.J.R. & MONCADA, S. (1992c). Induction of colonic nitric oxide synthase in a rat model of colitis. *Gastroenterology*, **102**, A598.
- BOUGHTON-SMITH, N.K., EVANS, S.M., COLE, A.T., WHITTLE, B.J.R. & HAWKEY, C.J. (1992d). Increased nitric oxide synthase activity in inflamed colon from ulcerative colitis patients. *Gut*, **33**, S11.
- BOUGHTON-SMITH, N.K., HUTCHESON, I., DEAKIN, A.M., WHITTLE, B.J.R. & MONCADA, S. (1990). Protective effect of S-nitroso-N-acetyl-pencillamine in endotoxin-induced acute intestinal damage in the rat. *Eur. J. Pharmacol.*, **191**, 485–488.
- BRAIN, S.D., TIPPINS, J.R. & WILLIAMS, T.J. (1988). Endothelin induces potent microvascular constriction. *Br. J. Pharmacol.*, **95**, 1005–1007.
- BRAIN, S.D., WILLIAMS, T.J., TIPPINS, J.R., MORRIS, H.R. & MACINTYRE, I. (1985). Calcitonin gene-related peptide is a potent vasodilator. *Nature*, **313**, 54–56.
- BREDT, D.S., HWANG, P.M. & SNYDER, S.H. (1990). Localization of nitric oxide synthase indicating a neural role of nitric oxide. *Nature*, **347**, 768–770.
- BROWN, J.F., HANSON, P.J. & WHITTLE, B.J.R. (1992a). Nitric oxide donors increase mucus gel thickness in rat stomach. *Eur. J. Pharmacol.*, **223**, 103–104.
- BROWN, J.F., HANSON, P.J. & WHITTLE, B.J.R. (1993). Nitric oxide donors stimulate rat gastric mucus release and epithelial cyclic GMP. *Gastroenterology*, **104**, 47A.
- BROWN, J.F., TEPPERMAN, B.L., HANSON, P.J., WHITTLE, B.J.R. & MONCADA, S. (1992b). Differential distribution of nitric oxide synthase between cell fractions isolated from the rat gastric mucosa. *Biochem. Biophys. Res. Commun.*, **184**, 680–685.
- CHUNG, S.C.S., LEUNG, J.W.C. & LEUNG, F.W. (1990). Effect of submucosal epinephrine injection on local gastric blood flow: a study using laser Doppler flowmetry and reflectance spectrophotometry. *Dig. Dis. Sci.*, **35**, 1008–1011.
- COWLEY, D.J. & CODE, C.F. (1970). Effects of secretory inhibitors on mucosal blood flow in non-secreting stomach of conscious dogs. *Am. J. Physiol.*, **218**, 270–274.
- CUMMING, J.D., HAIGH, A.L., HARRIS, E.H.L. & NUTT, M.E. (1963). A study of gastric secretion and blood flow in the anaesthetized dog. *J. Physiol.*, **168**, 219–233.
- DEL MAESTRO, R., THAW, H.H., BJORK, J., PLANKER, M. & ARFORS, K.-E. (1980). Free radicals as mediators of tissue injury. *Acta. Physiol. Scand.*, **492**, 43–57.
- DE NUCCI, G., THOMAS, R., D'ORLEANS-JUSTE, P., ANTUNES, E., WALDER, C., WARNER, T.D. & VANE, J.R. (1988). Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc. Natl. Acad. Sci. USA*, **85**, 9797–9800.
- DESAI, K.M., SESSA W.C. & VANE, J.R. (1991). Involvement of nitric oxide in the reflex relaxation of the stomach to accommodate food or fluid. *Nature*, **351**, 477–479.
- DIPETTE, D.J., SCHWARZENBERGER, K., KERR, N. & HOLLAND, O.B. (1987). Systemic and regional hemodynamic effects of calcitonin gene-related peptide. *Hypertension*, **9**, Suppl. III, 142–146.
- DONI, M.G., WHITTLE, B.J.R., PALMER, R.M.J. & MONCADA, S. (1988). Actions of nitric oxide on the release of prostacyclin from bovine endothelial cells in culture. *Eur. J. Pharmacol.*, **151**, 19–25.
- DRAPIER, J.-C. & HIBBS, J.B. Jr. (1988). Differentiation of murine macrophages to express non-specific cytotoxicity for tumour cells results in L-arginine-dependent inhibition of mitochondrial iron-sulfur enzymes in the macrophage effector cells. *J. Immunol.*, **140**, 2829–2838.
- DRUGGEMAN, T.W., WOOD, J.G. & DAVENPORT, H.W. (1979). Local control of blood flow in the dog's stomach. Vasodilatation caused by acid back diffusion following topical application of salicylic acid. *Gastroenterology*, **77**, 736–744.
- EINZIG, S., RAO, G.H.R. & WHITE J.G. (1980). Differential sensitivity of regional vascular beds in the dog to low-dose prostacyclin infusion. *Can. J. Physiol. Pharmacol.*, **58**, 940–946.
- EKBLAD, E., EDVINSSON, L., WAHLESTEDT, C., UDDMAN, R., HAKANSON, R. & SUNDLER, F. (1984). Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibres. *Regul. Pept.*, **8**, 225–235.
- EKBLAD, E., EKELUND, M., GRAFFNER, H., HAKANSON, R. & SUNDLER, M. (1985). Peptide-containing nerve fibres in the stomach wall of rat and mouse. *Gastroenterology*, **89**, 73–85.
- EL-SALHY, M., WILANDER, E., JUNTU-BERGREN, L. & GRIMELIUS, L. (1983). The distribution and ontogeny of polypeptide YY (PYY) and pancreatic polypeptide (PP)-immunoreactive cells in the gastrointestinal tract of rat. *Histochemistry*, **78**, 53–60.
- ESPLUGUES, J.V., BARRACHINA, M.D., CALATAYUD, S., PIQUE, J.M. & WHITTLE, B.J.R. (1993a). Nitric oxide mediates the inhibition by interleukin- 1β of pentagastrin-stimulated rat gastric acid secretion. *Br. J. Pharmacol.*, **108**, 9–10.
- ESPLUGUES, J.V., MARTINEZ-CUESTA, M.A., BARRACHINA, M.D., MORENO, L., PIQUE, J.M. & WHITTLE, B.J.R. (1993b). Reversal by tetrodotoxin and nitric oxide-inhibition of the reduction by endotoxin of pentagastrin-stimulated gastric acid secretion. *Gastroenterology*, **104**, A882.
- ESPLUGUES, J.V. & WHITTLE, B.J.R. (1988a). Close-arterial administration of the thromboxane mimetic U-46619 induces damage to the rat gastric mucosa. *Prostaglandins*, **35**, 137–148.
- ESPLUGUES, J.V. & WHITTLE, B.J.R. (1988b). Gastric mucosal damage induced by local intra-arterial administration of Paf in the rat. *Br. J. Pharmacol.*, **93**, 222–228.
- ESPLUGUES, J.V. & WHITTLE, B.J.R. (1989a). Gastric damage following local intra-arterial administration of reactive oxygen metabolites in the rat. *Br. J. Pharmacol.*, **97**, 1085–1092.
- ESPLUGUES, J.V. & WHITTLE, B.J.R. (1989b). Mechanisms contributing to gastric motility changes induced by PAF-acether and endotoxin in rats. *Am. J. Physiol.*, **256**, G275–G282.
- ESPLUGUES, J.V. & WHITTLE, B.J.R. (1990). Morphine potentiation of ethanol-induced gastric mucosal damage in the rat. Role of local sensory afferent neurons. *Gastroenterology*, **98**, 82–89.
