Upsala J Med Sci 102: 137–174, 1997

Duodenal Mucosal Permeability, Bicarbonate Secretion and Motility

Aspects of regulation and integration of duodenal function in the rat
Minireview based on a doctoral thesis

Anneli Hällgren

Department of Physiology and Medical Biophysics, Uppsala University

INTRODUCTION

The gastrointestinal tract has two major tasks: to efficiently absorb nutrients, fluid and electrolytes, and at the same time prevent potentially noxious substances from entering the body. To optimize the gastrointestinal function a multitude of mechanisms are regulated and integrated in a complex pattern. This study deals with the regulation and integration of some of the duodenal functions in anesthetized rats.

Aggressive factors and defense mechanisms

The potentially injurious factors to the duodenum can be divided into endogenous aggressors, such as hydrochloric acid (HCl), proteolytic enzymes and bile salts, and extrinsic factors including *Helicobacter pylori*, smoking, ethanol and some anti-inflammatory drugs.

The pathophysiological significance of acid in ulcer disease was expressed by Karl Schwarz in 1910 in his famous dictum "without acidic gastric juice, no peptic ulcer". In recent years the dictum has been rephrased to "no Helicobacter, no ulcer". Acid is still, however, considered a prerequisite for the development of peptic ulcers. The treatment of ulcer disease is largely targeted both at eradication of the bacterium and inhibition of acid secretion.

The duodenum is regularly exposed to the acidic gastric juice. It has been shown that 10-15% of the time the pH in the duodenal bulb decreases below pH 2 and more than 30% of the time below pH 3 in healthy subjects (12). Low pH, together with the proteolytic enzyme pepsin, poses a potential threat to the duodenal mucosa. However, multiple nervous reflexes, activated for instance by acidity in the duodenal lumen, protect the duodenum by regulating stomach emptying and the secretion of acid.

The first line of duodenal mucosal defense is provided by the bicarbonate containing viscoelastic mucus gel adhering to and covering the epithelium. Although the buffering capacity of mucus itself is negligible (5), the mucus is of great importance in epithelial protection. The mucus gel lubricates the epithelium and provides a stable unstirred layer that keeps the secreted bicarbonate close to the epithelium (5). The rate of diffusion of bicarbonate is 11 times less through duodenal

mucus than through saline (108). Furthermore, the mucus creates a physical barrier against large molecular luminal contents, such as bacterial toxins and enzymes. Another important function of mucus is to aid in the repair process of damaged mucosa. Following acute damage, a thick gelatinous protective coat, "the mucoid cap", is formed over re-epithelializing mucosa (166, 189).

The secretion of bicarbonate into the mucus provides for the formation of a standing pH gradient within the gel. The pH at the cell surface remains near neutral, despite acidities in the luminal bulk solution down to pH 2.0 (45, 151). The ability of the epithelium to secrete bicarbonate is probably a major mechanism in duodenal mucosal protection against acid. However, the secretion of water may also contribute to the establishment and maintenance of the pH gradient by conveying acid away from the epithelium (108).

Apart from the ability to secrete mucus and bicarbonate, other properties of the duodenal epithelium provide a "second line of defense". One of the suggested protective mechanisms, believed to prevent hydrogen ions from entering the tissue, is the restriction of junctional cation permeability induced by pH alterations or by changes in osmolarity (118). In rat small intestine, a switch from cation to anion selectivity of the paracellular pathway, due to neutralization of the negative charges lining the tight junctions, occurs at luminal pH 2.7 (170). Occasionally the protective mechanisms are insufficient to prevent mucosal damage. However, if superficial damage occurs, the surface epithelium is rapidly repaired by restitution. Restitution is a process in which re-epithelialization is accomplished within minutes or hours by the migration of remaining vital cells to cover the damage surface (38, 101, 110).

Duodenal motility and secretion of fluid dilute and remove potentially noxious agents and may therefore also be important in the mucosal defense. The secreted fluid contains secretory immunoglobulins which prevent adhesion of bacteria and toxins to the epithelial surface (132). Furthermore, the secretion of immunoglobulin is coordinated with interdigestive motility (127), and abnormal motility patterns may lead to bacterial overgrowth (75).

Duodenal mucosal bicarbonate secretion

The first report of an alkaline secretion from the duodenum dates back almost a century to Ponomarew at Pavlovs laboratory, who in 1902 described the duodenal juice from two dogs to be alkaline (148). Thirty years later Florey and Harding demonstrated that fluid from mammalian duodenal pouches have a pH in the alkaline range (46). The alkaline secretion was assumed to originate from Brunner's glands. Secretion of HCO_3^- by Brunner's glands is still unconfirmed, whereas the duodenal epithelium (devoid of Brunner's glands) secretes bicarbonate at high rates in all species tested (4, 39).

Mechanisms of transcellular transport

In the unstimulated situation in vivo the secretion of bicarbonate has been shown to be mainly transcellular and metabolically dependent (4, 39, 68, 143) (Fig. 1). The transcellular transport process includes several subcellular mechanisms. At the apical cell membrane, there are at least two bicarbonate transporting mechanisms: the electroneutral HCO_3^-/Cl^- exchange and an electrogenic anion transport

mechanism (41, 43, 80). The latter has been suggested to be identical with the cystic fibrosis transmembrane conductance regulator (CFTR) (66), which is abundant in duodenocytes (174, 184). In a recent study using transgenic mice, the CFTR was shown to be involved in basal as well as cAMP and Ca^{2+}-activated duodenal bicarbonate secretion (70). Whether the CFTR is mainly a luminal Cl^- supplier for the HCO_3^-/Cl^- exchange, or actually transports bicarbonate ions, remains to be shown. At the basolateral membrane, bicarbonate is taken up into the cell by a $NaHCO_3$ co-transporter (80, 196). Furthermore, bicarbonate (and H^+) can be formed from carbon dioxide within the duodenocyte, a reaction which is aided by carbonic anhydrase (71, 130). Hydrogen ions are extruded from the cells by Na^+/H^+ exchange at the basolateral membrane (80).

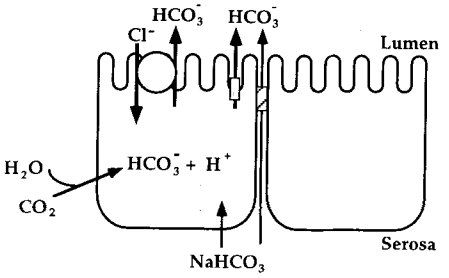

Fig. 1 Proposed mechanisms of paracellular and transcellular transport of bicarbonate in duodenal epithelium. Adapted from Flemström (1994).

In general, crypt cells are thought to have a secretory function whereas cells situated in the villi are mainly absorptive (26). However, in the rat duodenum, immunostaining for carbonic anhydrase increases from the base to the tip of the villi and is absent in crypt cells (113). This suggests that at least carbonic anhydrase-dependent bicarbonate secretion originates from the villi. Furthermore, Säfsten and Flemström found that vasoactive intestinal peptide (VIP) and dopamine stimulate cAMP formation in both villus and crypt cells (179) and suggested that duodenal epithelial bicarbonate secretion occurs along the entire crypt-villous axis.

Physiological control of mucosal bicarbonate secretion

The intracellular events leading to the secretion of bicarbonate are not fully understood. Increased intracellular calcium has been proposed as a "second messenger" to muscarinic receptor stimulation (32, 178). The levels of cAMP, a second messenger known to stimulate bicarbonate secretion by activation of both the HCO_3^-/Cl^- exchange (35) and the CFTR channel (70), increases in intestinal cells in response to VIP and prostaglandin E_2 (PGE_2) (154, 179). However, whether the increase in cAMP mediates the alkaline response to these agents is a matter of dispute (196). Recently, cGMP has been implicated as an intracellular messenger regulating electrogenic bicarbonate secretion in response to guanylin (59).

At the extracellular level, mucosal bicarbonate secretion is controlled by enteric secretomotor neurons. These neurons have their cell bodies located in the submucosal plexus of the enteric nervous system (ENS) (48). In the guinea pig, there are at least two types of secretomotor neurons: one that is cholinergic and one of the non-cholinergic kind (19). VIP, which is a known stimulant of duodenal mucosal bicarbonate secretion (44, 81), is a plausible primary transmitter in the non-cholinergic type. A rich supply of intrinsic VIP-immunoreactive nerve fibers extends

from the submucosal plexus into the mucosa, to form a non-ganglionated subepithelial network (88). Both types of secretomotor neurons of the submucous plexus receive excitatory input from myenteric neurons or other submucous neurons, whereas the non-cholinergic secretomotor neurons also get inhibitory signals from myenteric neurons and sympathetic axons (19).

In addition to the extensively studied excitatory transmitters acetylcholine (ACh) and VIP, there are a number of other candidates that may mediate or modulate the neural regulation of bicarbonate transport. Nitric oxide (NO), produced from L-arginine by the enzyme nitric oxide synthase (NOS), is a recently discovered mediator of a large variety of biological responses. Immunoreactivity for NOS has been found in intestinal myenteric and submucosal neurons (21, 111, 133). Regarding the role of NO as a regulator of duodenal bicarbonate secretion conflicting results have been presented. In anesthetized rats NOS-inhibitors stimulate the secretion of bicarbonate (77, 181) whereas the alkaline secretion was decreased by NO-depletion in conscious dogs (16).

The myenteric plexus of the proximal duodenum is densely innervated by efferent preganglionic vagal fibers, whereas virtually no such fibers terminate in the submucosal plexus or within the mucosa (14, 93). Thus, the vagal influence on secretion is probably relayed within the myenteric plexus. It has been proposed that the vagal nerves innervate a restricted number of "command" neurons, regulating enteric microcircuits (194). Electric stimulation of vagal nerves increases mucosal bicarbonate secretion in cats (49, 138), rats (50, 84) and pigs (52), an effect which is mediated by nicotinic transmission. Sympathetic neural activity, on the other hand, is mainly inhibitory (50). At least in the rat, the postganglionic secretomotor neurons stimulated by vagal activity appear to be mainly non-cholinergic (40, 84), with VIP as a possible transmitter (105).

It is clear that the secretion of bicarbonate is influenced by the central nervous system (CNS). Sham-feeding stimulates duodenal bicarbonate secretion (8, 96) whereas electric stimulation within the hypothalamic region of the brain decreases the secretion (51). Local administration within the CNS of several neuropeptides and drugs have further substantiated the role of central nervous control in the regulation of mucosal bicarbonate secretion (39). In most studies, the secretion of bicarbonate is reduced by physical stress. The mechanism is most probably an α_2-adrenoceptor (sympathetic)-mediated inhibition of basal and vagally stimulated duodenal bicarbonate secretion (50). The duodenal mucosal alkaline secretion is also influenced by circulating catecholamines released from the adrenal glands (84). A variety of other hormones have also been implicated as regulators of duodenal bicarbonate secretion (39, 95).

In the late 1960s the prostaglandins PGE_1 and PGE_2, produced from arachidonic acid by cyclo-oxygenase, were reported to have gastric antisecretory and protective antiulcer effects (155). Since then, the biological actions of prostaglandins in gastrointestinal physiology and pathology have been extensively studied (for review see ref. 192). In several species, endogenous prostanoid synthesis, as well as exogenously applied prostaglandins of the E series, have been shown to stimulate duodenal mucosal bicarbonate secretion both in vitro (41) and in vivo (42, 68, 79). However, it has also been reported that cyclo-oxygenase inhibitors such as indomethacin increase the secretion of bicarbonate (94, 162). This discrepancy has

been attributed to the induction of duodenal motility by inhibition of the synthesis of another prostaglandin, PGI_2 (159, 162).

HCl in the duodenal lumen is a stimulant of duodenal epithelial bicarbonate secretion in several species (39). This response is mediated by neural reflexes, local mucosal production of prostaglandins and possibly the release of humoral factors. The nerve reflex involves capsaicin-sensitive sensory afferents (64, 180) and muscarinic and nicotinic transmission (169), and is modulated by sympathetic tone (85). Recently, nitric oxide has also been suggested as a mediator of this response (16, 72). The presence of acid within the lumen may increase active metabolism-dependent secretion as well as passive migration/ultrafiltration of bicarbonate ions (39).

Duodenal motility

Electric activity of the smooth muscle

There are two types of electrical activity in smooth muscle cells: slow waves and spike potentials. The slow waves, which are believed to be generated by specialized pacemaker cells, the so-called interstitial cells of Cajal (176, 183), are oscillations of the resting membrane potential, while the spike potentials are action potentials superimposed on the slow waves. In general, the slow waves determine the maximal frequency of contractions, whereas the number of spike potentials per slow wave determines the contractile force (11). Voltage dependent Ca^{2+} channels and voltage dependent Ca^{2+} activated K^+ channels are the main pathways of current flow during electric activity of intestinal smooth muscle (163). The activity of these channels can be modulated by excitatory and inhibitory neurotransmitters, resulting in either depolarization or hyperpolarization. In the inner lamella of the circular muscle layer and in the longitudinal muscle, the occurrence of spike potentials is a prerequisite for the development of phasic contractions (122).

Motility patterns

Smooth muscle of the intestine exhibits a variable degree of tone (i.e. sustained contraction) on which are superimposed rhythmic contractions driven by the cyclic oscillations in the membrane potential mentioned above. These rhythmic contractions are usually suppressed by a predominantly inhibitory neural input. Removal of all neural input, both excitatory and inhibitory, restores the intrinsic myogenic rhythm of phasic contractions at the frequency of the slow waves (15, 193).

In the intact animal, the frequency and pattern of contractions differs in different regions of the gastrointestinal tract. Furthermore, in most species the motility is quite dissimilar in the fed state as compared to the interdigestive period. In the fasting state, motor activity changes cyclically and is characterized by long periods of quiescence interrupted by bursts of intense contractile activity. This pattern, proposed to be generated by cyclic disinhibition of smooth muscle activity, is known as the migrating motor complex or MMC (see ref. 190 for review). The motor complexes start in the stomach or in the proximal part of the small intestine and propagate down the intestine, sometimes as far as to the ileocecalic valve. As soon as one complex reaches the terminal ileum, the next one is initiated. In the rat, MMC occur at 15 min intervals (22, 115), whereas in man the mean duration of a full cycle

is 100 min (90, 126). The MMC may be regarded as an intestinal "housekeeper" which propels residual contents, sloughed off cells and intestinal bacteria towards the colon (177).

