Cellular basis of diarrhoea

The Croonian Lecture 1989

Diarrhoea is characterised by an increased loss of fluid, largely salt and water, in the stool and it is possible to speculate on the several mechanisms by which this may occur. For example, enhanced gastrointestinal motor activity may speed luminal contents through the gut; malabsorption of a number of solutes may induce an 'osmotic' type of diarrhoea; and the intestinal mucosa may be provoked into secreting salt and water as, for example, in cholera toxin-induced diarrhoea.

Ionic basis of intestinal secretion

Absorption of salt and water in the intestine is the net result of large fluxes of ions and water in both directions across the mucosa. Even small shifts in the balance between these two opposing fluxes can result in net secretion. The flux of water is a passive response to the movement of solute across the mucosa since water flows in response to osmotic and hydraulic pressure gradients. It is unnecessary to postulate a specific active transport process for water.

Absorption of sodium chloride in the small intestine involves an uptake mechanism across the apical brush border membrane, at least partly by ion exchange processes. Sodium is absorbed in exchange for hydrogen, and chloride in exchange for bicarbonate (Fig. 1). The presence of these two ion exchangers, originally postulated on the basis of information derived from *in vivo* perfusion studies in man [1], has been confirmed by studies of isolated brush border membrane vesicles [2,3]. Evidence from *in vitro* experiments suggests that the sodium/proton exchanger is probably inhibited during intestinal secretion [4], and it is possible that the anion exchanger is also inhibited.

The other major mechanism for sodium absorption, namely glucose or amino acid coupled sodium absorption, is unaffected during intestinal secretion. This process depends on the maintenance of a low intracellular sodium concentration so that sodium, linked to glucose, is encouraged to enter the cell across its apical membrane [5]. This concentration gradient for sodium is maintained by extrusion by a 'sodium pump' (the sodium/potassium ATPase) at the basolateral membrane, and this too is unaffected during secretion. This observation has had considerable implications for treatment of secretory diarrhoeal diseases such as cholera, where it is clear that sodium and

water absorption can be stimulated by this glucose-linked mechanism. Treatment of severe diarrhoea with oral rehydration solutions has been a major advance although its use predated an understanding of the underlying mechanisms. The administration of simple glucose/saline mixtures by mouth allows good rehydration of patients and reduces the need for expensive and scarce intravenous solutions (except in the most severely affected patients). The wide promulgation of advice on oral rehydration therapy by the World Health Organisation has been a major plank in its efforts to reduce the high mortality from infantile diarrhoea in Third World countries.

A number of separate ionic events occur during secretion (Fig. 2). An important change is the increase in permeability to anions which occurs in the apical membrane of epithelial cells. Normally this membrane is relatively impermeable to the major anions, chloride

Fig. 1. *Mechanisms of ion absorption in the small intestine.* Sodium coupled glucose uptake in the apical membrane is a major route for sodium transport. It relies on a low intracellular sodium concentration achieved by the sodium pump (Na,K,ATPase) sited on the basolateral membrane. In addition, the electrically neutral uptake of sodium and chloride is probably achieved by two ion exchangers, Na/H and Cl/HCO$_3$. The Cl leaves the basolateral side of the cell by mechanisms which may be multiple and could include an anion channel, an anion exchanger (Cl/HCO$_3$) or a coupled Na CLO$_3$ process. Evidence in favour of the latter processes is sketchy.
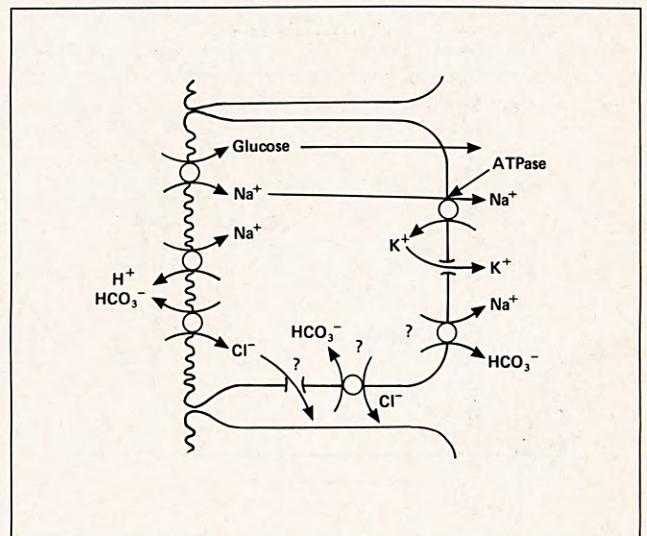
LESLIE TURNBERG, MD, FRCP
Professor of Medicine, University of Manchester