- ESPLUGUES, J.V. & WHITTLE, B.J.R. (1991). Peripheral opioid-sensitive mechanisms of mucosal injury and protection. In *Mechanisms of Injury, Protection and Repair of the Upper Gastrointestinal Tract*. ed. Garner, A. & O'Brien, P.E. pp. 115–125. Chichester: John Wiley & Sons.
- ESPLUGUES, J.V., WHITTLE, B.J.R. & MONCADA, S. (1989). Local opioid-sensitive afferent sensory neurones in the modulation of gastric damage induced by Paf. *Br. J. Pharmacol.*, **97**, 579–585.

- ESPLUGUES, J.V., WHITTLE, B.J.R. & MONCADA, S. (1992). Modulation by opioids and by afferent sensory neurones of prostanoid protection of the rat gastric mucosa. *Br. J. Pharmacol.*, **106**, 846–852.
- EULER, U.S. VON & GADDUM, J.H. (1931). An unidentified depressor substance in certain tissue extracts. *J. Physiol.*, **72**, 74–87.
- FEELISCH, M. (1991). Correlation between nitric oxide formation during degradation of organic nitrates and activation of guanylate cyclase. *Eur. J. Pharmacol.*, **139**, 19–30.
- FEELISCH, M. & NOACK, E.A. (1987). The biochemical pathways of nitric oxide formation from nitrovasodilators: appropriate choice of exogenous NO donors and aspects of preparation and handling of aqueous NO solutions. *J. Cardiovasc. Pharmacol.*, **17**, (Suppl. 3), S25–S33.
- FERREIRA, S.H. & NAKAMURA, M. (1979). Prostaglandin hyperalgesia. The peripheral analgesic activity of morphine, enkephalins and opioid antagonists. *Prostaglandins*, **18**, 191–200.
- FLEMING, I., JULOU-SCHAEFER, G., GRAY, G.A., PARRAT, J.R. & STOCKLET, J.-C. (1991). Evidence that an L-arginine/nitric oxide dependent elevation of tissue cyclic GMP content is involved in depression of vascular reactivity by endotoxin. *Br. J. Pharmacol.*, **103**, 1047–1052.
- FLEMSTROM, G. (1987). Gastric and duodenal mucosal bicarbonate secretion. In: *Physiology of the Gastrointestinal Tract*. ed. Johnson, L.R. Second Edition, Vol. II pp. 1011–1029, New York: Raven Press.
- FURCHGOTT, R.F. (1984). The role of endothelium in the responses of vascular smooth muscle to drugs. *Annu. Rev. Pharmacol. Toxicol.*, **24**, 175–197.
- FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373–376.
- FURNESS, V.B., COSTA, M., EMSON, P.C., HAKANSON, R., MOHIM-ZADEH, E., SUNDLER, F., TAYLOR, I.L. & CHANCE, R.E. (1983). Distribution, pathways and reactions to drug treatment of nerves with neuropeptide Y and pancreatic polypeptide like immunoreactivity in the guinea-pig digestive tract. *Cell Tissue Res.*, **234**, 71–92.
- GADDUM, J.H. & SCHILD, H. (1934). Depressor substances in extracts of intestine. *J. Physiol.*, **83**, 1–14.
- GADDUM, J.H., JANG, C.S. & KWIAKOWSKI, H. (1939). The effect on the intestine of the substance liberated by adrenergic nerves in a rabbit's ear. *J. Physiol.*, **96**, 104–108.
- GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1989). Regional haemodynamic effects of endothelin-1 in conscious, unrestrained, Wistar rats. *J. Cardiovasc. Pharmacol.*, **13**, Suppl. 5, S202–204.
- GARDINER, S.M., COMPTON, M., BENNETT, T., KEMP, P.A. & NEY, U. (1990a). Synergistic internal carotid vasodilator effects of human α -calcitonin gene related peptide and nimodipine in conscious rats. *Br. J. Pharmacol.*, **99**, 830–834.
- GARDINER, S.M., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1990b). Control of regional blood flow by endothelium-derived nitric oxide. *Hypertension*, **15**, 486–492.
- GASKILL, H.V., SIRINEK, K.R. & LEVINE, B.A. (1982). Prostacyclin-mediated gastric cytoprotection is dependent on mucosal blood flow. *Surgery*, **92**, 220–225.
- GERBER, J.G. & GUTH, P.H. (1989). Role of adenosine in the gastric blood flow response to pentagastrin in the rat. *J. Pharmacol. Exp. Ther.*, **251**, 550–556.
- GERBER, J.G. & NIES, A.S. (1982). Canine gastric mucosal vasodilation with prostaglandins and histamine analogs. *Dig. Dis. Sci.*, **27**, 870–874.
- GERKENS, J.F., GERBER, J.G., SHAND, D.G. & BRANCH, R.A. (1978). Effect of PGI₂, PGE₂ and 6-keto-PGF_{1 α} on canine gastric blood flow and acid secretion. *Prostaglandins*, **16**, 815–823.
- GIAID, A., GIBSON, S.J., IBRAHIM, N.B.N., LEGON, S., BLOOM, S.R., YANAGISAWA, M., MASAKI, T., VARNDELL, I.M. & POLAK, J.M. (1989). Endothelin 1, an endothelium-derived peptide is expressed in neurons of the human spinal cord and dorsal root ganglia. *Proc. Natl. Acad. Sci. USA*, **86**, 7634–7638.
- GREEN, T. & DOCKRAY, G.J. (1988). Characterisation of the peptidergic afferent innervation of the stomach in rat, mouse and guinea-pig. *Neuroscience*, **25**, 181–193.
- GRUETTER, C.A., BARRY, B.K., MCNAMARA, D.B., GRUETTER, D.Y., KADOWITZ, P.J. & IGNARRO, L.J. (1979). Relaxation of bovine coronary artery and activation of coronary guanylate cyclase by nitric oxide, nitroprusside and a carcinogenic nitrosoamine. *J. Cyclic. Nucl. Res.*, **5**, 211–224.
- GRYGLEWSKI, R.J., PALMER, R.M.J. & MONCADA, S. (1986). Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature*, **320**, 454–456.
- GUTH, P.H. & LEUNG, F.W. (1987). Physiology of the gastric circulation. In *Physiology of the Gastrointestinal Tract*. Second edition. Vol. II. ed. Johnson, L.R. pp. 1031–1053. New York: Raven Press.
- GUTH, P.H. & SMITH, E. (1975a). Escape from vasoconstriction in the gastric microcirculation. *Am. J. Physiol.*, **228**, 1893–1895.
- GUTH, P.H. & SMITH, E. (1975b). Neural control of gastric mucosal blood flow in the rat. *Gastroenterology*, **69**, 935–940.
- GUTH, P.H., PAULSEN, G. & NAGATA, H. (1984). Histologic and microcirculatory changes in alcohol-induced gastric lesions in the rat. Effect of prostaglandin cytoprotection. *Gastroenterology*, **87**, 1083–1090.
- GYIRES, K., FURST, S., FARZADI, E. & MARTON, A. (1985). Morphine potentiates the gastrulcerogenic effect of indomethacin. *Pharmacology*, **30**, 25–31.
- HAKANSON, R., WAHLSTEDT, C., EKBLAD, E., EDVINSSON, L. & SUNDLER, R. (1986). Neuropeptide Y: co-existence with noradrenaline. Functional implications. *Prog. Brain. Res.*, **68**, 279–287.
- HARPER, A.A., REED, J.D. & SMY, J.R. (1968). Gastric blood flow in anaesthetized cats. *J. Physiol.*, **194**, 795–807.
- HELLSTROM, P.M. (1987). Mechanisms involved in colonic vasoconstriction and inhibition of motility induced by neuropeptide Y. *Acta Physiol. Scand.*, **129**, 549–556.