In contrast to the pattern in the fasted state, postprandial motility is irregular and spread almost uniformly over time (158). The purpose of this type of motility is most probably to improve the mixing of ingested food with fluid and enzymes and to propel the resulting chyme further down the intestine. Increased intestinal motor activity is also believed to reduce the unstirred water layer, thereby facilitating diffusion of nutrients and electrolytes to the absorbing epithelium (26).

Regulation of intestinal motility

The primary unit of contractile activity is a bundle of smooth muscle cells. This unit can contract on its own, and because of the cyclic changes of the membrane potential, its contractions can be quite regular. However, in the intact organism, contractions of the smooth muscle cells are modulated in several ways, for instance by neurotransmitters, or by circulating or locally released factors.

Most of the innervation of intestinal smooth muscle emanates from the myenteric plexus, which is situated between the longitudinal and circular muscle layers. However, the inner lamella of circular muscle receive numerous fibers from the submucous plexus as well (122). There are two types of intrinsic neuronal input regulating intestinal motility; one is excitatory and the other is inhibitory. A majority of excitatory motor neurons contain both ACh and tachykinins (48). Normally the tonic inhibition dominates, and the inhibitory input has to be withdrawn for contractions to occur (193). The nature of the inhibitory neural control of intestinal smooth muscle is non-adrenergic non-cholinergic (NANC) (23). Several putative transmitters have been proposed. The first to be considered was ATP (24), followed by VIP a few years later (31, 37). More recent evidence from an extensive number of pharmacological and electrophysiological studies, along with the demonstration of NOS in myenteric neurons, strongly implicates the involvement of NO in NANC neurotransmission (162, 164, 171). Contribution to inhibitory transmission by pituitary adenylate cyclase activating peptide (PACAP) is also possible (87, 175), as is the involvement of carbon monoxide (137, 152, 186). Other neurotransmitters, such as Neuropeptide Y, somatostatin, opioids and GABA for example, are able to modulate transmitter release either from excitatory or inhibitory motor neurons or both, thereby adjusting contractile activity (122). At present VIP and NO are the strongest candidates to be the primary transmitters of NANC-induced tonic relaxation of intestinal smooth muscle (57, 122, 164). Neural NOS is co-localized with VIP (29) and in some tissues neurally derived NO may primarily relax intestinal smooth muscle by facilitating VIP-release (56). In the rat duodenum both NO and VIP are involved in the NANC-induced relaxation, but the effect of VIP appears to be independent of NO-synthesis (78).

The smooth muscle of the digestive tract operates essentially under enteric nervous control and functions adequately also in the absence of extrinsic influence. However, the extrinsic nerves, which primarily innervate the intramural plexa, play a modulatory role on enteric reflexes and are involved in the integration of motor activity in separate regions of the gut. In general, the motility of the small intestine is predominantly stimulated by activation of vagal nerves and inhibited by sympathetic

activity (91). However, vagal fibers also innervate inhibitory NANC nerves. Hence, in some circumstances stimulation of the vagus inhibits intestinal motor activity (see ref. 157 for review).

Mucosal permeability

One of the major roles of the intestinal mucosa is to limit passive movement of solutes across the epithelium. The capacity to impede such movement is generally referred to as the barrier function of the mucosa. Physiological and morphological studies indicate that the epithelial barrier effectively restricts the passage of some solutes with a molecular radius as small as 3 Å yet allows for limited movement of large molecules such as albumin (36 Å radius) (30, 131). There are two possible pathways by which solutes passively penetrate the epithelium: the transcellular and paracellular pathways. Because of the lipophilic nature of biological membranes, hydrophilic solutes, such as the tracer molecule used throughout this study (^{51}Cr-EDTA), mainly pass the epithelium through the paracellular pathway (120, 185).

The paracellular pathway

The gastrointestinal epithelium consists of a monolayer of cells, each attached to adjacent cells by the junctional complex. This complex is composed of the tight junction and the adherens junction (60). The tight junctions, which consist of a number of "strands" encircling the apices of epithelial cells, are the main structures that restrict the movement of solutes and ions by the paracellular route (149). The "tightness" of the epithelium has been proposed to correlate to the number of junctional strands (28). Hence, the relatively low number of strands in the tight junctions of crypt cells may indicate that the epithelium of the crypts is less tight than that of the villi. Moreover, since the apical part of the crypt cells are narrower than the apices of the absorptive villus cells, the junctional area per unit surface area is larger in the crypts than in the villi. Based on the structural differences between villi and crypt, Marcial et al. predicts that 73% of the paracellular conductance in the ileum should be localized to the crypt region of the epithelium (123).

Factors influencing paracellular transport

There are several factors that influence the passive paracellular transport of a hydrophilic solute across the intestinal epithelium; for instance the concentration gradient, the hydrostatic pressure gradient and the area available for diffusion. The area of the paracellular pathways represents only about 0.01-0.1% of the total surface area of intestinal epithelium (146).

The permeability of the paracellular pathway can be physiologically regulated by alterations of the structure and the electrical charge of the tight junction (120). Although most of the classical second messengers and intracellular signaling pathways have been shown to influence the permeability of the tight junctions (6), the mechanisms involved are not yet fully understood. However, it is believed that the cytoskeleton, which is anatomically and functionally tied to the junctional structure (119), may participate in the process (121). Presumably, elevations of intracellular Ca^{2+} may increase paracellular permeability by contracting actin/myosin filaments (89). Cyclic AMP, on the other hand, has been shown to decrease epithelial

permeability via changes in cytoskeletal organization (34). Mechanisms not involving the cytoskeleton, such as the degree of phosphorylation of tight junctional proteins, may also regulate paracellular permeability (7).

AIMS OF THE STUDY

The general objective of this study was to examine the regulation, with emphasis on neural influences, of some duodenal functions in vivo.
The specific aims were:
-to investigate the relationships between duodenal mucosal alkaline secretion and duodenal motility, mucosal permeability and blood flow,
-to study the involvement of nitric oxide in the regulation of the aforementioned duodenal functions,
-to examine the role of nitric oxide in duodenal protection against acid,
-to investigate the effect of the tachykinin neurokinin A on duodenal mucosal alkaline secretion, mucosal permeability and motility,
-to establish a model for computerized recording and analysis of motility.

METHODS

Animals

All experiments were approved by the Uppsala Ethical Committee for Animal Experiments. Male Sprague-Dawley rats, weighing 200-400 g, were used. The animals were kept under standardized conditions of temperature (21-22° C) and photoperiod (12 h light and 12 h dark). For a period of approximately 18 hours before the start of the experiments, the rats were deprived of food but had free access to drinking water. The rats were anesthetized by an intraperitoneal injection of 120 mg · kg^{-1} body weight Na-5-ethyl-1-(1'-methyl-propyl)-2-thiobarbituric acid (INACTIN®). To avoid stress, the animals were always kept in groups of at least two, and anesthesia was induced by familiar staff. Furthermore, physical restraint was minimized before the anesthetic agent was administered.

During surgical anesthesia the body temperature regulating mechanisms are suppressed. To maintain the temperature of the animal at 37-38° C a heating pad controlled by an intrarectal thermistor was used.

Surgical procedure

To facilitate spontaneous breathing, the rats were tracheotomized by inserting a cannula just below the thyroid gland. The carotid and/or the femoral artery were catheterized for continuous recordings of arterial pressure and for blood sampling. In one series of experiments, a thin catheter was introduced via the left common carotid artery and gently pushed into the thoracic aorta. The tip was positioned proximal to the coeliac trunk and the catheter was used for intraarterial infusion of VIP. One or both femoral veins, and in some experiments also the left external jugular vein, were cannulated for intravenous injection or infusion of various drugs and for infusion of Ringer's bicarbonate solution mixed with the permeability marker

^{51}Cr-EDTA. The composition of the Ringer's solution was: 25 mM $NaHCO_3$, 120 mM NaCl, 2.5 mM KCl and 0.75 mM $CaCl_2$. The Ringer's (1 ml/h) was infused to prevent dehydration and acid-base disturbances which may influence the results.

Laparotomy was performed in all animals. The common bile duct was catheterized very close to its entrance into the duodenum (102) to exteriorize pancreatico-biliary secretions, which would otherwise interfere with the determinations of mucosal bicarbonate output.

A 3 cm segment of the proximal duodenum was cannulated (Fig. 2) by gently introducing a soft plastic tubing via the mouth and esophagus into the stomach and

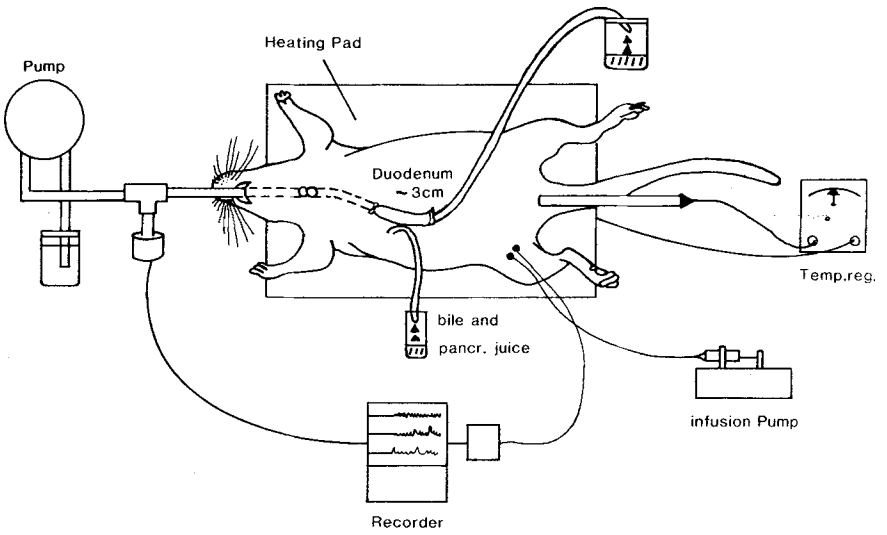

Fig. 2 Experimental setup.

then through the pylorus into the duodenum. This tubing was secured by a ligature 2-4 mm distal to the pylorus. Another cannula was inserted into the duodenum through an incision about 3 cm distal to the pylorus, and secured by ligatures. Care was taken not to tie off blood vessels supplying the segment under study and the adjacent tissues. The orally introduced tubing was connected to a peristaltic pump for perfusion of the duodenal segment. The abdominal cavity was closed with sutures or covered with plastic foil (blood flow experiments) to limit fluid loss from the wound. After surgery, the animals were allowed to recover for at least 1 h to stabilize cardiovascular and gastrointestinal functions.

Experimental design

The duodenal segment was continuously perfused with isotonic saline at a rate of 0.35-0.50 ml/min, during the hour of recovery and then throughout the experiments. In some experiments the saline was substituted for hypotonic saline or supplemented with N-nitro-L-arginine (L-NNA) or lidocaine. In one of the studies the segment was exposed to 50 mM HCl (adjusted to isotonicity) for 5 min. The effluent was collected in 10 min aliquots for analysis. After each experiment the duodenal segment was excised, rinsed and weighed on an electric precision balance for determination of wet tissue weight. The length of the excised tissue was

measured during a standardized stretch, i.e. a fixed weight was attached to one of the ends of the segment.

Duodenal mucosal bicarbonate secretion

The rate of luminal alkalinization was determined by back-titration with 50 mM HCl of the collected luminal effluent samples to the pH of the infused saline (~pH 6.0). The rate of alkalinization was expressed as the amount (micromoles) of base secreted per cm of intestine per hour. The samples were gassed with 100% N_2 for effective mixing and to remove initially present CO_2 as well as that formed during the titration procedure. Even though this technique does not distinguish between different types of alkali, it is most likely that all measured alkalinity in the gassed samples represents HCO_3^-. In an aqueous solution, bicarbonate ions and protons are in equilibrium with carbon dioxide and water and the amount present as HCO_3^- depends on the pH and temperature of the solution. Very high levels of P_{CO_2} have been recorded in duodenal pouches after instillation of HCl (65), reflecting intraduodenal neutralization of acid by bicarbonate.

Fluid flux

The technique used provides information about relative changes of fluid flux across the duodenal mucosa. The effluent weights were determined as weight difference for each vial, empty and with the collected effluent. The mean weight of the effluent samples collected during the control period was subtracted from the weight of each subsequent sample, and the difference was expressed as gram of fluid per gram of wet tissue weight per hour. The method does not distinguish between decreased absorption and increased secretion (positive flux) or vice versa, and does not provide any information on the secretory state during basal conditions. However, Nylander et al. previously reported net absorption of fluid from a duodenal segment perfused with saline (141).