and bicarbonate; increased permeability, probably brought about by the opening of specific anion channels, allows chloride (or bicarbonate) to diffuse out of the cytoplasm and into the lumen down an electrochemical gradient [6]. Although intracellular chloride concentrations are lower than those in the lumen, there is a strong electrical gradient (inside of the cell approximately −70 mV compared with the lumen) which prompts anion transport into the lumen. For chloride to be secreted across the whole epithelium, however, it has to enter the cell from the basolateral side and a specific uptake process is stimulated at this membrane in response to secretory stimuli [7]. In many cell types it appears that chloride entry mainly depends on both sodium and potassium, probably involving a coupled sodium/potassium/2 chloride transport mechanism [7].

The sodium and potassium which enter the cell with the chloride have to be dissipated. Sodium is pumped out via the sodium/potassium ATPase while potassium leaves via specific potassium channels in the basolateral membrane, completing a potassium circuit across that membrane. It can be seen that an increase in potassi-

Fig. 2. *Ion transport processes involved in small intestinal secretion.* Secretion of chloride ions is achieved by the opening of an anion channel in the apical membrane achieved by phosphorylation under the influence of second messengers (cyclic AMP, calcium, cyclic GMP). This allows chloride to flow down its electrochemical gradient into the lumen and sodium and water follow through the paracellular spaces. Chloride is stimulated to enter the cell, ready for secretion, via a triple ion uptake mechanism on the basolateral side of the cell (Na.K.2Cl). Such uptake processes have been demonstrated on a number of epithelia. The potassium which enters with chloride in this uptake mechanism escapes back through the basolateral membrane when a potassium conductance is opened in this membrane.
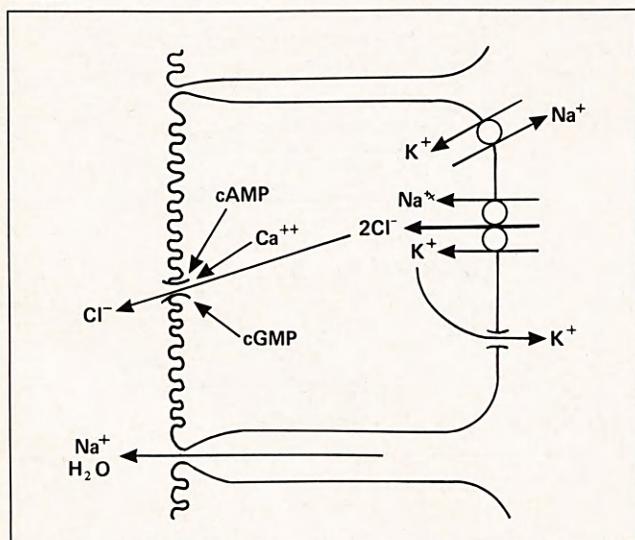
um permeability at the basolateral membrane, which would lower intracellular potassium, could be one mechanism by which the coupled sodium/potassium/2 chloride uptake process could be stimulated. Indeed, there is evidence that blockers of potassium channels in the basolateral membrane inhibit chloride secretion [8]. Potassium accumulation within the cell inhibits further uptake via the coupled uptake mechanism.

Stimulation of uptake at the basolateral side, linked to an increased permeability at the apical side of the cell, is the major driving force for anion secretion. Sodium and water follow the anion, probably passing between rather than through the cells via the intercellular spaces and tight junctions.

The nature of the anion channel in the apical membrane remains to be clarified. It is likely to be a protein which bridges the membrane and is stimulated to alter its configuration to allow anions to pass through it. The existence of such anion channels has been demonstrated in so called 'patch-clamp' studies in which small patches of cell membrane, about 2 or 3 microns in diameter, can be studied in isolation [9]. A number of cell types have anion channels, and recent studies of cultured epithelial cell lines derived from human colonic and gastric adenocarcinomas have confirmed the presence of similar channels which can be shown to open and close [10].