- HIBBS, J.B. JR., VAVRIN, Z. & TAINTOR, R.R. (1987). L-arginine is required for expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells. *J. Immunol.*, **138**, 550–565.
- HIBBS, J.B. JR., TAINTOR, R.R., VAVRIN, Z. & RACHLIN, E.M. (1988). Nitric oxide: a cytotoxic activated macrophage effector molecule. *Biochem. Biophys. Res. Commun.*, **157**, 87–94.
- HO, M., DAI, S. & OGLE, W. (1984). Decreased acid secretion and gastric lesion production by morphine in rats. *Eur. J. Pharmacol.*, **102**, 117–121.
- HOLM-RUTILI, L. & BERGLINDH, T. (1986). Pentagastrin and gastric mucosal blood flow. *Am. J. Physiol.*, **250**, G525–G580.
- HOLZER, P. (1991). Capsaicin: Cellular targets, mechanism of action, and selectivity for thin sensory neurones. *Pharmacol. Rev.*, **43**, 143–201.
- HOLZER, P. & GUTH, P.H. (1991). Neuropeptide control of rat gastric mucosal blood flow: Increase by calcitonin gene-related peptide and vasoactive intestinal polypeptide, but not substance P and Neurokinin A. *Circ. Res.*, **68**, 100–105.
- HOLZER, P. & LIPPE, I. Th. (1988). Stimulation of afferent nerve endings by intragastric capsaicin protects against ethanol-induced damage of gastric mucosa. *Neuroscience*, **27**, 981–987.
- HOLZER, P. & LIPPE, I. Th. (1992). Gastric mucosal hyperemia due to acid back diffusion depends on splanchnic nerve activity. *Am. J. Physiol.*, **262**, G505–G509.
- HOLZER, P., LIPPE, I. Th., BARTHO, L. & SARIA, A. (1987). Neuropeptide Y, inhibits excitatory enteric neurons supplying the circular muscle of the guinea-pig small intestine. *Gastroenterology*, **92**, 1944–1950.
- HOLZER, P., LIVINGSTON, E.H. & GUTH, P.H. (1991a). Sensory neurons signal for an increase in rat gastric mucosal blood flow in the face of pending acid injury. *Gastroenterology*, **101**, 416–423.
- HOLZER, P., LIVINGSTON, E.H., SARIA, A. & GUTH, P.H. (1991b). Sensory neurons mediate protective vasodilatation in rat gastric mucosa. *Am. J. Physiol.*, **260**, G363–G370.
- HOLZER, P. & PABST, M.A., LIPPE, I. Th., PESKAR, B.M., PESKAR, B.A., LIVINGSTON, E.H. & GUTH, P.H. (1990). Afferent nerve-mediated protection against deep mucosal damage in the rat stomach. *Gastroenterology*, **98**, 838–848.
- HOLZER, P. & SAMETZ, W. (1986). Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurones. *Gastroenterology*, **91**, 975–981.
- HUMPHREYS, G.A., DAVISON, J.S. & VEALE, W.L. (1992). Hypothalamic neuropeptide Y inhibits gastric acid output in rat: role of the autonomic nervous system. *Am. J. Physiol.*, **263**, G726–G732.
- HUTCHESON, I.R., WHITTLE, B.J.R. & BOUGHTON-SMITH, N.K. (1990). Role of nitric oxide in maintaining vascular integrity in endotoxin-induced acute intestinal damage in the rat. *Br. J. Pharmacol.*, **101**, 815–820.
- IGNARRO, L.J., BUGA, G.M., WOOD, K.S., BYRNS, R.E. & CHAUDHURI, G. (1987). Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci. USA*, **81**, 9265–9269.

- IGNARRO, L.J., LIPPTON, H., EDWARDS, J.C., BARICOS, W.H., HYMAN, A.L., KADOWITZ, P.J. & GRUETTER, C.A. (1981). Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: evidence for the involvement of S-nitrosothiols as active intermediates. *J. Pharmacol. Exp. Ther.*, **218**, 739-749.
- INOUE, A., YANAGISAWA, M., KIMURA, S., KASUYA, Y., MIYAUCHI, T., GOTO, K. & MASAKI, T. (1989). The human endothelin family: Three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc. Natl. Acad. Sci. USA*, **86**, 2863-2867.
- ITOH, M. & GUTH, P.H. (1985). Role of oxygen-derived free radicals in hemorrhagic shock-induced gastric lesions in the rat. *Gastroenterology*, **88**, 1162-1167.
- JACOBSON, E.D. (1970). Comparison of prostaglandin E_1 and norepinephrine on the gastric mucosal circulation. *Proc. Soc. Exp. Biol. Med.*, **133**, 516-519.
- JACOBSON, E.D., LINFORD, R.H. & GROSSMAN, M.I. (1966). Gastric secretion in relation to mucosal blood flow studied by a clearance technique. *J. Clin. Invest.*, **45**, 1-13.
- KAUFFMAN, G.L. (1982). Blood flow and gastric secretion. *Fed. Proc.*, **41**, 2080-2083.
- KAUFFMAN, G.L. & WHITTLE, B.J.R. (1982). Gastric vascular actions of prostanoids and the dual effect of arachidonic acid. *Am. J. Physiol.*, **242**, G582-G587.
- KAUFFMAN, G.L., WHITTLE, B.J.R., AURES, D., VANE, J.R. & GROSSMAN, M.I. (1979a). Effects of prostacyclin and a stable analogue 6 β -PGI $_1$ on gastric acid secretion, mucosal blood flow and blood pressure in conscious dogs. *Gastroenterology*, **77**, 1301-1306.
- KAUFFMAN, G.L., WHITTLE, B.J.R., AURES, D. & GROSSMAN, M.I. (1979b). Gastric antisecretory and cardiovascular actions of a stable 16-phenoxy prostacyclin analogue in the dog. In: *Advances in Prostaglandin and Thromboxane Research*, ed. Samuelsson, B., Ramwell, P.W. & Paoletti, R. pp. 1521-1524. Raven Press: New York.
- KELM, M., FEELISCH, M., SPAHR, R., PIPER, H.-M., NOACK, E. & SCHRADER, J. (1988). Quantitative and kinetic characterization of nitric oxide and EDRF released from cultured endothelial cells. *Biochem. Biophys. Res. Commun.*, **154**, 236-244.
- KHAN, M.T. & FURCHGOTT, R.F. (1987). Additional evidence that endothelium derived relaxing factor is nitric oxide. In *Pharmacology* ed. Rand, M.J. & Raper, C. pp. 341-344. New York: Elsevier.
- KILBOURN, R.G., JUBRAN, A.N., GROSS, S.S., GRIFFITH, O.W., LEVI, R., ADAMS, J. & LODATO, R.F. (1990). Reversal of endotoxin-mediated shock by N G -methyl-L-arginine, an inhibitor of nitric oxide synthesis. *Biochem. Biophys. Res. Commun.*, **172**, 1132-1138.
- KITAGAWA, H., TAKEDA, F. & KOHEI, H. (1987). Endothelium-dependent increases in rat gastric mucosal haemodynamics induced by acetylcholine and vagal stimulation. *Eur. J. Pharmacol.*, **133**, 57-63.
- KITAGAWA, H., TAKEDA, F., KOHEI, H. (1990). Effect of endothelium-derived relaxing factor on the gastric lesions induced by HCl in rats. *J. Pharmacol. Exp. Ther.*, **253**, 1133-1137.
- KIVILAAKSO, E., FROMM, D. & SILEN, W. (1978). Relationship between ulceration and intramural pH of gastric mucosa during hemorrhagic shock. *Surgery*, **84**, 70-78.