Mucosal permeability

The permeability of the duodenal epithelium was assessed as passage of the radioactive isotope ^{51}Chromium-labelled ethylenediaminetetraacetate (^{51}Cr-EDTA) from blood to lumen. The diffusion of ^{51}Cr-EDTA across capillary walls is unrestricted, and within 10 min after intravenous injection the concentration in interstitial fluid equals that measured in plasma (30). Thus, the rate-limiting barrier for the permeation of the isotope from blood to lumen is the mucosal epithelium (Fig. 3). The hydrophilic properties and the cross-sectional diameter (6.8 Å) of ^{51}Cr-EDTA (82, 112) implies that the water-filled paracellular shunts are the predominant route of epithelial passage (17, 30). ^{51}Cr-EDTA is neither metabolized in the tissue (188) nor taken up by cells (18) and is non-toxic even at high plasma concentrations (2). Duodenal ^{51}Cr-EDTA clearance is neither affected by changes in electrolyte and water transport across the mucosa, nor by duodenal blood flow (139-142). A prerequisite for accurate assessments of mucosal permeability as ^{51}Cr-EDTA passage from blood-to-lumen is the maintenance of a constant concentration gradient of the marker across the epithelium. ^{51}Cr-EDTA is eliminated by the kidneys with a $t_{1/2}$ of less than 40 min (103). To achieve a fairly constant plasma level of ^{51}Cr-EDTA, renal loss was either prevented by ligation of both renal pedicles, or

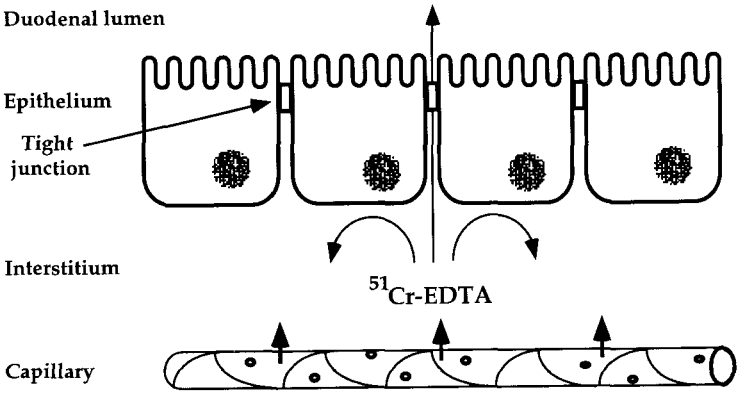

Fig. 3 The permeation of ^{51}Cr-EDTA from blood to duodenal lumen.

compensated for by a corresponding continuous intravenous infusion of the isotope. One hour was permitted for tissue equilibration of the ^{51}Cr-EDTA and for the animal to recover from surgery. Two (ligated kidneys) or 3-4 (continuous infusion experiments) blood samples of ~0.2 ml were collected at regular time intervals during the experiment, and the blood volume loss was compensated for by the injection of a 5-10% albumin or Ficoll® solution. After centrifugation of the samples, 50-100 µl of the plasma was removed for measurement of radioactivity. The luminal perfusate and the blood plasma were analyzed for ^{51}Cr-activity in a gamma counter. The plasma samples were extrapolated by linear regression to obtain a corresponding plasma value for each effluent sample. The clearance of ^{51}Cr-EDTA from blood to lumen, expressed as ml · min^{-1} · 100 g^{-1} wet tissue weight, was calculated according to the following formula:

$$^{51}\text{Cr-EDTA clearance} = \frac{\text{effluent (cpm/ml) x perfusion rate (ml/min)}}{\text{plasma (cpm/ml) x tissue weight (grams)}} \times 100\text{g}$$

Duodenal motility

The motility of the duodenum was assessed by measurement of intraluminal hydrostatic pressure. The inlet cannula of the perfusion system was connected to a pressure transducer by a T-tube. Circular contractions of the perfused segment will increase the resistance to flow. Since the flow rate is constant (set by the pump) the hydrostatic pressure will increase proportionally to the increased resistance (Ohm's law). The motor response was recorded on a Grass polygraph and quantified as the fraction of time occupied by contractions (fractional contraction time, FCT). A positive deflection ≥2 mm Hg was defined as a contraction. In an attempt to further develop the monitoring and analysis of motility the pressure profile was

continuously recorded, via a digitizer, into a computer and further analyzed using the SUPERSCOPE® software. Using the computer, the duodenal motility could be quantified in a number of ways; as FCT, amplitude or frequency of contractions, mean intraluminal pressure and area under the curve.

Previous experiments have shown that intraluminal pressure and myoelectric activity are highly correlated (10, 11). Also in the perfusion model used in the present investigation (Nylander et al., unpublished observation) correlation was excellent. The method of monitoring intraluminal pressure to assess duodenal motility is thereby validated. Furthermore, it was shown that the intermittent flow of the peristaltic pump or the depth and frequency of respiration does not affect the recording of intraluminal pressure (Hällgren et al, unpublished observation).

Mucosal blood flow

In some experiments the blood flow of the duodenal wall was determined by laser-Doppler flowmetry (LDF). The operating principle of LDF is based on the fact that monochromatic laser light is spectrally broadened when scattered by moving objects such as red blood cells, whereas light beams scattered in static structures remain unshifted in frequency (135). The laser-Doppler method permits continuous linear measurements of blood flow and is highly correlated to estimates by other techniques (1, 99). The laser light is guided to the tissue by an optical fiber of 0.7 mm diameter and the back-scattered light is picked up by a pair of equal-sized fibers. The magnitude of the Doppler shift from the illuminated tissue depends on the velocity and number of moving blood cells (134, 136). With this technique, a voltage output is obtained and blood flow is expressed in relative terms, as percent of baseline values. The probe is attached to a micromanipulator and adjusted to a distance estimated at ~1 mm from the serosal side of the duodenum. The blood flow is most probably measured through the entire thickness of the duodenal wall. However, using the microsphere technique, Jönson et al. showed that the muscle layers of the duodenum receive less than 5% of total duodenal blood flow (83). It follows that the laser Doppler signal, even when monitored from the serosal side, mainly estimates the blood flow of the duodenal mucosa and submucosa.

There are some drawbacks to the method, however. For instance, recordings of artifacts due to motion between probe tip and tissue surface and loss of optical coupling between probe and bowel are problems that arise when using LDF.

Histological evaluation

To assess mucosal damage after acid exposure a morphological examination was performed. Promptly upon termination of the experiment the duodenal segment was excised and fixed in a 10 % neutral buffered formalin solution. Longitudinal specimens were taken from the duodenal segment. The specimens were embedded in paraffin, dehydrated, cut in sections and stained with hematoxylin-eosin. Mucosal damage was assessed both quantitatively and qualitatively using light microscopy. The structural changes were quantified by scoring the damage on a five-graded scale (106). The total lesion score for each specimen was determined by multiplying each grade with the number of villi scoring that particular grade. The morphological examination was performed by an experienced pathologist who was unaware of the experimental procedure.

RESULTS

Relationship between duodenal mucosal alkaline secretion, permeability and blood flow

The secretion of bicarbonate may depend on the delivery of bicarbonate to the interstitium by the blood. Hence, there is a possibility that marked changes in duodenal blood flow influences luminal alkalinization. Furthermore, an increased paracellular permeability may result in increased luminal alkalinization by filtration and/or passive diffusion of bicarbonate.

The NOS inhibitor L-NNA, given as an intravenous infusion or intraluminally, significantly increased the rate of alkalinization of the duodenal lumen. A concomitant rise in fluid secretion was also observed. In intravenously infused animals, arterial blood pressure was significantly elevated, whereas mucosal blood flow decreased. These changes reflect increased vascular resistance within the mucosa. However, in the group given L-NNA intraluminally, the vascular effects were delayed and less pronounced. Arterial pressure increased slightly and slowly, and was significantly elevated only towards the end of the experiment, while a small, but not statistically significant, decrease in blood flow was obtained in response to intraluminal L-NNA.

The secretagogue VIP increased both fluid and bicarbonate secretion, whereas mucosal permeability decreased in response to the peptide. Arterial blood pressure and vascular resistance were also decreased by VIP, but the changes in mucosal blood flow were inconsistent. In four out of ten animals there was a clear (>10%) decrease in duodenal mucosal blood flow. In the group as a whole, only small and irregular decreases were obtained.

The concentration of base in the net fluid output was calculated and compared to that in plasma (assumed to be 24 mM). L-NNA, administered systemically or locally, as well as VIP, increased fluid secretion. The resulting net fluid output contained a higher concentration of bicarbonate than in interstitial fluid. Interestingly, in rats given a lower dose of VIP (2.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$), not affecting the vasculature, the concentration of bicarbonate in the secreted fluid was almost twice that obtained in response to 13.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$ VIP.

Both the nicotinic receptor antagonist hexamethonium and the α_1-adrenoceptor antagonist prazosin, significantly decreased arterial pressure, mucosal permeability and mucosal bicarbonate secretion. In addition, treatment with hexamethonium resulted in a slight and irregular increase in duodenal mucosal blood flow, whereas prazosin substantially decreased blood flow. Neither drug altered fluid output.

In a final series of experiments, blood was withdrawn to decrease arterial blood pressure in two steps, to 20 and 40 mm Hg below the initial pressure. Blood withdrawal resulted in decreases in both mucosal permeability and alkaline secretion.

No causal relationships between bicarbonate secretion and blood flow, or bicarbonate secretion and mucosal permeability were found. A positive linear relationship between mucosal permeability and arterial blood pressure, however, is suggested by these findings.

Effects of nitric oxide inhibition on duodenal function

The presence of the NO-synthesizing enzyme in a variety of neurons within the enteric nervous system implies a role for NO in the regulatory control of the intestine. To investigate the involvement of endogenous NO in duodenal function, the competitive NOS-inhibitor N-nitro-L-arginine methyl ester (L-NAME) was given intravenously in three consecutively increasing doses. The reversibility of the response was examined by injection of L-arginine, the natural substrate for NOS.

L-NAME induced duodenal motility and significantly increased arterial blood pressure, mucosal permeability to ^{51}Cr-EDTA, and the secretions of bicarbonate and fluid. Injection of L-arginine abolished the L-NAME induced motility and significantly diminished both bicarbonate and fluid secretion. On the other hand, the effect of L-arginine on arterial pressure and mucosal permeability was transient, and in the case of permeability, not statistically significant.

Pretreatment with hexamethonium almost totally abolished the response to L-NAME on duodenal motility and prevented the effect on luminal alkalinization. In contrast, although hexamethonium decreased arterial pressure the NOS-inhibitor still caused a net increase in mucosal permeability and arterial pressure similar to the net increases in the group given L-NAME alone.

The noradrenalin-depleting drug guanethidine did not affect any of the responses to L-NAME. In contrast, the muscarinic antagonist atropine significantly attenuated the response to L-NAME on mucosal permeability. The effects on bicarbonate secretion and arterial pressure were unaltered. The duration of the motility response after a single dose of L-NAME was reduced in rats pretreated with atropine.

In conclusion, duodenal smooth muscle activity in rats subjected to abdominal surgery may be tonically suppressed by NO. The removal of NO reveals an excitatory input to the intestinal smooth muscle, involving both cholinergic and non-cholinergic nerves. NO inhibits bicarbonate secretion by suppression of an excitatory, nicotinic receptor-dependent, neural mechanism. Finally, stimulation of muscarinic receptors mediates part of the L-NAME induced increase in mucosal permeability and fluid secretion.

Acid-induced increases in mucosal permeability

Acidic gastric juice is regularly disposed into the duodenum. To investigate whether NO alters the susceptibility of the duodenal epithelium to luminal acid, rats were treated with the NOS-inhibitor L-NAME. Mucosal integrity was assessed as the permeation of ^{51}Cr-EDTA from blood to lumen and by histological evaluation. Exposure of the duodenal segment to 50 mM HCl markedly but reversibly increased mucosal permeability to ^{51}Cr-EDTA. This increase was substantially augmented by L-NAME- or vasopressin treatment. Both these treatments are believed to interfere with vascular integrity. Furthermore, the increase in response to acid was markedly greater in the vasopressin-treated rats than in the group given L-NAME. Neither hexamethonium nor atropine altered the response to acid. However, combined with atropine or hexamethonium, L-NAME failed to augment the acid-induced increase in mucosal permeability. Lastly, perfusion of the duodenal segment with hypotonic

(25 mM) saline did not alter the response to acid.

Only very subtle changes, or no changes at all, were observed in the morphological examination of the acid-exposed tissue. Mild damage only, and to less than 12% of the villi, was observed in three groups: L-NAME alone, L-NAME and hexamethonium, and in the vasopressin-treated group.

Mucosal secretion of bicarbonate was also determined. Two of the groups, one treated with L-NAME and another treated with L-NAME in combination with atropine, differed from the control group with significantly elevated alkaline output.

It is concluded that endogenous NO is involved in the protection of the duodenal mucosa possibly by the regulation of vascular permeability. The mechanism may involve the suppression of cholinergic nerves. A moderate increase in mucosal permeability *per se* does not necessarily increase the susceptibility to acid-induced damage. The duodenal mucosal bicarbonate secretion, basal or stimulated by L-NAME, appears to be insufficient in preventing disturbances of duodenal mucosal integrity induced by 50 mM HCl in anesthetized rats.

Neurokinin A stimulates bicarbonate and fluid secretion and increases mucosal permeability

The tachykinins, of which substance P is the most extensively studied, are involved in the control of intestinal motility and the secretion of electrolytes. To examine the effects of one of the tachykinins, neurokinin A (NKA), on duodenal function, NKA was infused i three different doses. NKA induced duodenal motility of dose-dependent intensity. Duodenal mucosal bicarbonate secretion and the output of fluid also increased in response to the tachykinin although these responses were not dose-related. Rather, the net increase in bicarbonate output tended to decrease with larger doses of NKA. However, mucosal permeability was dose-dependently increased in response to NKA. Pretreatment with hexamethonium did not alter any of the effects of NKA, whereas the neurokinin-2 (NK-2) receptor antagonist MEN 10,627 efficiently inhibited all studied effects of the tachykinin.

The previously reported ability of indomethacin to induce duodenal motility and to stimulate duodenal mucosal alkaline secretion in anesthetized rats (162) was confirmed. In rats treated with indomethacin, the infusion of NKA further increased motility (Fig. 4), but decreased indomethacin-stimulated bicarbonate secretion. The NKA-induced increase in mucosal permeability was unaltered by indomethacin.

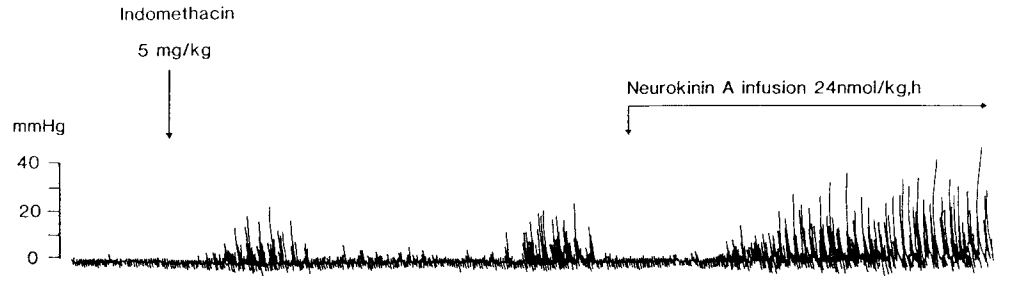

Fig. 4 Recording of intraluminal pressure in a rat given indomethacin followed by NKA.

These results suggest that NKA, apart from inducing duodenal motility, may have both a stimulative and an inhibitory action on duodenal epithelial bicarbonate secretion. The mechanism responsible for the NKA-induced increase in mucosal permeability may involve alterations of vascular permeability. All responses to NKA are dependent on activation of NK-2 receptors, but are not mediated by nicotinic transmission.