The recent cloning of the defective cystic fibrosis gene has been followed by the exciting demonstration that the specific protein for which this gene codes is a transmembrane regulator protein concerned with chloride ion transport [11]. This protein probably represents the anion channel in respiratory epithelia, and possibly also intestinal epithelium. The defect in most cystic fibrosis patients appears to reside in a deletion of a phenylalanine in an ATP-binding region on the intracytoplasmic portion of this transmembrane protein (Fig. 3). It is conceivable that this is the protein in the apical membrane of epithelial cells which is concerned with chloride secretion, and this would fit well with the functional changes known to be present in cystic fibrosis epithelium. That is, although a chloride channel is demonstrable in cystic fibrosis epithelium, its control by second messengers which cause phosphorylation (see later) is defective. It remains to be confirmed that this transmembrane regulator protein is indeed the chloride channel concerned with intestinal secretion. It is of interest in this regard that patients with cystic fibrosis have some resistance to developing secretory diarrhoea when exposed to enteric infections. This resistance may have offered an evolutionary advantage in the survival of the cystic fibrosis gene.

Anatomical site of secretion

There is much indirect evidence to suggest that secretion largely resides in the crypts while absorption occurs from the villi [12]. Thus, selective damage to

villous epithelium impairs absorption but does not inhibit secretion, while intestinal epithelium that does not contain crypts, for example that of the flounder intestine, is incapable of secretion. However, recent more direct studies from our own laboratories have revealed that villous epithelium responds to secretory stimuli by a depolarisation of the apical membrane compatible with the opening of a chloride channel [13]. Furthermore, brush border membrane vesicles derived from villous and crypt epithelium both appear to have an anion channel [14].

To summarise, the ionic basis of intestinal secretion includes an increase in apical membrane permeability to chloride and an associated increase in uptake of chloride via a sodium/potassium/2 chloride transporter at the basolateral membrane. The latter is stimulated by the opening of a potassium channel in the basolateral membrane. Sodium and water follow the electro-osmotic gradient created by the movement of chloride into the lumen. Sodium and chloride absorption via the two ion exchangers in the apical membrane (sodium/hydrogen and chloride/bicarbonate) is inhibited, but glucose-linked sodium absorption is unaffected.

Fig. 3. *Diagrammatic representation of the 'transmembrane regulator protein' which is believed to be defective in cystic fibrosis, as predicted by the recent cloning of the gene.* The chloride channel, set in the plane of the membrane, consists of two similar series of polypeptide chains which cross and recross the membrane six times each. This membrane domain is joined to three intra-cytoplasmic moieties of the molecule which can be phosphorylated (by ATP) by a number of protein kinases thus causing the chloride channel to open. In one of these moieties, a deletion of one phenylalanine residue renders it incapable of binding ATP in its nucleotide binding fold (NBF) and thus the channel is unaffected by agonists which normally open it via this mechanism (redrawn from Riordan *et al*) [11].
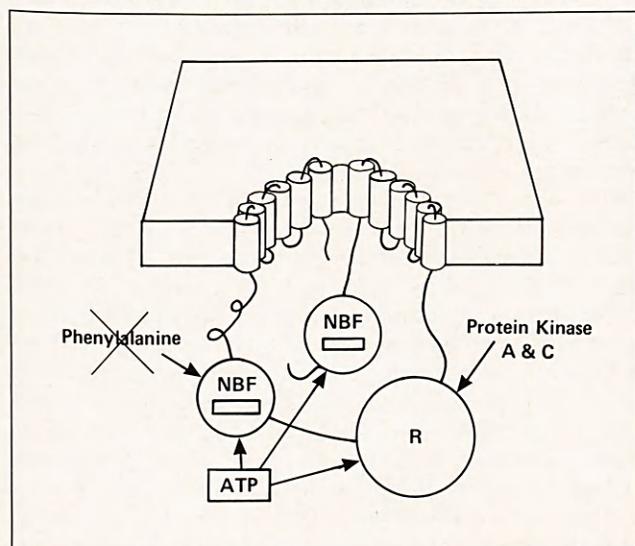
Second messenger systems

Externally perceived signals to the epithelium are transmitted to the ion transporting mechanisms operating at the cell membranes via a number of intracellular messenger systems. The best known of these involves the generation of intracellular cyclic adenosine monophosphate (cyclic AMP).