- KNOWLES, R.G., PALACIOS, M., PALMER, R.M.J. & MONCADA, S. (1989). Formation of nitric oxide from L-arginine in the central nervous system: a transduction mechanism for stimulation of the soluble guanylate cyclase. *Proc. Natl. Acad. Sci. USA*, **86**, 5159-5162.
- KONTUREK, S.J., BROZOZOWSKI, T., MAJKA, J.K. & CZARNOBILSKI, K. (1992). Role of nitric oxide and prostaglandins in sucralate-induced gastroprotection. *Eur. J. Pharmacol.*, **211**, 277-279.
- KONTUREK, S.J., BRZOZOWSKI, T., RADECKI, T. & PIASTUCKI, I. (1984). Comparison of gastric and intestinal antisecretory and protective effects of prostacyclin and its stable thia-imino-analogue (HOE 892) in conscious rats. *Prostaglandins*, **151**, 19-25.
- KONTUREK, S.J., ROBERT, A., HANCHER, A.J. & NEZAMIS, J.E. (1980). Comparison of prostacyclin and prostaglandin E_2 on gastric acid secretion, gastrin release and mucosal blood flow in dogs. *Dig. Dis. Sci.*, **25**, 673-679.
- KONTUREK, S.J., RADECKI, T., BROZOZOWSKI, T., PIASTUCKI, I., DEMBINSKA-KIEC, A. & ZMUDA, A. (1981). Aspirin-induced gastric ulcers in cats. Prevention by prostacyclin. *Dig. Dis. Sci.*, **26**, 1003-1012.
- KUBES, P. & GRANGER, D.N. (1992). Nitric oxide modulates microvascular permeability. *Am. J. Physiol.*, **262**, H611-H615.
- KUBES, P., SUZUKI, M. & GRANGER, D.N. (1990). Modulation of PAF-induced leukocyte adherence and increased microvascular permeability. *Am. J. Physiol.*, **259**, G859-G864.
- KUBES, P., SUZUKI, M. & GRANGER, D.N. (1991). Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc. Natl. Acad. Sci. USA*, **88**, 4651-4655.
- KUROSE, I., MIURA, S., FUKUMURA, D., TASHIRO, H., IMAEDA, H., SHIOZAKI, H., SUEMATSU, M., NAGATA, H., SEKIZUKA, E. & TSUCHIYA, M. (1992). Role of platelet activating factor on the fibrinolytic activation in the pathogenesis of gastric mucosal damage induced by endothelin-1. *Gut*, **33**, 868-871.
- KVIETYS, P.B., TWOHIG, B., DANZELL, J. & SPECIAN, R.D. (1990). Ethanol-induced injury to the rat gastric mucosa. Role of neutrophils and xanthine oxidase-derived radicals. *Gastroenterology*, **98**, 909-920.
- LEE, Y., SHIOSAKA, S., EMSON, P. C., POWELL, J.F., SMITH, A.D. & TOHYAMA, M. (1985). Neuropeptide Y-like immunoreactive structure in the rat stomach with special reference to the nor-adrenaline neuron system. *Gastroenterology*, **89**, 118-126.
- LEMBECK, F. & DONNERER, J. (1985). Opioid control of the function of primary afferent substance P fibres. *Eur. J. Pharmacol.*, **114**, 241-246.
- LEONE, A.M., PALMER, R.M.J., KNOWLES, R.G., FRANCIS, P.L., ASHTON, D.S. & MONCADA, S. (1991). Constitutive and inducible nitric oxide synthases are L-arginine N G -C G -dioxigenases. *J. Biol. Chem.*, **266**, 23790-23795.
- LEUNG, F.W. (1992). Modulation of autoregulatory escape by capsaicin-sensitive afferent nerves in rat stomach. *Am. J. Physiol.*, **262**, H562-H567.
- LEUNG, F.W., GUTH, P.H., SCREMIN, O.U., GOLANSKA, E.M., KAUFFMAN, G.L. Jr. (1984). Regional gastric mucosal blood flow measurements by hydrogen gas clearance in the anaesthetized rat and rabbit. *Gastroenterology*, **87**, 28-36.
- LEUNG, F.W., TALLOS, E.G., TACHE, Y.F. & GUTH, P.H. (1987). Calcitonin gene-related peptide inhibits acid secretion without modifying blood flow. *Am. J. Physiol.*, **252**, G215-G218.
- LI, C.G. & RAND, M.J. (1990). Nitric oxide and vasoactive intestinal polypeptide mediate non-adrenergic, non-cholinergic inhibitory transmission to smooth muscle of the rat gastric fundus. *Eur. J. Pharmacol.*, **191**, 303-309.
- LI, D.S., RAYBOULD, H.E., QUINTERO, E. & GUTH, P.H. (1991). Role of calcitonin gene-related peptide in gastric hyperemic response to intragastric capsaicin. *Am. J. Physiol.*, **261**, G657-G661.
- LI, D.S., RAYBOULD, H.E., QUINTERO, E. & GUTH, P.H. (1992). Calcitonin gene-related peptide mediates the gastric hyperemic response to acid back-diffusion. *Gastroenterology*, **102**, 1124-1128.
- LIPPE, I.Th. & HOLZER, P. (1992). Participation of endothelium-derived nitric oxide but not prostacyclin in the gastric mucosal hyperaemia due to acid back diffusion. *Br. J. Pharmacol.*, **105**, 708-714.
- LIPPE, I.Th., LORBACH, M. & HOLZER, P. (1989a). Close arterial infusion of calcitonin gene-related peptide into the rat stomach inhibits aspirin- and ethanol-induced hemorrhagic damage. *Regul. Pept.*, **26**, 35-46.
- LIPPE, I.Th., PABST, M.A. & HOLZER, P. (1989b). Intragastric capsaicin enhances rat gastric acid elimination and mucosal blood flow by afferent nerve stimulation. *Br. J. Pharmacol.*, **96**, 91-100.
- LOPEZ-BELMONTE, J. & WHITTLE, B.J.R. (1993). Paradoxical interactions between endothelin-1 and calcitonin gene-related peptide in the rat gastric microcirculation. *Br. J. Pharmacol.*, **108**, 113P.
- LOPEZ-BELMONTE, J., WHITTLE, B.J.R. & MONCADA, S. (1993). The actions of nitric oxide donors in the prevention or induction of injury to the rat gastric mucosa. *Br. J. Pharmacol.*, **108**, 73-78.
- LUNDBERG, J.M. & TATEMOTO, K. (1982). Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction resistant to α -adrenoceptor blockade. *Acta Physiol. Scand.*, **116**, 393-402.
- LUNDBERG, J.M., TATEMOTO, K., TERENIUS, L., HELLSTROM, P.M., MITT, V., HOKFELT, T. & HAMBERGER, B. (1982). Localisation of peptide YY (PYY) in gastrointestinal endocrine cells and effects on intestinal blood flow. *Proc. Natl. Acad. Sci. USA*, **79**, 4471-4475.
- LUNDBERG, J.M., TERENIUS, L., HOKFELT, T., MARTLING, C.R., TATEMOTO, K., MUTT, V., POLAK, J., BLOOM, S. & GOLDSTEIN, M. (1984). Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. *Acta Physiol. Scand.*, **116**, 447-480.

- LUNDBERG, J.M., PERNOW, J., FRANCO-CERECEDA, A. & RUDEHILL, A. (1987). Effect of antihypertensive drugs on sympathetic vascular control in relation to neuropeptide Y. *J. Cardiovasc. Pharmacol.*, **10** (Suppl. 12), S51–S68.
- MAIN, I.H.M. & WHITTLE, B.J.R. (1973). Gastric mucosal blood flow during pentagastrin- and histamine-stimulated acid secretion in the rat. *Br. J. Pharmacol.*, **49**, 534–542.