Modulation of neurokinin A-induced increases in mucosal permeability and alkaline secretion

In an attempt to further investigate the mechanisms involved in the NKA-induced increase in mucosal permeability, the duodenum was perfused with the local anesthetic lidocaine. Lidocaine did not alter basal permeation of ^{51}Cr-EDTA across the epithelium, but significantly potentiated the response to NKA. Furthermore, the effect of NKA on mucosal permeability was diminished by VIP. Elevation of intraluminal pressure, either alone or combined with indomethacin treatment, did not alter the permeability response to the tachykinin.

The stimulative effect of NKA on mucosal bicarbonate secretion was potentiated in rats subjected to elevated intraluminal pressure. In contrast, in the group subjected to increased intraluminal pressure in combination with indomethacin, NKA diminished the bicarbonate output. Likewise, but to a lesser degree, the alkaline secretion stimulated by VIP was also reduced by the tachykinin. Luminal lidocaine decreased basal secretion of bicarbonate, whereas the response to NKA was unaltered.

The duodenal motility induced by NKA was slightly decreased by VIP or lidocaine, but these changes did not attain statistical significance. The motor response was, however, diminished by elevation of intraluminal pressure. The capacity of NKA to induce motility in rats subjected to elevated intraluminal pressure was partly restored by indomethacin.

It is concluded that the NKA-induced increase in duodenal mucosal alkaline secretion is independent of mucosal neurons and possibly mediated by prostanoids. In contrast, the inhibitory effect is probably exerted within the enteric nervous system. The increase in mucosal permeability in response to NKA is suppressed by mucosal nerves, possibly utilizing VIP as a transmitter.

DISCUSSION

Duodenal mucosal bicarbonate secretion

Active transcellular or passive paracellular transport of bicarbonate?
Experiments performed in amphibian duodenal mucosa in vitro indicate that the alkaline secretion is due partly to active, energy-consuming transcellular transport, but also involves passive diffusion or filtration of bicarbonate (41, 167). In mammals in vivo, however, basal (unstimulated) secretion of bicarbonate is predominantly due to active transport (68, 143). In the first study the concentration of bicarbonate in the net fluid secreted in response to VIP was well above that in plasma, indicating

that this secretagogue mainly stimulates active bicarbonate transport. Interestingly, the concentration of bicarbonate was more than twofold higher in response to a low dose of VIP, as compared to a higher dose of the peptide. It is possible that in small amounts VIP activates an electroneutral transport process, i. e. HCO_3^-/Cl^- exchange, whereas in response to higher doses, electrogenic anion transport, accompanied by fluid transport, is also stimulated. In fact, VIP has been reported to increase bicarbonate output via simultaneous activation of both the electrogenic transporter CFTR and HCO_3^-/Cl^- exchange (69, 196).

Further evidence that VIP stimulates an active rather than passive transport process is provided by the finding that the paracellular permeability to ^{51}Cr-EDTA decreases in response to VIP. In contrast, inhibition of NOS either by L-NNA or L-NAME increased bicarbonate secretion and mucosal permeability in parallel. However, a major contribution by passive paracellular transport of bicarbonate to the secretory response to L-NAME is unlikely for the following reasons: 1. The calculated concentration of bicarbonate in the net fluid secreted in response to L-NNA or L-NAME was significantly higher than that in plasma; 2. Intraluminal administration of L-NNA increased the rate of alkalinization to a similar extent as did systemic administration, but mucosal permeability was unaltered; 3. The nicotinic receptor antagonist hexamethonium did not prevent the L-NAME-induced increase in mucosal permeability but abolished the alkaline response, whereas the muscarinic receptor antagonist atropine diminished the increase in mucosal permeability without altering the L-NAME-induced secretion of bicarbonate.

NKA, like L-NAME, increased mucosal permeability and bicarbonate secretion concomitantly, but the concentration of bicarbonate in the net secreted fluid was less than that in plasma. However, the rate of alkalinization and the permeability to ^{51}Cr-EDTA did not correlate inasmuch as increasing doses of NKA further increased permeability while the secretory response tended to decrease. Furthermore, in rats receiving indomethacin, NKA decreased the alkaline secretion but increased mucosal permeability. A dissociation between permeability and alkaline secretion was also noted in animals subjected to elevated intraluminal pressure or luminal lidocaine. Hence, active transcellular transport of bicarbonate is implicated as the mechanism responsible for the stimulative effect of NKA on duodenal mucosal alkalinization. Possibly, NKA may also stimulate electrogenic secretion of Cl^-, thereby influencing fluid transport.

In summary, a dissociation in the control of active transcellular transport of bicarbonate from that of passive paracellular diffusion of solutes is suggested. Under physiological circumstances, the relative importance of passive paracellular transport of bicarbonate may be insignificant. However, in epithelial damage the ultrafiltration/diffusion of bicarbonate is increased and is probably important in the repair process (172).

Dependency on local blood flow

Some previous studies have shown that duodenal mucosal bicarbonate secretion is independent of mucosal blood flow (71, 83) whereas other investigators have suggested that local perfusion, and thus the delivery of bicarbonate, is a limiting factor in epithelial alkaline secretion (172). We demonstrate that a high rate of duodenal bicarbonate secretion, induced by the secretagogue VIP or by NOS

inhibition, can be maintained despite a reduction of mucosal blood flow. Furthermore, a decreased rate of alkalinization, such as that induced byhexamethonium, is not necessarily related to changes in vascular perfusion. A lack of causal relationship between moderate changes in blood flow and bicarbonate secretion is indicated. Yet, under extreme conditions, such as severe acidosis or hemorrhagic shock, active secretion of bicarbonate may be compromised (173).

Is there a link between duodenal motility and bicarbonate secretion?
Sababi and Nylander recently proposed that distension of the duodenum or the induction of duodenal motility (by cyclo-oxygenase inhibition), stimulates duodenal mucosal bicarbonate secretion by a nicotinic receptor-dependent mechanism (162). It has also been noted that the few rats exhibiting spontaneous motility have a significantly elevated basal rate of alkalinization (159). As demonstrated in the present investigation, NOS-inhibition induced duodenal motility and increased bicarbonate secretion concomitantly, effects which were abolished by hexamethonium. Accordingly, the L-NAME-stimulated alkalinization may also be related to duodenal contractile activity. It is proposed that duodenal motility stimulates a mechanoreceptor, thereby activating a secretory reflex resulting in increased secretion of bicarbonate. Similar motility-linked secretory reflexes have previously been postulated as occurring in various parts of the intestine (54). In proximal duodenum of conscious dogs, for example, basal mucosal bicarbonate secretion varies with the phases of the migrating motor complex, and late phase II and/or phase III of the MMC are associated with increased bicarbonate secretion (96). An increased phase III-related bicarbonate secretion has also been reported in human duodenum (187). However, in the distal duodenum of humans the motility-related electrogenic anion secretion is probably due to active secretion of chloride rather than bicarbonate (126, 168). Variations in the potential difference over the mucosa, indicative of electrogenic ion transport, also co-varies with myoelectric activity or intraluminal pressure in the jejunum (55, 153).

Although a growing body of evidence supports a relationship between duodenal motility and the secretion of bicarbonate it is not known whether this relation is due to simultaneous activation of parallel but separate mechanisms or sequential events. Evidence for parallel stimulation of motility and secretion comes from the experiments with L-NAME, which slightly but significantly increased the rate of alkalinization even at the lowest dose tested although no motility was registered. Furthermore, in atropine-treated rats the motility response to a single dose of L-NAME was transient (~20 min), while the alkaline response was unaltered by the muscarinic receptor antagonist and remained elevated for one hour. It should be mentioned, however, that our findings conflict with those of Takeuchi et al., who reported that atropine diminished the alkaline secretory response to L-NAME by ~50% (182). In a preparation where the duodenum was opened and the lumen exposed to a fixed (atmospheric) pressure, Sababi et al. demonstrated a 40% increase in mucosal alkalinization in response to L-NNA (160). Possibly, two separate excitatory nicotinic receptor-dependent neural pathways are operative in the NO-depleted rat, one that is related to induction of duodenal motility and one that is independent of motility. Both these neural pathways may stimulate a common non-cholinergic secretomotor neuron (Fig. 5).

To further investigate the relationship between motility and bicarbonate secretion, the tachykinin NKA, known to stimulate duodenal motility in the rat in

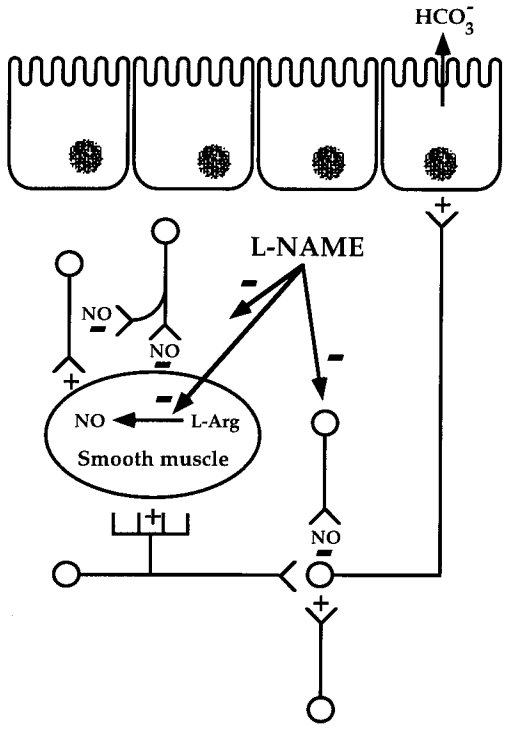

Fig. 5 Schematic drawing of some of the possible sites of action of the NOS-inhibitor L-NAME. L-NAME is suggested to stimulate duodenal epithelial bicarbonate secretion partly via the induction of duodenal motility but also by removal of inhibitory influences exerted by NO at excitatory, nicotinic receptor-dependent, secreto-motor neurons. To suppress duodenal motility, NO may be synthesized from L-arginine (L-arg) within the smooth muscle cells, or released from inhibitory motor neurons, or from interneurons interfering with excitatory transmission to the muscle.

vivo (115), was used. As expected, NKA dose-dependently stimulated motility. Duodenal bicarbonate secretion was also stimulated, but only moderately and with a tendency to decrease with an increase in dose. Neither hexamethonium nor lidocaine altered the response to NKA. These results indicate that the bicarbonate-stimulating action of the tachykinin is mediated by a non-neural mechanism and not activated by a motility-induced reflex. Should the general hypothesis of a causal relationship between duodenal motility and mucosal bicarbonate secretion be rejected based on the findings with NKA? To reject this hypothesis, it has to be assumed that NKA only influences bicarbonate secretion via induction of motility. However, the effect of NKA on luminal alkalinization includes both a stimulative effect and an inhibitory action. The latter was observed in rats treated with indomethacin, or indomethacin combined with elevated intraluminal pressure. In contrast, in rats subjected to elevated intraluminal pressure but not treated with indomethacin, the effect of NKA on luminal alkalinization was significantly potentiated. We speculate that NKA inhibits the motility-activated secretory reflex but not the one activated by distension (Fig. 6). Some differences between the two assumed reflexes have in fact been demonstrated. The distension-induced secretion is vagally mediated whereas the effect of indomethacin was unaltered by vagotomy (162). Moreover, distension did not further increase indomethacin-stimulated alkaline secretion. Although not suggested at the time, this may indicate that the distension-induced secretion involves a nicotinic receptor-mediated increase in prostaglandin synthesis (Fig. 6). There are at least two

types of sensory receptors that may be involved in secretory reflexes: in series tension receptors located within the intestinal muscle layer, and mucosal pressure sensitive mechanoreceptors (58). Possibly, one population of sensory receptors is stimulated by distension and the other by motility.

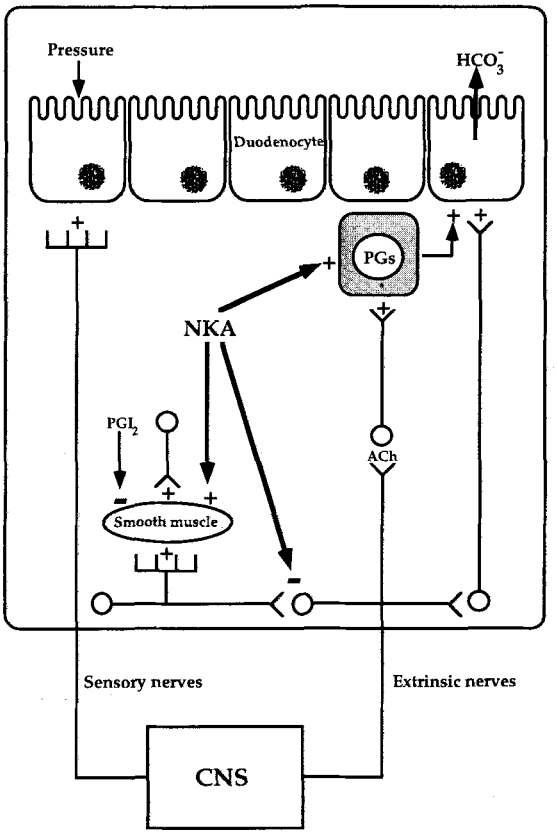

Fig. 6 Schematic drawing of the proposed mechanism of action of NKA on duodenal mucosal bicarbonate secretion. NKA stimulates duodenal motility and thereby activates the proposed reflex to the bicarbonate-secreting enterocytes. However, the signal transmission within the reflex arc is concomitantly inhibited by the tachykinin. The stimulative action of NKA may at least in part be attributed to the release of endogenous prostaglandins. The distension-induced secretion, which is not inhibited by NKA, may also involve a prostaglandin-dependent step. Hence, in indomethacin-treated animals neither NKA nor distension stimulates the bicarbonate secretion, and the inhibitory action of the tachykinin, presumably exerted on the motility-activated secretory reflex, is uncovered.