Cyclic adenosine monophosphate and G protein

Cyclic AMP is produced from ATP by the enzyme adenylyl cyclase which is sited on the basolateral membrane. The adenylyl cyclase complex consists of (a) a catalytic subunit responsible for generating cyclic AMP, (b) a G protein responsible for coupling the signal from (c) the receptor unit (Fig. 4) [15]. Activation of the receptor from the extracellular environment induces the G protein to dissociate into two components—the alpha and the beta/gamma components. The alpha component binds to GTP (hence the acronym 'G' protein) which then stimulates the catalytic subunit of adenylyl cyclase to produce cyclic AMP [16]. Once activated, the GTP bound to the alpha component of the G protein is split to yield GDP, which then dissociates from the alpha subunit, thus switching off the stimulus for cAMP generation and allowing the alpha to recombine with the beta/gamma components. The G protein has an intrinsic GTPase activity which releases GDP once it has activated the catalytic unit.

This stimulatory G protein (G_s) is a mobile component of the cell membrane in both the basolateral and apical brush border. If activated in the latter, it may

Fig. 4. *Diagrammatic representation of the structure of adenylyl cyclase on the basolateral epithelial cell membrane.* The receptor (R) is activated by specific agonists and causes the G protein to bind GTP and dissociate into its α and β/γ subunits. GTP bound to α subunits activates the catalytic unit to produce cyclic AMP. Once this has been achieved the intrinsic GTPase activity of the α subunit splits its GTP. It is this latter activity which is inhibited by cholera toxin thus causing an irreversible activation of the catalytic unit.
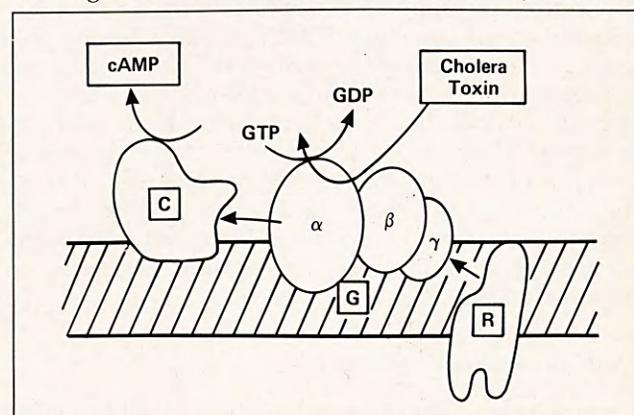
move round to activate the adenylyl cyclase catalytic subunit which is restricted to the basolateral membrane. It is possible too that the alpha subunit could pass across the cytoplasm once liberated. This may occur, for example, in cholera toxin-induced secretion [17].

Apart from this G_s protein, which is one of a family of similar proteins, there are inhibitory G proteins (G_i) which inhibit activity of the adenylyl cyclase [16].

In the intestine the best known receptor-mediated activators of the adenylyl cyclase complex are vasoactive intestinal peptide (VIP) [18] and certain prostaglandins [19]. Activators of G_i in the intestine are alpha-2-adrenergic agonists and somatostatin [20] while pertussis toxin inhibits it.

Activation of adenylyl cyclase by cholera toxin involves a mechanism not requiring the specific adenylyl cyclase receptor. In this case the toxin attaches to the apical membrane via its B subunits (see later), and the active component of the toxin, the A subunit, then enters the cell membrane. There it is split at its disulphide bond, and the A1 component of this subunit then activates the G_s protein directly, thus bypassing the receptor component [21]. It has not yet been possible to demonstrate that the A1 component of the toxin passes into the cytoplasm where it may activate that G_s protein present in the apical membrane, which then migrates round to the catalytic subunit in the basolateral membrane [17]. In any event, it appears to activate G_s by causing ribosylation of its ADP which irreversibly inhibits GTPase activity. Thus, once GTP binds to the alpha subunit of G_s, it remains in this activated, GTP-bound form throughout the remainder of that cell's life cycle. The catalytic subunit of adenylyl cyclase remains 'switched on' until that particular epithelial cell migrates up the side of the villus and dies as it falls off the tip. This prolonged activation of adenylyl cyclase, characteristic of cholera toxin, lasts about 2–4 days and accounts in part for the length of time that secretory diarrhoea occurs after exposure to cholera.