- MAIN, I.H.M. & WHITTLE, B.J.R. (1975). Investigation of the vasodilator and antisecretory role of prostaglandins in the rat gastric mucosa by use of non-steroidal anti-inflammatory drugs. *Br. J. Pharmacol.*, **53**, 217–225.
- MACLEAN, M.R. & HILEY, C.R. (1990). Effect of neuropeptide Y on cardiac output, its distribution, regional blood flow and organ vascular resistance in the pithed rat. *Br. J. Pharmacol.*, **99**, 340–342.
- MACNAUGHTON, W.K., CIRINO, G. & WALLACE, J.L. (1989a). Endothelium derived relaxing factor (nitric oxide) has protective actions in the stomach. *Life Sci.*, **45**, 1869–1876.
- MACNAUGHTON, W.K., KEENAN, C.M., MCKNIGHT, G. & WALLACE, J.L. (1989b). The modulation of gastric mucosal integrity endothelin-1 and prostacyclin. *J. Cardiovasc. Pharmacol.*, (Suppl. 5), S118–S122.
- MAGGI, C.A., EVANGELISTA, S., GIULIANI, S. & MELI, A. (1987). Anti-ulcer activity of calcitonin gene-related peptide in rats. *Gen. Pharmacol.*, **18**, 33–34.
- MARLETTA, M.A., YOON, P.S., IYENGAR, R., LEAF, C.D. & WISHNOK, J.S. (1988). Macrophage oxidation of L-arginine to nitrite and nitrate: Nitric oxide is an intermediate. *Biochemistry*, **27**, 8706–8711.
- MARTINEZ-CUESTA, M.A., BARRACHINA, D., PIQUE, J.M., WHITTLE, B.J.R. & ESPLUGUES, J.V. (1992). The role of nitric oxide and platelet-activating factor in the inhibition by endotoxin of pentagastrin-stimulated gastric acid secretion. *Eur. J. Pharmacol.*, **218**, 351–354.
- MARTINSON, J. (1965). The effect of graded vagal stimulation on gastric motility, secretion and blood flow in the cat. *Acta Physiol. Scand.*, **62**, 256–262.
- MASAKI, T., KIMURA, S., YANAGISAWA, M. & GOTO, K. (1991). Molecular and cellular mechanism of endothelin regulation. Implication for vascular function. *Circulation*, **84**, 1457–1468.
- MASUDA, E., KAWANO, S., NAGANO, K., TSUJI, S., ISHIGAMI, Y., TSUJII, M., HAYASHI, N., FUSAMOTO, H. & KAMADA, T. (1992). Effect of intravascular ethanol on modulation of gastric mucosal integrity, possible role of endothelin-1. *Am. J. Physiol.*, **262**, G785–G790.
- MATSUDA, M., AONO, M., MORIGA, M. & OKUMA, M. (1991). Centrally administered NPY stimulated gastric acid and pepsin secretion by a vagally mediated mechanism. *Regul. Pept.*, **35**, 31–41.
- MATSUMOTO, H., SUZUKI, N., ONDA, H. & FUJINO, M. (1989). Abundance of endothelin-3 in rat intestine, pituitary gland and brain. *Biochem. Biophys. Res. Commun.*, **164**, 74–80.
- MCCALL, T.B., BOUGHTON-SMITH, N.K., PALMER, R.M.J., WHITTLE, B.J.R. & MONCADA, S. (1989). Synthesis of nitric oxide from L-arginine by neutrophils. Release and interaction with superoxide anion. *Biochem. J.*, **261**, 293–296.
- MCINTOSH, C.H.S., DADGAR, A. & KWOK, Y.N. (1992). Cholinergic stimulation of neuropeptide Y secretion from the isolated perfused rat stomach. *Regul. Pept.*, **39**, 83–94.
- MONCADA, S., GRYGLEWSKI, R.J., BUNTING, S. & VANE, J.R. (1976). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature*, **263**, 663–665.
- MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991) Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev.*, **43**, 109–142.
- MONCADA, S. & VANE, J.R. (1979). Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A₂, and prostacyclin. *Pharmacol. Rev.*, **30**, 293–331.
- MORALES, R.W., JOHNSON, B.R. & SZABO, S. (1992). Endothelin induces vascular and mucosal lesions, enhances the injury by HCl/ethanol, and the antibody exerts gastroprotection. *FASEB J.*, **6**, 2354–2360.
- MORLEY, J.E., LEVINE, A.S. & SILVIS, S.E. (1982). Endogenous opiates and stress ulceration. *Life Sci.*, **31**, 693–699.
- MORRIS, M.J., KAPOOR, V. & CHALMERS, J.P. (1987). Plasma neuropeptide Y concentration is increased after hemorrhage in conscious rats: Relative contributions of sympathetic nerves and the adrenal medulla. *J. Cardiovasc. Pharmacol.*, **9**, 541–545.
- MULDERRY, P.K., GHATEI, M.A., SPOKES, R.A., JONES, P., PIERSON, A.M., HAMID, Q.A., KAUSE, S., AMARA, S.G., BURR, J.M., LEGON, S., POLAK, J.M. & BLOOM, S.R. (1988). Different expression of α -CGRP and β -CGRP by primary sensory neurones and enteric autonomic neurons of the rat. *Neuroscience*, **25**, 195–206.
- MURAKAMI, M., MORIGA, M., MIYAKE, T. & UCHINO, H. (1982). Contact electrode method in hydrogen gas clearance technique: a new method for determination of regional gastric mucosal blood flow in animals and humans. *Gastroenterology*, **82**, 457–467.
- NAVA, E., PALMER, R.M.J. & MONCADA, S. (1991). Inhibition of nitric oxide synthesis in septic shock: how much is beneficial? *Lancet*, **338**, 1555–1557.
- NICOLOFF, D.M., PETER, E.T., STONE, N.H. & WANGENSTEEN, O.H. (1964). Effect of catecholamines on gastric secretion and blood flow. *Ann. Surg.*, **159**, 32–36.
- O'CONNOR, K.J. & MONCADA, S. (1991). Glucocorticoids inhibit the induction of nitric oxide synthase and the related cell damage in adenocarcinoma cells. *Biochim. Biophys. Acta*, **1097**, 227–231.
- OATES, P.J. & HAKKINEN, J.P. (1988). Studies on the mechanism of ethanol-induced gastric damage in rats. *Gastroenterology*, **94**, 10–21.
- OREN-WOLMAN, N. & GUTH, P.H. (1984). Adrenergic sensitivity of different-sized gastric submucosal arterioles. *Microvasc. Res.*, **28**, 345–351.
- PALMER, R.M.J. & MONCADA, S. (1989). A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. *Biochem. Biophys. Res. Commun.*, **158**, 348–352.
- PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327**, 524–526.
- PALMER, R.M.J., ASHTON, D.S. & MONCADA, S. (1988a). Vascular endothelial cells synthesise nitric oxide from L-arginine. *Nature*, **333**, 664–666.
- PALMER, R.M.J., BRIDGE, L., FOXWELL, N.A. & MONCADA, S. (1992). The role of nitric oxide in endothelial cell damage and its inhibition by glucocorticoids. *Br. J. Pharmacol.*, **105**, 11–12.
- PALMER, R.M.J., REES, D.D., ASHTON, D.S. & MONCADA, S. (1988b). L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Biochem. Biophys. Res. Commun.*, **153**, 1251–1256.
- PANES, J., CASADEVALL, M., PIQUE, J.M., BOSCH, J., WHITTLE, B.J.R. & TERES, J. (1992). Effects of acute normovolemic anemia on gastric mucosal blood flow in rats: role of nitric oxide. *Gastroenterology*, **103**, 407–413.