Neutralization of luminal acid

Mucosal secretion of bicarbonate has been shown to prevent or limit damage and to facilitate the repair process once damage has occurred (3, 142, 147, 172). However, despite a significantly elevated bicarbonate secretion in L-NAME-treated animals the acid-induced increase in mucosal permeability and the histology score were augmented. Furthermore, in L-NAME-treated rats pretreatment with hexamethonium normalized both the alkaline secretion and the response to acid. Thus, neither basal nor stimulated bicarbonate secretion is sufficient to prevent disturbances of mucosal integrity induced by 50 mM acid. This is consistent with the finding that H^+ concentrations above 10 mM acidify the villus tip epithelium (145). Although the increased bicarbonate secretion in response to L-NAME may be expected to protect the mucosa against acid, it should be remembered that inhibition

of NO synthesis results in various, potentially harmful, changes in other functions. Furthermore, the concentration used (50 mM=pH 1.3) is more acidic than the postprandial pH (range 1.5-4.0, mean 2.4) in the proximal duodenum of conscious rats (107).

Duodenal motility

Postoperative ileus

In the fasting state, conscious rats exhibit MMC-like myoelectric activity (115). In contrast, in the anesthetized rat during the hours immediately after abdominal surgery, the contractile activity of duodenal circular muscle is sparse, or, in most cases, completely absent. The mechanisms behind this condition, which is commonly referred to as postoperative ileus, are not completely understood (109). The anesthesia is inhibitory but this effect seems to be of rather short duration (22). In fact, intraperitoneally injected Inactin® (the means of anesthesia used throughout this work) inhibited myoelectric activity (spike potentials), but only for 20 min (Sababi and Nylander, personal communication). Instead, the paralysis is probably induced by the abdominal surgery. Laparotomy, exposure of the intestine to air and handling of the gut all contribute to inhibit intestinal motility for several hours (22). In the present study, L-NAME was shown to induce duodenal motility. It follows that excessive NO-formation, possibly related to the surgical trauma, may be participatory in the postoperative paralysis of the intestine. The relative contribution of NO to the development of this condition is probably of less relevance than that of endogenous prostaglandins since the motility induced by indomethacin exceeds that induced by L-NAME (161). Other factors, such as upregulation of the sympathetic activity (47), or increased VIP release (36), may also contribute to postoperative ileus.

Induction of motility

The duodenal motility induced by L-NAME is entirely dependent on neural activity. The excitatory mechanism, uncovered by NO-depletion, involves nicotinic transmission and is partly dependent on muscarinic receptor activation. However, the muscarinic component was not apparent during the first 20 min after a single dose of L-NAME. Thus the motility induced by NOS-inhibition may consist of two phases, an initial atropine resistant phase followed by an atropine sensitive phase. Possibly, the atropine resistant excitatory mechanism involves a tachykinin, as indicated by experiments on rat duodenum in vitro (125). The involvement of cholinergic motor neurons in the excitatory response to NOS-inhibitors is supported by a study by Calignano et al., who reported that atropine in fairly high doses reduces, but does not abolish, the contractile effects of L-NAME in the rat jejunum in vivo (25). In contrast, Gustafsson and Delbro showed that in vagotomized rats treated with guanethidine and α- and β-adrenoceptor antagonists, the duodenal "hypermotility" induced by L-NNA was not altered by subsequent administration of atropine (61). Atropine was also able to induce duodenal motility, and the existence of non-cholinergic excitatory motor neurons, suppressed by ACh and NO, was suggested.

NKA is the dominant and most potent of the tachykinins in stimulating rat duodenal contractions (92, 115, 129, 144). The effect of NKA has been reported to be due mostly to direct activation of smooth muscle cells, but also partly to stimulation

of cholinergic neurons (9). In our experimental setup, the contractile response was not altered by either hexamethonium or lidocaine, indicating that in the rat duodenum NKA exerts most of its effect directly at the smooth muscle.

To summarize, the existence of both cholinergic and non-cholinergic excitatory motor neurons, which are dependent on nicotinic transmission and suppressed by NO, is proposed. Interestingly, NO has been shown to suppress the release of excitatory transmitters: the release of substance P from enteric nerves of the guinea pig ileum is inhibited by NO (62) and NO suppresses the release of ACh from neurons of canine deep muscular plexa (73).

Motility patterns

An interesting observation is that the motility unmasked by L-NAME (or indomethacin) resembles the interdigestive pattern usually observed in conscious rats (i.e. MMC-like). However, the mean duration of a full cycle is considerably shorter than in conscious untreated animals (~6 min as compared to 15 min) (115, 159). Interestingly, inhibition of NO-synthesis shortens the duration of MMC cycles in the conscious dog (117, 165) and in the rat (67, 156) whereas the length of a full cycle was prolonged by NO (67). At higher doses of NO, a fed-like irregular pattern is induced (67, 156). Based on these data NO has been suggested to be involved in the regulation of the MMC rhythm.

The maximal frequency of phasic contractions is governed by, and can not exceed, the frequency of the slow waves. The highest dose of NKA induced intense motility with some contractions generating an intraluminal pressure above 40 mm Hg. The mean frequency was 16±1 min^{-1} which is well below the reported frequency of duodenal slow waves (43±1 min^{-1}) in conscious rats (74). It is not known whether the slow wave frequency is affected by anesthesia. Nevertheless, the contraction frequency induced by NKA is clearly submaximal compared to that induced by indomethacin, which occasionally exceeds 30 min^{-1} (Hällgren et al., unpublished observation).

In the last study, the recording and analysis of motility was performed on a computer enabling quantification in a number of ways: as FCT, amplitude or frequency of contractions, mean intraluminal pressure and area under the pressure curve. FCT was significantly lower when assessed by the computer ($P<0.001$) as compared to manual measurements. This is due to a considerably higher resolution of the pressure profile, revealing "gaps" between contractions otherwise overlooked (Fig. 7). The computerized model constitutes a potentially useful tool in future quantitative and qualitative analysis of intestinal motility.

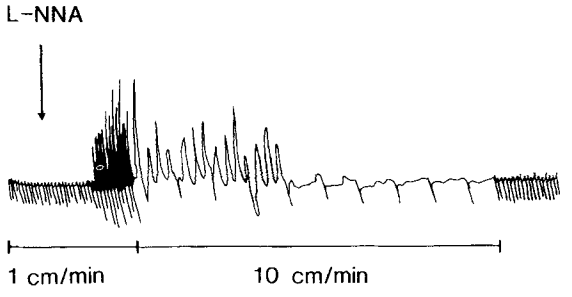

Fig. 7 A recording of intraluminal pressure, using two different recording speeds, in a rat treated with L-NNA.

Mucosal permeability

Microcirculation and epithelial paracellular permeability

In the first study a positive correlation between the relative change in mucosal permeability to ^{51}Cr-EDTA clearance and the change in mean arterial blood pressure was obtained. However, alterations of arterial blood pressure *per se* does not explain all of the changes in mucosal permeability. For instance, the L-NAME-induced increase in paracellular permeability was diminished by atropine despite a sustained elevation of arterial pressure. Moreover, after a single dose of L-NNA, mucosal permeability was transiently increased while arterial pressure remained elevated.

Perhaps a more plausible relationship between microcirculatory events and epithelial permeability is that any factor that markedly changes the vascular fluid filtration rate will modulate epithelial permeability. The transcapillary fluid flux is determined by the hydrostatic pressure gradient across the capillary wall and the difference in oncotic pressure between blood and interstitial fluid. The agents which increased mucosal permeability in the present study may all increase the filtration of fluid across the capillaries. For instance, in the feline ileum, L-NAME increases the vascular permeability (98). Likewise, in the rat duodenum, the extravasation of plasma proteins is increased in response to NKA (114). It was recently shown that a vasopressin antagonist partly prevents the endotoxin-induced plasma leakage in L-NAME-treated rats (104), thus providing indirect evidence that vasopressin increases vascular permeability. Luminal acid has also been shown to increase extravasation of plasma (114). In contrast, hypovolemia, which decreases capillary filtration and dehydrates the interstitium (53), diminished mucosal permeability.

How does an increase in vascular filtration translate into increased epithelial permeability? According to the literature, a small change in interstitial fluid volume has a great impact on interstitial fluid pressure. In vitro, excess serosal pressures (above 4 cm H_2O) can induce fluid filtration (63). However, convective forces, moving solutes and fluid down a hydrostatic pressure gradient, or by solvent drag in actively secreting epithelia, are not likely to be involved in the observed alterations of epithelial permeability for the following reasons: Firstly, in paper V, NKA increased mucosal permeability to a similar extent also in rats subjected to an elevated intraluminal "counterpressure". Secondly, Kubes showed that L-NAME increases mucosal permeation of ^{51}Cr-EDTA in both directions i. e. also from lumen to blood (97). Kubes also noted that a net fluid absorption was maintained during L-NAME-infusion. Moreover, Nylander et al. demonstrated that mucosal permeability to ^{51}Cr-EDTA is independent of the osmolarity of the luminal solution (141) and hence the net fluid transport across the mucosa (128).

Another explanation to the proposed relation between vascular and epithelial permeability has to be sought. Bentzel et al. have demonstrated that the permeability of the tight junctions is altered upon pressure/volume changes in the intercellular space. This process may be purely mechanical, pulling the junctional structures apart, or involve intracellular mechanisms triggered by the pressure or mechanical tension at the basolateral membrane (13). We propose that an increased vascular filtration elevates the hydrostatic pressure of the interstitium and, by altering the epithelial junctional structures, facilitates paracellular diffusion. Further support for a relationship between interstitial fluid pressure and epithelial permeability is

provided by a study by Yablonski et al., showing that elevation of venous pressure increases the epithelial permeability for inulin and plasma proteins (195). Another possibility, although very speculative, is that the filtration of a blood born factor may affect epithelial permeability. It has been reported that a protein present in plasma has the ability to increase tight junctional permeability of cultured kidney cells (124).

Neural regulation of paracellular permeability?

The fact that tight junctional permeability is influenced by intracellular events gives rise to the possibility that physiological processes, including neural mechanisms, control paracellular permeability. Several findings presented in this thesis indicate neural involvement in the regulation of mucosal permeability. The first evidence is the finding that atropine diminished the L-NAME-induced increase in mucosal permeability. Cholinergic transmission was also involved in the aggravated response to luminal HCl in L-NAME-treated rats. Possibly, NO suppresses cholinergic neurons that either directly or indirectly affect mucosal permeability. The strongest evidence of neural involvement, however, is the finding that lidocaine augmented the increase in mucosal permeability in response to NKA. Hence, the existence of neurons able to counteract increases in mucosal permeability is suggested.

At present, the nature of the proposed inhibitory or permeability stabilizing neurons is not known. However, VIP is a plausible transmitter candidate for this population of nerves. The demonstration of VIP receptors on the basolateral membrane of rat enterocytes (150) and VIP-immunoreactive nerve fibers in proximity to the duodenal epithelium (88) provide the anatomical basis for VIP as a regulator of epithelial permeability. Furthermore, VIP is known to increase cAMP levels within the enterocytes (100) and cAMP has been reported to decrease tight junctional permeability (13, 34). In the present study exogenous VIP decreased basal permeability and prevented the NKA-induced increase. Moreover, in a study by Nylander et al., VIP attenuated the increase in mucosal permeability in response to luminal acid (142). However, this effect may partly reflect the increase in mucosal alkalinization in response to VIP.

Paracellular permeability and mucosal integrity

The nitric oxide formed by constitutively expressed NOS has been suggested to be important in mucosal protection in the stomach (116, 191) and in the small intestine (20, 76). In papers I and II it was shown that inhibition of NO biosynthesis increases duodenal epithelial permeability. An increased mucosal permeability has also been demonstrated in the rat jejunum, possibly due to loss of inhibitory influence on mast cells upon NO-depletion (86), and in the feline ileum (97). However, L-NAME did not alter the permeability of a monolayer of cultured intestinal epithelial cells (86).

As mentioned above, inhibition of NOS greatly augments the acid-induced increase in mucosal permeability to ^{51}Cr-EDTA. It seems reasonable that an elevated basal mucosal permeability could promote the diffusion of H^+ into the lateral intercellular space, thereby making the mucosa more susceptible to acid. In fact, Chen et al. have shown that the sensitivity of gastric monolayers to apical acidification is greatly enhanced by increases in tight junctional permeability (27). Vasopressin, which increases basal mucosal permeability by 70%, also potentiated the effect of HCl. However, the evidence that moderate changes in epithelial permeability augment

the mucosal susceptibility to acid are ambiguous. Perfusion of the duodenal segment with hypotonic saline (25 mM) which, like L-NAME and vasopressin, also increases paracellular permeability, did not alter the acid-induced response. In contrast to L-NAME and vasopressin, however, luminal hypotonicity promotes net absorption of fluid across the epithelium and across the capillary wall (53, 128). We speculate that the enhanced susceptibility to acid in L-NAME and vasopressin-treated animals involves interference with the regulation of vascular permeability.

Despite large increases in mucosal permeability in response to HCl, no, or only very superficial damage, limited to the villus tips only, was observed by histological examination of tissue samples. Possibly, the changes seen in ^{51}Cr-EDTA clearance, but not visible by light microscopy, involve functional alterations or reversible damage of the tight junctional structures. Interestingly, perfusion of the duodenum with EGTA, an agent that removes extracellular Ca^{2+} and thereby dissociates the junctional complex (33), increased duodenal mucosal permeability to ^{51}Cr-EDTA (141). Taken as a whole, the results presented provide further support for the use of mucosal permeability to ^{51}Cr-EDTA to detect functional changes and/or mild reversible damage to the epithelial cells and/or the junctional structures.

CONCLUSIONS

Luminal alkalinization is not affected by moderate changes in blood flow and is not related to epithelial paracellular permeability to solutes the size of ^{51}Cr-EDTA. A close relationship between duodenal motility and the secretion of bicarbonate is proposed.
Endogenous NO is suggested to inhibit duodenal mucosal bicarbonate secretion and motility by suppression of nicotinic receptor-dependent excitatory pathways. Part of the alkaline secretory response to NO-depletion may be related to the induction of duodenal motility. NO may be involved in the regulation of duodenal mucosal permeability and blood flow.

Inhibition of endogenous NO synthesis increased both basal mucosal permeability and the susceptibility to acid. Hence, endogenous NO may be involved in duodenal mucosal protection. However, moderate changes in mucosal permeability *per se* do not necessarily affect the mucosal sensitivity to HCl. The mechanism involved may instead be related to changes of the Starling forces in the microcirculatory bed upon NO-depletion.