Protein kinase A

Cyclic AMP generated in the cytoplasm activates an enzyme (protein kinase A) which is responsible for causing the phosphorylation of a number of cytoplasmic and membrane-bound proteins [22]. Among the latter it is likely that a component of the anion channel in the apical membrane is phosphorylated. Once phosphorylated, the channel may change its configuration to allow anions to pass [11]. Other proteins phosphorylated by cyclic AMP presumably change their function, but their roles have yet to be clearly defined. Inhibition of a sodium/hydrogen exchanger in the apical membrane may be part of the total response to cyclic AMP.

Cyclic guanosine monophosphate

Less is known about a second cyclic nucleotide, cyclic guanosine monophosphate (cyclic GMP). This is generated in response to stimulation of the enzyme guanylyl cyclase, a particulate enzyme found predominantly in the apical membrane of epithelial cells and in the adjacent cytoplasm [23]. Stimulation of this cyclase is likely to occur from the luminal side of epithelial cells although a basolateral stimulatory component cannot be eliminated. It, too, consists of a catalytic subunit, stimulatory and inhibitory G proteins, and a specific guanylyl cyclase-linked receptor. Activation of the receptor from the luminal side occurs in response to the heat-stable toxin of *E. coli* [24]. It is conceivable that atrial natriuretic peptide also activates GTP production, although how that is achieved is not clear [25]. The G protein which stimulates guanylyl cyclase is probably different from that which stimulates adenylyl cyclase, but it is unclear whether a different inhibitory G protein is associated with this cyclase.

Cyclic GMP activates a different protein kinase, protein kinase G, which then phosphorylates and opens the same, or a similar, component of the transmembrane ion channel [26]. It is as yet unclear whether the cyclic GMP-dependent protein kinase has any other effects on ion transport mechanisms in the epithelium.

Second messenger systems involving calcium

Although both cyclic AMP and cyclic GMP-dependent systems are influenced to some extent by intracellular calcium, secretion can be provoked without the involvement of either of the cyclic nucleotides. For example, acetyl choline and 5-hydroxytryptamine (serotonin) stimulate secretion without activating adenylyl or guanylyl cyclases. In these instances, secretion is associated with a rise in intracellular free calcium concentration. In recent years much interest has been displayed in such calcium-dependent mechanisms [27]. In normal circumstances, cytoplasmic calcium is held at the very low concentration of about 10^{-7}M or lower. Even slight transient increases in intracellular calcium to, say, 10^{-7} or 10^{-6} M will induce secretion. In most instances, the additional calcium appears to be derived from an intracellular source, probably the smooth endoplasmic reticulum, but extracellular calcium is necessary to regenerate intracellular calcium stores.

The signal to stimulate release of intracellular calcium is inositol triphosphate (IP_3) (Fig. 5), a metabolite of a membrane-bound phospholipid [28]. The breakdown of this phospholipid, with IP_3 release, is achieved by an enzyme, phospholipase C. This is activated by externally perceived signals via specific receptors [29]. A G protein is involved in linking the activated receptor to the phospholipase. The enzyme then preferentially splits phosphatidyl inositol bisphosphate, liberating water-soluble IP_3 into the cytoplasm where it activates release of calcium from the endoplasmic reticulum.

The rise in calcium concentration occurs in an oscillatory manner, with oscillations varying in periodicity

from about 20 to 60 seconds. Furthermore, these oscillations occur as waves generated in one part of the cell and spread right across the cell [28]. It is possible that the period and amplitude of these waves of increase in calcium concentration determine the response.

Once liberated, calcium is bound to the calcium-dependent regulator protein, calmodulin, and this combination of calcium calmodulin activates a protein kinase [30] which again causes the phosphorylation of proteins, including the anion channel in the apical membrane.

The IP_3 is but one of a family of several similar inositol polyphosphates, but most of them are formed very transiently and their roles and relative importance remain to be discovered [28]. Once IP_3 has activated calcium release it is dephosphorylated and recycled to the cell membrane. Here it is taken up, together with diacyl glycerol, to form phosphatidyl inositol and further phosphorylated to re-form phosphatidyl inositol bisphosphate.