- PARKS, D.A., BULKLEY, G.B.M., GRANGER, D.N., HAMILTON, S.R. & MCCORD, J.M. (1982). Ischemic injury in the cat small intestine: role of superoxide radicals. *Gastroenterology*, **82**, 9–15.
- PASCAUD, X.B., CHOVET, M., ROZE, C. & JUNIEN, J.L. (1993). Neuropeptide Y and σ receptor agonists act through a common pathway to stimulate duodenal alkaline secretion in rats. *Eur. J. Pharmacol.*, **231**, 389–394.
- PERRY, M., HAEDICKE, G., BULKLEY, G., KVIETYS, P., GRANGER, D. (1983). Relationship between acid secretion and blood flow in the canine stomach, role of oxygen consumption. *Gastroenterology*, **85**, 529–534.
- PESKAR, B.M., NOWAK, P. & LAMBRECHT, N. (1992). Effect of prostaglandins and capsaicin on gastric vascular flow and mucosal injury in endothelin-1 treated rats. *Agents & Actions* (Suppl. 37), 89–91.
- PESKAR, B.M., RESPONDEK, M., MULLER, K.M. & PESKAR, B.A. (1991). A role of nitric oxide in capsaicin-induced gastroprotection. *Eur. J. Pharmacol.*, **198**, 113–114.
- PETROS, A., BENNETT, D. & VALLANCE, P. (1991). Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock. *Lancet*, **338**, 1557–1558.
- PIHAN, G., MAJZOUBI, D., HAUDENSCHILD, C., TRIER, J.S. & SZABO, S. (1986). Early microcirculatory stasis in acute gastric mucosal injury in the rat and prevention by 16,16-dimethyl prostaglandin E₂ or sodium thiosulfate. *Gastroenterology*, **91**, 1415–1426.
- PIQUE, J.M., ESPLUGUES, J.V. & WHITTLE, B.J.R. (1992a). Endogenous nitric oxide as a mediator of gastric mucosal vasodilatation during acid secretion. *Gastroenterology*, **102**, 168–174.
- PIQUE, J.M., ESPLUGUES, J.V. & WHITTLE, B.J.R. (1990). Influence of morphine or capsaicin pretreatment on rat gastric microcirculatory response to PAF. *Am. J. Physiol.*, **258**, G352–G357.

- PIQUE, J.M., LEUNG, F.W., TAN, H.W., LIVINGSTON, E., SCREMIN, O.U. & GUTH, P.H. (1988). Gastric mucosal blood flow response to stimulation and inhibition of gastric acid secretion. *Gastroenterology*, **95**, 642–650.
- PIQUE, J.M., PIZCUETA, M.P., BOSCH, J., FERNANDEZ, M., WHITTLE, B.J.R. & MONCADA, S. (1992b). Role of nitric oxide in the hyperdynamic splanchnic circulation of portal hypertensive rats. In: *The Biology of Nitric Oxide. 1. Physiological and Clinical Aspects*. eds. Moncada, S., Marletta, M.A., Hibbs, Jr, J.B. & Higgs, E.A. pp. 60–64. London & Chapel Hill, Portland Press.
- PIQUE, J.M., WHITTLE, B.J.R. & ESPLUGUES, J.V. (1989). The vasodilator role of endogenous nitric oxide in the rat gastric microcirculation. *Eur. J. Pharmacol.*, **174**, 293–296.
- PIZCUETA, M.P., PIQUE, J.M., BOSCH, J., WHITTLE, B.J.R. & MONCADA, S. (1992a). Effects of inhibiting nitric oxide biosynthesis on the systemic and splanchnic circulation of rats with portal hypertension. *Br. J. Pharmacol.*, **105**, 184–190.
- PIZCUETA, M.P., PIQUE, J.M., FERNANDEZ, M., BOSCH, J., RODES, J., WHITTLE, B.J.R. & MONCADA, S. (1992b). Modulation of the hyperdynamic circulation of cirrhotic rats by nitric oxide inhibition. *Gastroenterology*, **103**, 1909–1915.
- QUINTERO, E. & GUTH, P.H. (1992a). Nitric oxide-mediated gastric hyperemia decreases ethanol-induced gastric mucosal injury in uraemic rats. *Dig. Dis. Sci.*, **37**, 1324–1328.
- QUINTERO, E. & GUTH, P.H. (1992b). Renal failure increases gastric mucosal blood flow and acid secretion in rats: role of endothelium-derived nitric oxide. *Am. J. Physiol.*, **263**, G75–G80.
- REED, J.D., SANDERS, D.J. & THORPE, V. (1971). The effect of splanchnic nerve stimulation on gastric acid secretion and mucosal blood flow in the anaesthetized cat. *J. Physiol.*, **214**, 1–13.
- REES, D.D., PALMER, R.M.J., HODSON, H.F. & MONCADA, S. (1989b). A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. *Br. J. Pharmacol.*, **96**, 418–424.
- REES, D.D., PALMER, R.M.J. & MONCADA, S. (1989a). Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc. Natl. Acad. Sci. USA*, **86**, 3375–3378.
- REES, D.D., PALMER, R.M.J., SCHULZ, R., HODSON, H.F. & MONCADA, S. (1990). Characterisation of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. *Br. J. Pharmacol.*, **101**, 746–752.
- RITCHIE, W.P. (1975). Acute gastric mucosal damage induced by bile salts, acid and ischemia. *Gastroenterology*, **68**, 699–707.
- ROBERT, A., NEZAMIS, J.E., LANCASTER, C. & HANCHAR, A.J. (1979). Cytoprotection by prostaglandins in rats – Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. *Gastroenterology*, **77**, 433–443.
- ROSAM, A.C., WALLACE, J.L. & WHITTLE, B.J.R. (1986). Potent ulcerogenic actions of platelet-activating factor on the stomach. *Nature*, **319**, 54–56.
- RUBANYI, G.M. & VANHOUTTE, P.M. (1986). Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am. J. Physiol.*, **250**, H822–H827.
- RUSSELL, N.J.W., SCHAIBLE, H.G. & SCHMIDT, R.F. (1987). Opiates inhibit the discharges of fine afferent units from inflamed knee joint of the cat. *Neurosci. Lett.*, **76**, 107–112.
- SAKURAI, T., YANAGISAWA, M., TAKUWA, Y., MIYAZAKI, H., KIMURA, S., GOTTO, K. & MASAKI, T. (1990). Cloning of cDNA encoding a non-isopeptide selective subtype of the endothelin receptor. *Nature*, **348**, 732–735.
- SALTER, M., KNOWLES, R.G. & MONCADA, S. (1991). Widespread tissue distribution, species distribution and changes in activity of Ca^{2+} -dependent and Ca^{2+} -independent nitric oxide synthases. *FEBS. Lett.*, **291**, 145–149.
- SALVATI, P. & WHITTLE, B.J.R. (1981). Investigation of the vascular actions of arachidonate lipoxygenase and cyclo-oxygenase products on the isolated perfused stomach of rat and rabbit. *Prostaglandins*, **22**, 141–156.
- SARIA, A. & BEUBLER, E. (1985). Neuropeptide Y (NPY) and peptide YY (PYY) inhibit prostaglandin E_2 -induced intestinal fluid and electrolyte secretion in the rat jejunum *in vivo*. *Eur. J. Pharmacol.*, **119**, 47–52.
- SELYE, M. (1935). A syndrome produced by diverse noxious agents. *Nature*, **138**, 32–40.
- SHEA-DONOHUE, T., NOMPLEGGI, D., MYERS, L. & DUBOIS, A. (1982). A comparison of the effects of prostacyclin and the 15(S) 15-methyl analogs of PGE_2 and $\text{PGF}_{2\beta}$ on gastric parietal and non-parietal secretion. *Dig. Dis. Sci.*, **27**, 17–22.