NKA stimulates duodenal contractions by direct (non-neural) activation of the intestinal smooth muscle. The effect of NKA on luminal alkalinization includes both an inhibitory action, possibly exerted on enteric neurons, and a stimulative effect which may be mediated by endogenous prostaglandins. NKA increases mucosal permeability, an effect which may be related to increased vascular permeability. The NKA-induced increase in mucosal permeability is suppressed by mucosal nerves, possibly utilizing VIP as one of the transmitters.

Computerized collection and analysis of motility data enables detailed assessment of

intestinal motility. The high resolution and the concurrent analysis of several variables provides a powerful tool in the evaluation of intestinal motility profiles.

REFERENCES

1. Ahn, H., J. Lindhagen, G. E. Nilsson, E. G. Salerud, M. Jodal, and O. Lundgren. Evaluation of laser Doppler flowmetry in the assessment of intestinal blood flow in cat. Gastroenterol 88: 951-957, 1985.
2. Ahrens, F. A., and A. L. Aronson. A comparative study of the toxic effects of calcium and chromium chelates of ethylenediamine-tetraacetate in the dog. Toxicol Appl Pharmacol 18: 10-25, 1971.
3. Ainsworth, M. A., C. Fenger, P. Svendsen, and O. B. Schaffalitzky de Muckadell. Effect of stimulation of mucosal HCO3- secretion on acid-induced injury to porcine duodenal mucosa. Scand J Gastroenterol 28: 1091-1097, 1993.
4. Ainsworth, M. A., M. A. Koss, D. L. Hogan, and J. I. Isenberg. Higher proximal duodenal mucosal bicarbonate secretion is independent of Brunner's glands in rats and rabbits. Gastroenterol 109: 1160-1166, 1995.
5. Allen, A., G. Flemström, A. Garner, and E. Kivilaakso. Gastroduodenal mucosal protection. Physiol Rev 73: 823-857, 1993.
6. Anderson, J. M., and C. M. van Itallie. Tight junctions and the molecular basis for regulation of paracellular permeability. Am J Physiol 269: G467-G475, 1995.
7. Balda, M. S. Intracellular signals in the assembly and sealing of tight junctions. In: Tight junctions, edited by M. Cereijido. Boca Raton, Florida: CRC Press, Inc., 1992, p. 121-137.
8. Ballesteros, M. A., J. D. Wolosin, D. L. Hogan, M. A. Koss, and J. I. Isenberg. Cholinergic regulation of human proximal duodenal bicarbonate secretion. Am J Physiol 261: G327-G331, 1991.
9. Bartho, L., and P. Holzer. Search for a physiological role of substance P in gastrointestinal motility. Neuroscience 16: 1-32, 1985.
10. Bass, P., C. F. Code, and E. H. Lambert. Motor and electric activity of the duodenum. Am J Physiol 201: 287-291, 1961.
11. Bass, P., and J. N. Wiley. Effects of ligation and morphine on electric and motor activity of dog duodenum. Am J Physiol 208: 908-913, 1965.
12. Bendtsen, F., B. Rosenkilde-Gram, U. Tage-Jensen, L. Ovesen, and S. J. Rune. Duodenal bulb acidity in patients with duodenal ulcer. Gastroenterol 93: 1263-1269, 1987.
13. Bentzel, C. J., C. E. Palant, and M. Fromm. Physiological and pathological factors affecting the tight junction. In: Tight Junctions, edited by M. Cereijido. Boca Raton, Florida: CRC Press, Inc., 1992, p. 151-173.
14. Berthoud, H. R., A. Jedrzejewska, and T. L. Powley. Simultaneous labeling of vagal innervation of the gut and afferent projections from the visceral forebrain with dil injected into the dorsal vagal complex in the rat. J Comp Neurol 301: 65-79, 1990.
15. Biber, B., and J. Fara. Intestinal motility increased by tetrodotoxin, lidocaine, and procaine. Experientia 29: 551-552, 1973.
16. Bilski, J., and S. J. Konturek. Role of nitric oxide in gastroduodenal alkaline

secretion. J Physiol Pharmacol 45: 541-553, 1994.
17. Bjarnason, I., A. Macpherson, and D. Hollander. Intestinal permeability: an overview. Gastroenterol 108: 1566-1581, 1995.
18. Bjarnason, I., P. Smethurst, A. J. Levi, and T. J. Peters. Intestinal permeability to ^{51}Cr-EDTA in rats with experimentally induced enteropathy. Gut 26: 579-585, 1985.
19. Bornstein, J. C., and J. B. Furness. Correlated electrophysiological and histochemical studies of submucous neurons and their contribution to understanding enteric neural circuits. J Autonom Nerv Syst 25: 1-13, 1988.
20. Boughton-Smith, N. K., I. R. Hutcheson, A. M. Deakin, B. J. R. Whittle, and S. Moncada. Protective effect of S-nitroso-N-acetyl-penicillamine in endotoxin-induced acute intestinal damage in the rat. Eur J Pharmacol 191: 485-8, 1990.
21. Bredt, D. S., P. M. Hwang, and S. H. Snyder. Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature 347: 768-770, 1990.
22. Bueno, L., J.-P. Ferre, and Y. Ruckebusch. Effects of anesthesia and surgical procedures on intestinal myoelectric activity in rats. Dig Dis 23: 690-695, 1978.
23. Burnstock, G. The non-adrenergic non-cholinergic nervous system. Arch Int Pharmacodyn Ther. 280: 1-15, 1986.
24. Burnstock, G. Purinergic nerves. Pharmacol Rev 24: 509-581, 1972.
25. Calignano, A., B. J. R. Whittle, M. Di Rosa, and S. Moncada. Involvement of endogenous nitric oxide in the regulation of rat intestinal motility in vivo. Eur J Pharmacol 229: 273-276, 1992.
26. Chang, E. B., and M. C. Rao. Intestinal water and electrolyte transport. Mechanisms of physiological and adaptive responses. In: Physiology of the Gastrointestinal Tract (3 ed.), edited by L. R. Johnson. New York: Raven Press, 1994, p. 2027-2081.
27. Chen, M. C., A. Chang, T. Buhl, M. Tanner, and A. H. Soll. Apical acidification induces paracellular injury in canine gastric mucosal monolayers. Am J Physiol 267: G1012-G1020, 1994.
28. Claude, P. Morphological factors influencing transepithelial permeability: a model for the resistance of the zonula occludens. J Membr Biol 39: 219-232, 1978.
29. Costa, M., J. B. Furness, S. J. H. Brookes, D. S. Bredt, and S. H. Snyder. Presence and chemical coding of neurons with nitric oxide synthase immunoreactivity in the guinea pig small intestine. Proc Natl Physiol: Pharmacol Soc 22: 97P, 1991.
30. Crissinger, K. D., P. R. Kvietys, and D. N. Granger. Pathophysiology of gastrointestinal mucosal permeability. J Internal Med 228, Suppl. 1: 145-154, 1990.
31. Daniel, E. E. Peptidergic nerves in the gut. Gastroenterol 75: 142-145, 1978.
32. Dharmsathaphorn, K., J. Cohn, and G. Beuerlein. Multiple calcium-mediated effector mechanisms regulate chloride secretory responses in T84-cells. Am J Physiol 256: C1224-C1230, 1989.
33. Donowitz, M., and J. L. Madara. Effect of extracellular calcium depletion on epithelial structure and function in rabbit ileum: a model for selective crypt or villus epithelial cell damage and suggestion of secretion by villus epithelial cells. Gastroenterol 83: 1231-43, 1982.
34. Duffey, M. E., B. Hainau, S. Ho, and C. J. Bentzel. Regulation of epithelial tight junction permeability by cyclic AMP. Nature 294: 451-453, 1981.
35. Dunk, C. R., C. D. A. Brown, and L. A. Turnberg. Stimulation of Cl/HCO_3

exchange in rat duodenal brush border membrane vesicles by cAMP. Pflugers Arch 414: 701-705, 1989.
36. Espat, N. J., G. Cheng, M. C. Kelley, S. B. Vogel, C. A. Sninsky, and M. P. Hocking. Vasoactive intestinal peptide and substance P receptor antagonists improve postoperative ileus. J Surg Res 58: 719-723, 1995.
37. Fahrenkrug, J. Vasoactive intestinal polypeptide: measurement, distribution and putative neurotransmitter function. Digestion 19: 149-169, 1979.
38. Feil, W., E. Wenzl, P. Vattay, M. Starlinger, T. Sogukoglu, and R. Schiessel. Repair of rabbit duodenal mucosa after acid injury in vivo and in vitro. Gastroenterol 92: 1973-1986, 1987.
39. Flemström, G. Gastric and duodenal mucosal secretion of bicarbonate. In: Physiology of the Gastrointestinal Tract (3 ed.), edited by L. R. Johnsson. New York: Raven Press, 1994, p. 1285-1310.
40. Flemström, G. Stimulation of duodenal mucosal bicarbonate secretion in the rat by brain peptides. Gastroenterol 97: 412-420, 1989.
41. Flemström, G. Stimulation of HCO3- transport in isolated proximal bullfrog duodenum by prostaglandins. Am J Physiol 239: G198-G203, 1980.
42. Flemström, G., A. Garner, O. Nylander, B. C. Hurst, and J. R. Heylings. Surface epithelial HCO3- transport by mammalian duodenum in vivo. Am J Physiol 243: G348-G358, 1982.
43. Flemström, G., J. R. Heylings, and A. Garner. Gastric and duodenal HCO_3^- transport in vitro: effects of hormones and local transmitters. Am J Physiol 242, G100-G110, 1982.
44. Flemström, G., G. Jedstedt, and O. Nylander. Effects of some opiates and vasoactive intestinal peptide (VIP) on duodenal surface epithelial bicarbonate secretion in the rat. Scand J Gastroenterol 20 (Suppl. 110): 49-53, 1985.
45. Flemström, G., and E. Kivilaakso. Demonstration of a pH gradient at the luminal surface of rat duodenum in vivo and its dependence on mucosal alkaline secretion. Gastroenterol 84: 787-794, 1983.
46. Florey, H. W., and H. E. Harding. The functions of Brunner's glands and the pyloric end of the stomach. J Pathol Bacteriol 37: 431-453, 1933.
47. Furness, J. B., and M. Costa. Adynamic ileus, its pathogenesis and treatment. Med Biol 52: 82-89, 1974.
48. Furness, J. B., H. M. Young, S. Pompolo, J. C. Bornstein, W. A. A. Kunze, and K. McConalogue. Plurichemical transmission and chemical coding of neurons in the digestive tract. Gastroenterol 108: 554-563, 1995.
49. Fändriks, L. Sympatho-adrenergic inhibition of vagally induced gastric motility and gastroduodenal HCO3- secretion in the cat. Acta Physiol Scand 128: 555-62, 1986.
50. Fändriks, L., and C. Jönson. Vagal and sympathetic control of gastric and duodenal bicarbonate secretion. J Intern Med 228: 103-107, 1990.
51. Fändriks, L., C. Jönson, and B. Lisander. Hypothalamic inhibition of duodenal alkaline secretion via a sympatho-adrenergic mechanism in the rat. Acta Physiol Scand 137: 357-363, 1989.
52. Glad, H., P. Svendsen, O. Olsen, and O. B. Schaffalitsky de Muckadell. Importance of vagus nerves in duodenal acid neutralization in anesthetized pigs. Am J Physiol 272: G154-G160, 1997.

53. Granger, D. N., P. R. Kvietys, M. A. Perry, and J. A. Barrowman. The microcirculation and intestinal transport. In: Physiology of the Gastrointestinal Tract (2 ed.), edited by L. R. Johnson. New York: Raven Press, 1987, p. 1671-1697.
54. Greenwood, B., and J. S. Davison. The relationship between gastrointestinal motility and secretion. Am J Physiol 252: G1-G7, 1987.
55. Greenwood, B., and J. S. Davison. Role of extrinsic and intrinsic nerves in the relationship between intestinal motility and transmural potential difference in the anesthetized ferret. Gastroenterol 89: 1286-1292, 1985.
56. Grider, J. R. Interplay of VIP and nitric oxide in regulation of the descending relaxation phase of peristalsis. Am J Physiol 264: G334-G340, 1993.
57. Grider, J. R., and G. M. Makhlouf. Vasoactive intestinal peptide. Transmitter of inhibitory motor neurons of the gut. Ann N Y Acad Sci 527: 369-77, 1988.
58. Grundy, D., and T. Scratcherd. Sensory afferents from the gastrointestinal tract. In: Handbook of Physiology: The Gastrointestinal System, edited by J. D. Wood. Washington DC: American Physiological Society, 1989, p. 593-620.
59. Guba, M., M. Kuhn, W.-G. Forssmann, M. Classen, M. Gregor, and U. Seidler. Guanylin strongly stimulates rat duodenal HCO3- secretion: proposed mechanism and comparison with other secretagogues. Gastroenterol 111: 1558-1568, 1996.
60. Gumbiner, B. Structure, biochemistry, and assembly of epithelial tight junctions. Am J Physiol 253: C749-C758, 1987.
61. Gustafsson, B. I., and D. S. Delbro. Neurogenic inhibition of duodenal and jejunal motility in the anaesthetized rat. Eur J Pharmacol 257: 227-33, 1994.
62. Gustafsson, L. E., C. U. Wiklund, N. P. Wiklund, M. G. Persson, and S. Moncada. Modulation of autonomic neuroeffector transmission by nitric oxide in guinea pig ileum. Biochem Biophys Res Commun 173: 106-10, 1990.
63. Hakim, A. A., and N. Lifson. Effects of pressure on water and solute transport by dog intestinal mucosa in vitro. Am J Physiol 216: 276-284, 1969.
64. Hamlet, A., C. Jönson, and L. Fändriks. The mediation of increased duodenal alkaline secretion in response to 10 mM HCl in the anaesthetized rat. Support for the involvement of capsaicin-sensitive nerve elements. Acta Physiol Scand 146: 519-525, 1992.
65. Harmon, J. W., M. Woods, and N. J. Gurll. Different mechanisms of hydrogen ion removal in stomach and duodenum. Am J Physiol 235: E692-E698, 1978.
66. Hedemark Poulsen, J., H. Fischer, B. Illek, and T. E. Machen. Bicarbonate conductance and pH regulatory capability of cystic fibrosis transmem-brane conductance regulator. Proc Natl Acad Sci U S A 91: 5340-5344, 1994.
67. Hellström, P. M., and T. Ljung. Nitrergic inhibition of migrating myoelectric complex in the rat is mediated by vasoactive intestinal peptide. Neurogastroenterol Motil 8: 299-306, 1996.
68. Heylings, J. R., and M. Feldman. Basal and PGE2-stimulated duodenal bicarbonate secretion in the rat in vivo. Am J Physiol 255: G470-G475, 1988.
69. Hogan, D. L., D. L. Crombie, J. I. Isenberg, P. Svendsen, O. B. Schaffalitzky de Muckadell, and M. A. Ainsworth. Acid-stimulated duodenal bicarbonate secretion involves a CFTR-mediated transport pathway in mice. Gastroenterol 113: 533-541, 1997.
70. Hogan, D. L., D. L. Crombie, J. I. Isenberg, P. Svendsen, d. M. O. Schaffalitzky, and