Phospholipase C activity not only releases IP_3 but also another metabolite, diacyl glycerol (Fig. 5). This is lipid soluble, stays within the cell membrane, and is far from inert. In the presence of calcium and phosphatidyl serine it activates yet another protein kinase, protein kinase C, which also causes phosphorylation of membrane-bound proteins [31]. The protein kinase C family is well known to have roles in cell multiplication and growth, and in the epithelium it appears to modulate ion secretion. For example, protein kinase C activates a potassium channel in the basolateral membrane of a colonic epithelial cell line [8]. The opening of this channel allows potassium exit from the cell, thus lowering intracellular potassium concentrations which in turn stimulate uptake via the sodium/potassium/2 chloride mechanism. This then provides the chloride for secretion through the apical membrane. In this case, protein kinase C potentiates the effect of the opening of the chloride channel by other protein kinases. Protein kinase C activation also turns off the secretory response provoked by protein kinase A, thus modulating the final response [32]. It is clear that, far from simply duplicating each other's activity, these protein kinases interact one with the other and modulate the final response to multiple secretagogues.

From this description, the impression is gained of a complex series of interacting second messengers which determines the final response to a range of secretory stimuli from both the luminal and interstitial fluid sides of the epithelial cell (Fig. 6).

Extracellular stimuli to secretion

The intestinal epithelium is stimulated to secrete salt and water in response to a range of stimuli perceived from either side of the epithelium. Luminal factors include bacterial toxins, while from the basolateral side stimuli include a range of inflammatory media-

Fig. 5. *Calcium and diacyl glycerol-mediated mechanisms involved in control of apical membrane chloride channels.* For explanation see text. ER=endoplasmic reticulum: IP_3=inositol triphosphate: DG=diacyl glycerol: P.I.2P=phosphatidyl inositol bis phosphate: P.I.1P.=phosphotidyl inositol monophosphate: P.I.=phosphatidyl inositol: P.L.C.=phospholipase C.

Fig. 6. *Diagram of three main types of receptor systems activating production of four types of intracellular second messengers.* Each second messenger activates a specific protein kinase which in turn causes the phosphorylation of membrane bound proteins. The opening of a chloride channel in the apical membrane is one of the major final common pathways leading to secretion.
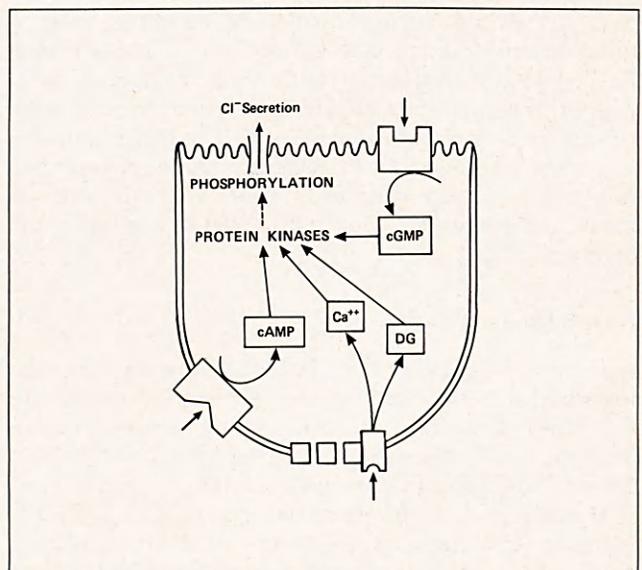
tors, neuropeptides and circulating hormones. Some of these stimuli are physiological and most are relevant to specific diarrhoeal diseases.

Toxins

The best known example of a bacterial toxin is that derived from the *Vibrio cholerae*. This toxin stimulates adenylyl cyclase by directly acting on the stimulatory G protein [21]. The toxin has a series of five B subunits surrounding a single A subunit. It is recognised by a specific receptor, the GM_1 receptor, for the B subunits on the epithelial apical membrane and even brief transient exposure to the toxin results in firm binding to the receptor. Cholera toxoid which has been used in prophylaxis also binds to the receptor but, lacking the A subunit, does not provoke secretion. Furthermore, it can prevent further binding by whole toxin molecules.