- SHEIKH, S.P. (1991). Neuropeptide Y and peptide YY: major modulators of gastrointestinal blood flow and function. *Am. J. Physiol.*, **261**, G701–G715.
- SMITH, T.W. & BUCHAN, P. (1984). Peripheral opioid receptors located on rat saphenous nerve. *Neuropeptides*, **5**, 217–220.
- SMITH, S.M., HOLM-RUTILI, L., PERRY, M.A., GRISHAM, M.N., ARFORS, K-E., GRANGER, D.N., KVIETYS, P.R. & RUSSEL, J.M. (1987). Role of neutrophils in haemorrhagic shock-induced gastric mucosal injury in the rat. *Gastroenterology*, **93**, 466–471.
- STERNINI, C., REEVE, J.R. & BRECHA, N. (1987). Distribution characterisation of calcitonin gene-related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats. *Gastroenterology*, **93**, 852–862.
- SU, H.C., BISHOP, A.E., POWER, R.F., HAMADA, Y. & POLAK, J.M. (1987). Dual intrinsic and extrinsic origins in CGRP- and NPY-immunoreactive nerves of rat gut and pancreas. *J. Neurosci.*, **7**, 2674–2687.
- SUNDLER, F., MOGHIMZADEH, E., HAKANSON, R., EKELAND, M. & EMSON, P. (1983). Nerve fibres in the gut and pancreas of the rat displaying neuropeptide-Y immunoreactivity. Intrinsic and extrinsic origin. *Cell Tissue Res.*, **280**, 487–493.
- SZABO, S., TRIER, J.S., BROWN, A. & SCHNOOR, J. (1985). Early vascular injury and increased vascular permeability in gastric mucosal injury caused by ethanol in the rat. *Gastroenterology*, **88**, 228–236.
- SZOLCSANYI, J. & BARTHO, L. (1981). Impaired defense mechanism to peptic ulcer in the capsaicin-desensitized rat. In *Gastrointestinal Defense Mechanisms*. ed. Mozsik, G., Hanninen, O. & Javor. *Adv. Physiol. Sci.*, Vol. 29, pp. 39–51. Oxford U.K. and Budapest, Hungary: Pergamon Press and Akademiai Kiado.
- TAKAHASHI, K., JONES, P.M., KANSE, S.M., LAM, H.-C., SPOKES, R.A., GHATEI, M.A. & BLOOM, S.R. (1990). Endothelin in the gastrointestinal tract: Presence of endothelin like immunoreactivity, endothelin-1 messenger RNA, endothelin receptors and pharmacological effect. *Gastroenterology*, **99**, 1660–1667.
- TEPPERMAN, B.L. & WHITTLE, B.J.R. (1991). Comparison of the effects of neuropeptide Y and noradrenaline on rat gastric mucosal blood flow and integrity. *Br. J. Pharmacol.*, **102**, 95–100.
- TEPPERMAN, B.L. & WHITTLE, B.J.R. (1992). Endogenous nitric oxide and sensory neuropeptides interact in the modulation of the rat gastric microcirculation. *Br. J. Pharmacol.*, **105**, 171–175.
- THIEMERMANN, C. & VANE, J. (1990). Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharides in the rat *in vivo*. *Eur. J. Pharmacol.*, **182**, 591–595.
- THOMPSON, J.E. & VANE, J.R. (1953). Gastric secretion induced by histamine and its relationship to the rate of blood flow. *J. Physiol.*, **121**, 433–444.
- TILL, M., GATI, T., RABAI, K., SZOMBATH, D. & SZEKELY, J.I. (1988). Effect of [D-Met²Pro⁵]enkephalinamide on gastric ulceration and transmucosal potential difference. *Eur. J. Pharmacol.*, **150**, 325–330.
- VALLANCE, P., COLLIER, J. & MONCADA, S. (1989). Effects of endothelium-derived nitric oxide on peripheral arteriole tone in man. *Lancet*, **ii**, 997–1000.
- VARRO, V., DOBRONTE, Z. & SAGI, I. (1978). Inter-relation between gastric blood flow and HCl secretion in dogs. *Acta Med. Acad. Sci. Hung.*, **35**, 1–20.
- WADHWA, S.S. & PERRY, M.A. (1987). Gastric injury induced by hemorrhage, local ischemia, and oxygen radical generation. *Am. J. Physiol.*, **253**, G129–G133.
- WAHLESTEDT, C., DEVINSSON, L., EKBLAD, G. & HAKANSON, R. (1985). Neuropeptide Y potentiates noradrenaline-evoked vasoconstriction: mode of action. *J. Pharmacol. Exp. Ther.*, **243**, 735–741.
- WALDER, C.E., THIEMERMANN, C. & VANE, J.R. (1990). Endothelium derived relaxing factor participates in the increased blood flow in response to pentagastrin in the rat stomach mucosa. *Proc. R. Soc.*, **241**, 195–200.
- WALLACE, J.L., ARFORS, K-E. & MCKNIGHT, G.W. (1991). A monoclonal antibody against the CD18 leukocyte adhesion molecule prevents indomethacin-induced gastric damage in the rabbit. *Gastroenterology*, **100**, 878–883.
- WALLACE, J.L., CIRINO, G., DE NUCCI, G., MCKNIGHT, W. & MACNAUGHTON, W.K. (1989a). Endothelin has potent ulcerogenic vasoconstrictor actions in the stomach. *Am. J. Physiol.*, **256**, G661–666.

- WALLACE, J.L., KEENAN, C.M., MACNAUGHTON, W.K. & MC-KNIGHT, G.W. (1989b). Comparison of the effects of endothelin-1 and endothelin-3 on the rat stomach. *Eur. J. Pharmacol.*, **167**, 41–47.
- WALLACE, J.L. & WHITTLE, B.J.R. (1986). Picomole doses of platelet-activating factor predispose the gastric mucosa to damage by topical irritants. *Prostaglandins*, **31**, 989–998.
- WALUS, K.M., PAWLIK, W. & KONTUREK, S.J. (1980). Prostacyclin-induced gastric mucosal vasodilatation and inhibition of acid secretion in the dog. *Proc. Soc. Exp. Biol. Med.*, **163**, 228–232.
- WANG, Y.N., McDONALD, J.K. & WYATT, R.J. (1987). Immunocytochemical localisation of neuropeptide Y-like immunoreactivity in adrenergic and non-adrenergic neurons of the rat gastro-intestinal tract. *Peptides*, **8**, 145–151.
- WESTFALL, T.C., CARPENTIER, S., CHEN, X., BEINFELD, M.C., NAES, L. & MELDRUM, M.J. (1987). Prejunctional and postjunctional effects of neuropeptide Y at the noradrenergic neuro-effector junction of the perfused mesenteric arterial bed of the rat. *J. Cardiovasc. Pharmacol.*, **10**, 716–722.
- WHITTLE, B.J.R. (1977). Mechanisms underlying gastric mucosal damage induced by indomethacin and bile salts and the actions of prostaglandins. *Br. J. Pharmacol.*, **61**, 455–460.
- WHITTLE, B.J.R. (1980). Actions of prostaglandins on gastric mucosal blood flow. In: *Gastro-Intestinal Mucosal Blood Flow*, ed. Fielding, L.P. pp. 180–191. Edinburgh, New York: Churchill Livingstone.
- WHITTLE, B.J.R. (1981). Antisecretory actions of prostacyclin and its analogues on the gastric mucosa. In: *Clinical Pharmacology of Prostacyclin*, ed. Lewis P.J. O'Grady, J. pp. 219–232. New York: Raven Press.