M. A. Ainsworth. CFTR mediates cAMP- and Ca2+-activated duodenal epithelial HCO3- secretion. Am J Physiol 272: G872-G878, 1997.
71. Holm, L., G. Flemström, and O. Nylander. Duodenal alkaline secretion in rabbits: influence of artificial ventilation. Acta Physiol Scand 138: 471-478, 1990.
72. Holm, M., B. Johansson, C. von Bothmer, C. Jönson, A. Pettersson, and L. Fändriks. Acid-induced increase in duodenal mucosal alkaline secretion in the rat involves the L-arginine/NO pathway. Acta Physiol Scand in press, 1997.
73. Hryhorenko, L. M., Z. Woskowska, and J. E. T. Fox-Threlkeld. Nitric oxide (NO) inhibits release of acetylcholine from nerves of isolated circular muscle of the canine ileum: relationship to motility and release of nitric oxide. J Pharmacol Exp Ther 271: 918-926, 1994.
74. Husebye, E., P. M. Hellström, and T. Midtvedt. Intestinal microflora stimulates myoelectric activity of rat small intestine by promoting cyclic initiation and aboral propagation of migrating myoelectric complex. Dig Dis Sci 39: 946-956, 1994.
75. Husebye, E., V. Skar, T. Hoverstad, T. Iversen, and K. Melby. Abnormal intestinal motor patterns explain enteric colonization with gram-negative bacilli in late radiation enteropathy. Gastroenterol 109: 1078-1089, 1995.
76. Hutcheson, I. R., B. J. R. Whittle, and N. K. Boughton-Smith. Role of nitric oxide in maintaining vascular integrity in endotoxin-induced acute intestinal damage in the rat. Br J Pharmacol 101: 815-820, 1990.
77. Hällgren, A., G. Flemström, and O. Nylander. Effects of nitric oxide synthase inhibitors on bicarbonate secretion, net fluid transport, mucosal permeability, blood flow and motility in rat duodenum. Gastroenterol 104: A93, 1993.
78. Irie, K., T. Muraki, K. Furukawa, and T. Nomoto. L-N-Nitro-arginine inhibits nicotine-induced relaxation of isolated rat duodenum. Eur J Pharmacol 202: 285-288, 1991.
79. Isenberg, J. I., D. L. Hogan, M. A. Koss, and J. A. Selling. Human duodenal bicarbonate secretion; Evidence for basal secretion and stimulated by hydrochloric acid and a synthetic prostaglandin E_1 analogue. Gastroenterol 91: 370-378, 1986.
80. Isenberg, J. I., M. Ljungström, B. Säfsten, and G. Flemström. Proximal duodenal enterocyte transport: evidence for Na(+)-H+ and Cl(-)-HCO3- exchange and NaHCO3 cotransport. Am J Physiol 265: G677-G685, 1993.
81. Isenberg, J. I., B. Wallin, C. Johansson, B. Smedfors, V. Mutt, K. Tatemoto, and S. Emås. Secretin, VIP, and PHI stimulate rat proximal duodenal surface epithelial bicarbonate secretion in vivo. Regul Pept 8: 315-320, 1984.
82. Jenkins, R. T., and R. A. Bell. Molecular radii of probes used in studies of intestinal permeability [letter]. Gut 28: 110-111, 1987.
83. Jönson, C., L. Holm, T. Jansson, and L. Fändriks. Effects of hypovolemia on blood flow, arterial [HCO3-], and HCO3- output in the rat duodenum. Am J Physiol 259: G179-G183, 1990.
84. Jönson, C., O. Nylander, G. Flemström, and L. Fändriks. Vagal stimulation of duodenal HCO3--secretion in anaesthetized rats. Acta Physiol Scand 128: 65-70, 1986.
85. Jönson, C., P. Tunbäck-Hanson, and L. Fändriks. Splanchnic nerve activation inhibits the increase in duodenal HCO3- secretion induced by luminal

acidification in the rat. Gastroenterol 96: 45-49, 1989.
86. Kanwar, S., J. L. Wallace, D. Befus, and P. Kubes. Nitric oxide synthesis inhibition increases epithelial permeability via mast cells. Am J Physiol 266: G222-G229, 1994.
87. Katsoulis, S., and W. E. Schmidt. Role of PACAP in the regulation of gastrointestinal motility. Ann N Y Acad Sci 805: 364-378, 1996.
88. Keast, J. R., J. B. Furness, and M. Costa. Distribution of certain peptide-containing nerve fibres and endocrine cells in the gastrointestinal mucosa in five mammalian species. J Comp Neurol 236: 403-422, 1985.
89. Keller, T. C. I., and M. S. Mooseker. Ca++-calmodulin-dependent phosphorylation of myosin, and its role in brush border contraction in vitro. J Cell Biol 95: 943-59, 1982.
90. Kerlin, P., and S. Phillips. Variability of motility of the ileum and jejunum in healthy humans. Gastroenterol. 82: 694-700, 1982.
91. Kewenter, J. The vagal control of the jejunal and ileal motility and blood flow. Acta Physiol Scand 251: 3-68, 1965.
92. Kim, Y. C., and P. M. Hellström. Identification of mechanisms for duodenal contraction induced by tachykinins in the rat. J Gastrointest Mot 5: 97-106, 1993.
93. Kirchgessner, A. L., and M. D. Gershon. Identification of vagal efferent fibers and putative target neurons in the enteric nervous system of the rat. J Comp Neurol 285: 38-53, 1989.
94. Knutson, L., C. Nimbratt, and G. Flemström. Effects of leukotriene D4, the antagonist L-649-923, and arachidonic acid on duodenal bicarbonate secretion in the rat in vivo. Scand J Gastroenterol 23: 1225-1231, 1988.
95. Konturek, S. J., J. Bilski, J. Tasler, and J. Laskiewicz. Gut hormones in stimulation of gastroduodenal alkaline secretion in conscious dogs. Am J Physiol 248: G687-G691, 1985.
96. Konturek, S. J., and P. Thor. Relation between duodenal alkaline secretion and motility in fasted and sham-fed dogs. Am J Physiol 251: G591-G596, 1986.
97. Kubes, P. Nitric oxide modulates epithelial permeability in the feline small intestine. Am J Physiol 262: G1138-G1142, 1992.
98. Kubes, P., and D. N. Granger. Nitric oxide modulates microvascular permeability. Am J Physiol 262: H611-H615, 1992.
99. Kvietys, P. R., A. P. Shepherd, and D. N. Granger. Laser-Doppler, H_2 clearance, and microsphere estimates of mucosal blood flow. Am J Physiol 249: G221-G227, 1985.
100. Laburthe, M., J. C. Prieto, B. Amiranoff, C. Dupont, D. Hui Bon Hoa, and G. Rosselin. Interaction of vasoactive intestinal peptide with isolated intestinal epithelial cells from rat. 2. Characterization and structural requirements of the stimulatory effect of vasoactive intestinal peptide on production of adenosine 3':5'-monophosphate. Eur J Biochem 96: 239-248, 1979.
101. Lacy, E. R., and S. Ito. Rapid epithelial restitution of the rat gastric mucosa after ethanol injury. Lab Invest 51: 573-83, 1984.
102. Lambert, R. The Pancreatic ducts in the rat. In: Surgery of the Digestive System in the Rat. Springfield: Charles C Thomas Publisher, 1965, p. 109-112.
103. Larsson, M., L. Johnson, G. Nylander, and U. Öhman. Kinetics of ^{51}Cr-EDTA in the rat. Acta Chir Scand 146: 477-481, 1980.

104. Laszlo, F., and B. J. R. Whittle. Constitutive nitric oxide modulates the injurious actions of vasopressin on rat intestinal microcirculation in acute endotoxaemia. Eur J Pharmacol 260: 265-8, 1994.
105. Lenz, H. J., W. W. Vale, and J. E. Rivier. TRH-induced vagal stimulation of duodenal HCO-3 mediated by VIP and muscarinic pathways. Am J Physiol 257: G677-G682, 1989.
106. Leung, F. W., J. C. Miller, T. J. Reedy, and P. H. Guth. Exogenous prostaglandin protects against acid-induced deep mucosal injury by stimulating alkaline secretion in rat duodenum. Dig Dis Sci 34: 1686-1691, 1989.
107. Li, P., K. Y. Lee, T. M. Chang, and W. Y. Chey. Mechanism of acid-induced release of secretin in rats. Presence of a secretin-releasing peptide. J Clin Invest 86: 1474-1479, 1990.
108. Livingston, E. H., J. Miller, and E. Engel. Bicarbonate diffusion through mucus. Am J Physiol 269: G453-G457, 1995.
109. Livingston, E. H., and E. J. Passaro. Postoperative ileus. Dig Dis Sci 35: 121-32, 1990.
110. Livingston, E. H., E. P. Passaro Jr., J. Miller, and P. H. Guth. Spectrum of injury produced in the duodenum by perfusion with luminal acid in the rat. Gastroenterol 103: 481-489, 1992.
111. Llewellyn-Smith, I. J., Z. M. Song, M. Costa, D. S. Bredt, and S. H. Snyder. Ultrastructural localization of nitric oxide synthase immunoreactivity in guinea-pig enteric neurons. Brain Res 577: 337-342, 1992.
112. Lökken, P. Studies on 51Cr-EDTA and its evaluation as a reference substance in gastrointestinal research. In: Norwegian Monog Med Sci. Oslo: Universitetsförlaget, 1970.
113. Lönnerholm, G., L. Knutson, P. J. Wistrand, and G. Flemström. Carbonic anhydrase in the normal rat stomach and duodenum and after treatment with omeprazole and ranitidine. Acta Physiol Scand 136: 253-262, 1989.
114. Lördal, M., A. Hällgren, O. Nylander, and P. M. Hellström. Tachykinins increase vascular permeability in the gastrointestinal tract of the rat. Acta Physiol Scand 156: 489-494, 1996.
115. Lördal, M., C. Johansson, and P. M. Hellström. Myoelectric pattern and effects of small bowel transit induced by the tachykinins neurokinin A, neurokinin B, substance P and neuropeptide K in the rat. J Gastrointest Mot 5: 33-40, 1993.
116. MacNaughton, W. K., G. Cirino, and J. L. Wallace. Endothelium-derived relaxing factor (nitric oxide) has protective actions in the stomach. Life Sci 45: 1869-1876, 1989.
117. Maczka, M., P. Thor, K. Lorens, and S. J. Konturek. Nitric oxide inhibits the myoelectric activity of the small intestine in dogs. J Physiol Pharmacol 44: 31-42, 1993.
118. Madara, J. L. Increases in guinea pig small intestinal transepithelial resistance induced by osmotic loads are accompanied by rapid alterations in absorptive-cell tight-junction structure. J Cell Biol 97: 125-36, 1983.
119. Madara, J. L. Intestinal absorptive cell tight junctions are linked to cytoskeleton. Am J Physiol 253: C171-C175, 1987.
120. Madara, J. L. Loosening tight junctions. Lessons from the intestine. J Clin Invest 83: 1089-1094, 1989.

121. Madara, J. L., R. Moore, and S. Carlson. Alteration of intestinal tight junction structure and permeability by cytoskeletal contraction. Am J Physiol 253: C854-C861, 1987.
122. Makhlouf, G. M. Neuromuscular function of the small intestine. In: Physiology of the Gastrointestinal Tract (3 ed.), edited by L. R. Johnson. New York: Raven Press, 1994, p. 977-990.
123. Marcial, M. A., S. L. Carlson, and J. L. Madara. Partitioning of paracellular conductance along the ileal crypt-villus axis: a hypothesis based on structural analysis with detailed consideration of tight junction structure-function relationships. J Membr Biol 80: 59-70, 1984.
124. Marmorstein, A. D., K. H. Mortell, D. R. Ratcliffe, and E. B. Cramer. Epithelial permeability factor: a serum protein that condenses actin and opens tight junctions. Am J Physiol 262: C1403-C1410, 1992.
125. Martinez-Cuesta, M. A., J. V. Esplugues, and B. J. R. Whittle. Modulation by nitric oxide of spontaneous motility of the rat isolated duodenum: role of tachykinins. Br J Pharmacol 118: 1335-1340, 1996.
126. Mellander, A., H. Abrahamsson, and H. Sjövall. Mechanisms behind cyclic changes in duodenal fluid transport in the fasting state. Acta Physiol Scand 149: 475-82, 1993.
127. Mellander, A., A. Mattsson, A. M. Svennerholm, and H. Sjövall. Relationship between interdigestive motility and secretion of immunoglobulin A in human proximal small intestine. Dig Dis Sci 42: 554-567, 1997.
128. Miller, D. L., S. A. Hamburger, and H. P. Schedl. Effects of osmotic gradients on water and solute transport: in vivo studies in rat duodenum and ileum. Am J Physiol 237: E389-E396, 1979.
129. Mizrahi, J., S. Dion, P. D'Orleans-Juste, E. Escher, G. Drapeau, and D. Regoli. Tachykinin receptors in smooth muscles: a study with agonists (substance P, neurokinin A) and antagonists. Eur J Pharmacol 118: 25-36, 1985.
130. Muallem, R., R. Reimer, H. S. Odes, M. Schwenk, W. Beil, and K.-F. Sewing. Role of carbonic anhydrase in basal and stimulated bicarbonate secretion by the guinea pig duodenum. Dig Dis Sci 39: 1078-84, 1994.
131. Naftalin, R. J., and S. Tripathi. Passive water flows driven across the isolated rabbit ileum by osmotic, hydrostatic and electrical gradients. J Physiol Lond 360: 27-50, 1985.
132. Neutra, M. R., P. Michetti, and J.-P. Kraehenbul. Secretory immunoglobulin A. Induction, biogenesis and function. In: Physiology of the Gastrointestinal Tract (3 ed.), edited by L. R. Johnson. New York: Raven Press, 1994, p. 685-708.
133. Nichols, K., W. Staines, and A. Krantis. Nitric oxide synthase distribution in the rat intestine: a histochemical analysis. Gastroenterol 105: 1651-1661, 1993.
134. Nilsson, G. E. Signal processor for laser Doppler tissue flowmeters. Med Biol Eng Comput 22: 343-8, 1984.
135. Nilsson, G. E., T. Tenland, and P. A. Öberg. A new instrument for continuous measurement of tissue blood flow by light beating spectroscopy. IEEE Trans Biomed Eng 27: 12-19, 1980.
136. Nilsson, G. E., T. Tenland, and P. Å. Öberg. Evaluation of a laser Doppler flowmeter for measurement of tissue blood flow. IEEE Trans Biomed Eng 27: 597-604, 1980.