- WHITTLE, B.J.R. (1983). The potentiation of taurocholate-induced rat gastric erosions following parental administration of cyclooxygenase inhibitors. *Br. J. Pharmacol.*, **80**, 545–551.
- WHITTLE, B.J.R. (1992). Unwanted effects of aspirin and related agents on the gastrointestinal tract. In: *Aspirin and other Salicylates*, ed. Vane, J.R. & Botting, R. pp. 465–509. London: Chapman & Hall.
- WHITTLE, B.J.R. & BOUGHTON-SMITH, N.K. (1979). 16-Phenoxy prostacyclin analogues potent, selective anti-ulcer compounds. In: *Prostacyclin*, ed. Vane, J.R. & Bergstrom, S. pp. 159–171. New York: Raven Press.
- WHITTLE, B.J.R., BOUGHTON-SMITH, N.K. & MONCADA, S. (1992a). Biosynthesis and role of the endothelium-derived vasodilator, nitric oxide, in the gastric mucosa. In: *Neuro-immuno-physiology of the Gastrointestinal Mucosa. Implications for Inflammatory Diseases*, ed. Stead, R.H., Perdue, M.H., Cooke, H., Powell, D.W. & Barrett, K.W. *Ann. NY Acad. Sci.*, **664**, 126–139.
- WHITTLE, B.J.R., BOUGHTON-SMITH, N.K., MONCADA, S. & VANE, J.R. (1978). Actions of prostacyclin (PGI₂) and its product 6-oxo-PGF_{1α} on the rat gastric mucosa *in vivo* and *in vitro*. *Prostaglandins*, **15**, 955–968.
- WHITTLE, B.J.R. & ESPLUGUES, J.V. (1988). Induction of rat gastric damage by the endothelium-derived peptide, endothelin. *Br. J. Pharmacol.*, **95**, 1011–1013.
- WHITTLE, B.J.R. & ESPLUGUES, J.V. (1989). Pro-ulcerogenic eicosanoids and related lipid mediators in gastro-intestinal damage. In: *Advances in Drug Therapy of Gastrointestinal Ulceration*, ed. Garner, A. & Whittle, B.J.R. pp. 165–188. Chichester: J. Wiley and Sons.
- WHITTLE, B.J.R. & ESPLUGUES, J.V. (1990). Vascular interactions between endogenous prostanoids and vasoactive peptide mediators in gastric damage. *Advances in Prostaglandin, Thromboxane and Leukotriene Research*, Vol. 21, ed. Samuelsson, B. pp. 761–765. New York: Raven Press.
- WHITTLE, B.J.R., HIGGS, G.A., EAKINS, K.E., MONCADA, S. & VANE, J.R. (1980). Selective inhibition of prostaglandin production in inflammatory exudates and gastric mucosa. *Nature*, **284**, 271–273.
- WHITTLE, B.J.R., KAUFFMAN, G.L. & BOUGHTON-SMITH, N.K. (1984). Stimulation of gastric alkaline secretion by stable prostacyclin analogues in rat and dog. *Eur. J. Pharmacol.*, **100**, 277–283.
- WHITTLE, B.J.R., KAUFFMAN, G.L. & MONCADA, S. (1981). Vasoconstriction with thromboxane A₂ induces ulceration of the gastric mucosa. *Nature*, **292**, 472–474.
- WHITTLE, B.J.R. & LOPEZ-BELMONTE, J. (1991). Interactions between the vascular peptide endothelin-1 and sensory neuropeptides in gastric mucosal injury. *Br. J. Pharmacol.*, **102**, 950–954.
- WHITTLE, B.J.R., LOPEZ-BELMONTE, J. & REES, D.D. (1989a). Modulation of the vasodepressor actions of acetylcholine, bradykinin, substance P and endothelin in the rat by a specific inhibitor of nitric oxide formation. *Br. J. Pharmacol.*, **98**, 646–652.
- WHITTLE, B.J.R., LOPEZ-BELMONTE, J. & MONCADA, S. (1990). Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory neuropeptides in the rat. *Br. J. Pharmacol.*, **99**, 607–611.
- WHITTLE, B.J.R., LOPEZ-BELMONTE, J. & MONCADA, S. (1992b). Nitric oxide mediates rat mucosal vasodilatation induced by intragastric capsaicin. *Eur. J. Pharmacol.*, **218**, 339–341.
- WHITTLE, B.J.R., OREN-WOLMAN, N. & GUTH, P.H. (1985). Gastric vasoconstrictor actions of leukotriene C₄, PGF_{2α} and thromboxane mimetic U-46619 on rat submucosal microcirculation *in vivo*. *Am. J. Physiol.*, **248**, G580–G586.
- WHITTLE, B.J.R., PAYNE, A.N. & ESPLUGUES, J.V. (1989b). Cardiopulmonary and gastric ulcerogenic actions of endothelin-1 in the guinea-pig and rat. *J. Cardiovasc. Pharmacol.*, **13** (Suppl 5) S103–S107.
- WHITTLE, B.J.R., MORISHITA, T., OHYA, Y., LEUNG, F.W. & GUTH, P.H. (1986). Microvascular actions of platelet-activating factor on the rat gastric mucosa and submucosa. *Am. J. Physiol.*, **251**, G772–G778.
- WHITTLE, B.J.R. & TEPPERMAN, B.L. (1991). Role of the endogenous vasoactive mediators, nitric oxide, prostanoids and sensory neuropeptides in the regulation of gastric blood flow and mucosal integrity. In: *Mechanism of Injury, Protection and Repair of the Upper Gastrointestinal Tract*, ed. Garner, A. & O'Brien, P.E. pp. 127–137. Chichester: J. Wiley & Sons.
- WHITTLE, B.J.R. & VANE, J.R. (1987). Prostanoids as regulators of gastrointestinal function. In: *Physiology of the Gastrointestinal Tract*, ed. Johnson, L.R. Second Edition, Vol. 1. pp. 143–180. New York: Raven Press.
- WOOD, J.G., YAN, Z.Y., CHEUNG, L.Y. (1992). Relative potency of endothelin analogues on changes in gastric vascular resistance. *Am. J. Physiol.*, **262**, G977–G982.
- WRIGHT, C.E. & FOZARD, J.R. (1988). Regional vasodilation is a prominent feature of the haemodynamic response to endothelin in anaesthetized spontaneously hypertensive rats. *Eur. J. Pharmacol.*, **155**, 201–203.
- WRIGHT, C.E., REES, D.D. & MONCADA, S. (1992). Protective and pathological roles of nitric oxide in endotoxin shock. *Cardiovasc. Res.*, **26**, 48–57.
- YANAGISAWA, M., KURIHARA, H., KIMURA, S., TOMOBE, Y., KOSASHI, M., YAZAKI, Y., GOTO, K. & MASAKI, T. (1988). A potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, **333**, 411–415.
- YANO, S.A., FUJIWARA, Y., OZAKI & HARADA, M. (1983). Gastric blood flow responses to autonomic nerve stimulation and related pharmacological studies in rats. *J. Pharm. Pharmacol.*, **35**, 641–646.
- YOSHIZAWA, T., KIMURA, S., KANAZAWA, I., UCHIYAMA, Y., YGISAWA, M. & MASAKI, T. (1989). Endothelin localizes in dorsal horn and acts on the spinal neurones: possible involvement of dihydropyridine-sensitive calcium channels and substance P release. *Neurosci. Lett.*, **102**, 179–184.
- ZINNER, M.J., KERR, J.C. & REYNOLDS, D.G. (1975). Hemodynamic effects of intra-arterial infusions of catecholamine on the canine gastric circulation. *Surgery*, **78**, 381–388.

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