137. Ny, L., P. Alm, P. Ekström, B. Larsson, L. Grundemar, and K.-E. Andersson. Localization and activity of haem oxygenase and functional effects of carbon monoxide in the feline lower oesophageal sphincter. Br J Pharmacol 118: 392-399, 1996.
138. Nylander, O., G. Flemström, D. Delbro, and L. Fändriks. Vagal influence on gastroduodenal HCO3- secretion in the cat in vivo. Am J Physiol 252: G522-G528, 1987.
139. Nylander, O., L. Holm, E. Wilander, and A. Hällgren. Exposure of the duodenum to high concentrations of hydrochloric acid. Effects on mucosal permeability, alkaline secretion, and blood flow. Scand. J. Gastroenterol 29: 437-444, 1994.
140. Nylander, O., P. Kvietys, and D. N. Granger. Effects of hydrochloric acid on duodenal and jejunal mucosal permeability in the rat. Am J Physiol 257: G653-G660, 1989.
141. Nylander, O., M. Sababí, and J. Bark. Characterization of 51Cr-EDTA as a marker of duodenal mucosa permeability. Acta Physiol Scand 143: 117-126, 1991.
142. Nylander, O., E. Wilander, G. M. Larson, and L. Holm. Vasoactive intestinal polypeptide reduces hydrochloric acid-induced duodenal mucosal permeability. Am J Physiol 264: G272-G279, 1993.
143. Odes, H. S., D. L. Hogan, M. A. Ballesteros, J. D. Wolosin, M. A. Koss, and J. I. Isenberg. Human duodenal mucosal bicarbonate secretion. Evidence suggesting active transport under basal and stimulated conditions. Gastroenterol 98: 867-72, 1990.
144. Osakada, F., K. Kubo, K. Goto, I. Kanazawa, and E. Munekata. The contractile activities of neurokinin A, B and related peptides on smooth muscles. Eur J Pharmacol 120: 201-208, 1986.
145. Paimela, H., T. Kiviluoto, H. Mustonen, P. Sipponen, and E. Kivilaakso. Tolerance of rat duodenum to luminal acid. Dig Dis Sci 35: 1244-1248, 1990.
146. Pappenheimer, J. R. Physiological regulation of transepithelial impedance in the intestinal mucosa of rats and hamsters. J Membr Biol 100: 137-48, 1987.
147. Pascaud, X. B., M. Chovet, P. Soulard, E. Chevalier, C. Roze, and J. L. Junien. Effects of a new sigma ligand, JO 1784, on cysteamine ulcers and duodenal alkaline secretion in rats. Gastroenterol 104: 427-434, 1993.
148. Ponomarew, S. J. The physiology of Brunner's glands (in Russian) Thesis, J Botskinshospital in St. Petersburg , 1-8, 1902.
149. Powell, D. W. Barrier function of epithelia. Am J Physiol 241: G275-G288, 1981.
150. Prieto, J. C., M. Laburthe, and G. Rosselin. Interaction of vasoactive intestinal peptide with isolated intestinal epithelial cells from rat. 1. Characterization, quantitative aspects and structural requirements of binding sites. Eur J Biochem 96: 229-237, 1979.
151. Quigley, E. M., and L. A. Turnberg. pH of the microclimate lining human gastric and duodenal mucosa in vivo. Studies in control subjects and in duodenal ulcer patients. Gastroenterol 92: 1876-1884, 1987.
152. Rattan, S., and S. Chakder. Inhibitory effect of CO on internal anal sphincter: heme oxygenase inhibitor inhibits NANC relaxation. Am J Physiol 265: G799-G804, 1993.
153. Read, N. W., R. H. Smallwood, R. J. Levin, C. D. Holdsworth, and B. H. Brown. Relationship between changes in intraluminal pressure and transmural

potential difference in the human and canine jejunum in vivo. Gut 18: 141-151, 1977.
154. Reimer, R., H. S. Odes, R. Muallem, M. Schwenk, W. Beil, and K.-F. Sewing. Cyclic adenosine monophosphate is the second messenger of prostaglandin E2- and vasoactive intestinal polypeptide-stimulated active bicarbonate secretion by guinea-pig duodenum. Scand. J. Gastroenterol 29: 153-9, 1994.
155. Robert, A., J. E. Nezamis, and J. P. Phillips. Effect of prostaglandin E1 on gastric secretion and ulcer formation in the rat. Gastroenterol 55: 481-487, 1968.
156. Rodriguez-Membrilla, A., V. Martinez, M. Jimenez, E. Gonalons, and P. Vergara. Is nitric oxide the final mediator regulating the migrating myoelectric complex cycle? Am J Physiol 268: G207-G214, 1995.
157. Roman, C., and J. Gonella. Extrinsic control of digestive tract motility. In: Physiology of the Gastrointestinal Tract (2 ed.), edited by L. R. Johnson. New York: Raven Press, 1987, p. 507-553.
158. Rukebusch, M., and J. Fioramonti. Electrical spiking activity and propulsion in small intestine in fed and fasted rats. Gastroenterol 68: 1500-08, 1975.
159. Sababi, M., A. Hällgren, and O. Nylander. Interaction between prostanoids, NO and VIP in modulation of duodenal alkaline secretion and motility. Am J Physiol 271: G582-G590, 1996.
160. Sababi, M., E. Nilsson, and L. Holm. Mucus and alkali secretion in the rat duodenum: effects of indomethacin, N omega-nitro-L-arginine, and luminal acid. Gastroenterol 109: 1526-1534, 1995.
161. Sababi, M., and O. Nylander. Comparative study of the effects of nitric oxide synthase and cyclo-oxygenase inhibition on duodenal functions in rats anaesthetized with inactin, urethane or alpha-chloralose. Acta Physiol Scand 158: 45-52, 1996.
162. Sababi, M., and O. Nylander. Elevation of intraluminal pressure and cyclooxygenase inhibitors increases duodenal alkaline secretion. Am J Physiol 266: G22-G30, 1994.
163. Sanders, K. M. Ionic mechanisms of electrical rhythmicity in gastrointestinal smooth muscles. Annu Rev Physiol 54: 439-453, 1992.
164. Sanders, K. M., and S. M. Ward. Nitric oxide as a mediator of nonadrenergic noncholinergic neurotransmission. Am J Physiol 262: G379-G392, 1992.
165. Sarna, S. K., M. F. Otterson, R. P. Ryan, and V. E. Cowles. Nitric oxide regulates migrating motor complex cycling and its postprandial disruption. Am J Physiol 265: G759-G766, 1993.
166. Sellers, L. A., A. Allen, and M. K. Bennett. Formation of a fibrin based gelatinous coat over repairing rat gastric epithelium after acute ethanol damage: interaction with adherent mucus. Gut 28: 835-843, 1987.
167. Simson, J. N., A. Merhav, and W. Silen. Alkaline secretion by amphibian duodenum. I. General characteristics. Am J Physiol 240: G401-G408, 1981.
168. Sjövall, H., I. Hagman, and H. Abrahamsson. Relationship between interdigestive duodenal motility and fluid transport in humans. Am J Physiol. 259: G348-G354, 1990.
169. Smedfors, B., and C. Johansson. Cholinergic influence on duodenal bicarbonate response to hydrochloric acid perfusion in the conscious rat. Scand J Gastroenterol 21: 809-815, 1986.

170. Smyth, D. H., and E. M. Wright. Streaming potentials in the rat small intestine. J Physiol Lond 182: 591-602, 1966.
171. Stark, M. E., and J. H. Szurszewski. Role of nitric oxide in gastrointestinal and hepatic function and disease. Gastroenterol 103: 1928-1949, 1992.
172. Starlinger, M. Role of alkaline secretion and cell restitution in duodenal mucosal protection. J Intern Med 228, suppl. 1: 109-112, 1990.
173. Starlinger, M., and R. Schiessel. Bicarbonate (HCO3) delivery to the gastroduodenal mucosa by the blood: its importance for mucosal integrity. Gut 29: 647-654, 1988.
174. Strong, T. V., K. Boehm, and F. S. Collins. Localization of cystic fibrosis transmembrane conductance regulator mRNA in the human gastrointestinal tract by in situ hybridization. J Clin Invest 93: 347-54, 1994.
175. Sundler, F., E. Ekblad, A. Absood, R. Håkanson, K. Köves, and A. Arimura. Pituitary adenylate cyclase activating peptide: a novel vasoactive intestinal peptide-like neuropeptide in the gut. Neuroscience 46: 439-454, 1992.
176. Suzuki, N., C. L. Prosser, and V. Dahms. Boundary cells between longitudinal and circular layers: essential for electrical slow waves in cat intestine. Am J Physiol 250: G287-G294, 1986.
177. Szurszewski, J. H. A migrating electric complex of canine small intestine. Am J Physiol 217: 1757-63, 1969.
178. Säfsten, B., C. S. Chew, and G. Flemström. Calcium signaling in a new cultured enterocyte model. Gastroenterol 106: A267, 1994.
179. Säfsten, B., and G. Flemström. Dopamine and vasoactive intestinal peptide stimulate cyclic adenosine-3',5'- monophosphate formation in isolated rat villus and crypt duodenocytes. Acta Physiol Scand 149: 67-75, 1993.
180. Takeuchi, K., J. Matsumoto, K. Ueshima, and S. Okabe. Role of capsaicin-sensitive afferent neurons in alkaline secretory response to luminal acid in the rat duodenum. Gastroenterol 101: 954-961, 1991.
181. Takeuchi, K., T. Ohuchi, H. Miyake, S. Niki, and S. Okabe. Effects of nitric oxide synthase inhibitors on duodenal alkaline secretion in anesthetized rats. Eur J Pharmacol 231: 135-138, 1993.
182. Takeuchi, K., T. Ohuchi, H. Miyake, and S. Okabe. Stimulation by nitric oxide synthase inhibitors of gastric and duodenal HCO3- secretion in rats. J Pharmacol Exp Ther 266: 1512-1519, 1993.
183. Thuneberg, L. Interstitial cells of Cajal: intestinal pacemaker cells? Adv Anat Embryol Cell Biol 71: 1-130, 1982.
184. Trezise, A. E. O., and M. Buchwald. In vivo cell-specific expression of the cystic fibrosis transmembrane conductance regulator. Nature 353: 434-437, 1991.
185. Turnheim, K. Intestinal permeation of water. In: Handbook of Experimental Pharmacology, edited by T. Z. Csaky. New York: Springer, 1984, p. 381-463.
186. Utz, J., and V. Ullrich. Carbon monoxide relaxes ileal smooth muscle through activation of guanylate cyclase. Biochem Pharmacol 41: 1195-201, 1991.
187. Vantrappen, G. R., T. L. Peeters, and J. Janssens. The secretory component of the interdigestive migrating motor complex in man. Scand J Gastroenterol. 14: 663-667, 1979.
188. Volf, V., M. Vladar, and A. Seidel. Distribution of labelled calcium-, yttrium and chromium-chelates of EDTA in rats. Arch Int Pharmacodyn 190: 110-123, 1971.

189. Wallace, J. L., and B. J. R. Whittle. Role of mucus in the repair of gastric epithelial damage in the rat. Inhibition of epithelial recovery by mucolytic agents. Gastroenterol 91: 603-611, 1986.
190. Weisbrodt, N. W. Motility of the small intestine. In: Physiology of the Gastrointestinal Tract, edited by L. R. Johnson. New York: Raven Press, 1987, p. 631-663.
191. Whittle, B. J. R., J. Lopez-Belmonte, and S. Moncada. Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory neuropeptides in the rat. Br J Pharmacol 99: 607-611, 1990.
192. Whittle, B. J. R., and J. R. Vane. Prostanoids as regulators of gastrointestinal function. In: Physiology of the Gastrointestinal Tract, edited by L. R. Johnson. New York: Raven Press, 1987, p. 143-180.
193. Wood, J. D. Excitation of intestinal muscle by atropine, tetrodotoxin, and xylocaine. Am J Physiol 222: 118-125, 1972.
194. Wood, J. D. Physiology of the enteric system. In: Physiology of the Gastrointestinal Tract (3 ed.), edited by L. R. Johnsson. New York: Raven Press, 1994, p. 423-482.
195. Yablonski, M. E., and N. Lifson. Mechanism of production of intestinal secretion by elevated venous pressure. J Clin Invest 57: 904-915, 1976.
196. Yao, B., D. L. Hogan, K. Bukhave, M. A. Koss, and J. I. Isenberg. Bicarbonate transport by rabbit duodenum in vitro: effect of vasoactive intestinal polypeptide, prostaglandin E2, and cyclic adenosine monophosphat. Gastroenterol 104: 732-740, 1993.

Correspondence address:
Anneli Hällgren
Department of Physiology and Medical Biophysics
Uppsala University
Box 572
S-571 23 Uppsala, Sweden