Other toxins of a similar type include the heat-labile toxin of *E. coli*, responsible for much of the 'travellers' diarrhoea' suffered by western visitors to Third World countries [33]. The diarrhoea is provoked by a similar second messenger system involving cyclic AMP but is rather shorter lived and less severe. A number of toxins derived from other organisms closely resemble the heat-labile toxin of *E. coli*, including those produced by some strains of *Klebsiella* and *Bacillus cereus*.

Another toxin, the heat-stable toxin derived from *E. coli*, is also responsible for some episodes of travellers' diarrhoea, but activates guanylyl cyclase to provoke intestinal secretion [24]. The A toxin derived from *Clostridium difficile* appears to require calcium and may activate the phospholipase C system [34].

Entamoeba histolytica is not generally thought of as causing a secretory diarrhoea. It is associated with mucosal ulceration, particularly in the colon, but it is now clear that this organism also produces agents capable of inducing secretion. Secretion is provoked by cell-free lysates of *E. histolytica* and requires the presence of calcium, suggesting that the phospho-inositol turnover mechanism is involved [35]. The surprising finding that *E. histolytica* produces a number of neuropeptides, including 5-hydroxytryptamine and neurotensin, is of some interest, and it appears that the secretion produced by *E. histolytica* lysates is probably due to their content of 5-HT [36]. The relevance of these observations to the manifestations of amoebic dysentery is not clear.

Inflammatory mediators

A number of mediators of inflammation can provoke intestinal secretion and may be relevant to some of the diarrhoea occurring in patients with a range of inflammatory bowel diseases, including ulcerative colitis, Crohn's disease, and infective enteritis [37].

Histamine is a potent secretagogue acting via H1 receptors and appears to rely on a calcium-mediated mechanism rather than cyclic nucleotides [38].

Various prostaglandins are also potent secretagogues. Prostaglandin E_2 probably activates secretion through adenylyl cyclase via a specific receptor [19]. On the other hand, prostacyclin (PGI_2) may induce secretion through a local neurological mechanism since its effects are markedly inhibited by neuroblockade with, for example, tetrodotoxin [39]. In contrast, prostaglandin D_2 may inhibit the secretory effects of PGE_2 [40]. These observations emphasise the complexity of the effects of this group of inflammatory mediators on the intestinal epithelium and point to the difficulties that have to be overcome if a specific anti-inflammatory agent, which can interfere with the secretory diarrhoea associated with inflammation, is to be developed.

Some leukotrienes, including LTB_4 and LTC_4, can also provoke secretion [41]. It is of some interest that LTB_4 production is inhibited by 5-aminosalicylic acid and sulphasalazine [42], and this may be responsible, at least in part, for the therapeutic effect of these agents in ulcerative colitis. The recent demonstration that a more specific inhibitor of leukotriene synthesis may have beneficial effects in a rat model of colitis is of particular interest [43].

A number of kinins, including bradykinin, can also induce secretion. However, it is unlikely that they act directly on the intestinal epithelium since there is a close correlation between their secretory effect and the generation of prostaglandin E_2 which they provoke [44]. It seems likely that kinins exert their action by stimulating phospholipase A_2 activity in subepithelial monocytes to liberate arachidonic acid. This in turn provides the substrate for prostanoid and leukotriene production which then secondarily stimulate secretion.

Thus, several of the inflammatory mediators, liberated close to the epithelium, provoke secretion, many of them acting one upon the other and some also depending on the intermediary effects of other cells, neurones and agonists [45]. Dissecting the various interrelationships between them may prove valuable in the search for specific inhibitors which could have therapeutic benefit in some diarrhoeal diseases. A recent uncontrolled trial of supplementing the diet with fish oil, which is known to alter the relative proportions of the various leukotrienes and prostanoids, showed an apparently beneficial effect on the inflammatory reaction in colitis [46].

Neuropeptides

Many neuropeptides are liberated from the enteric nervous system and many of them can stimulate intestinal secretion. Vasoactive intestinal peptide (VIP) is present in high concentrations in the subepithelial neural plexuses, and since this peptide is a potent secretagogue which acts via specific adenylyl cyclase linked receptors [18], it seems highly likely that it has an important physiological role. Unfortunately, such a

role has not yet been unequivocally demonstrated. The possibility that this local plexus is involved in secretory diarrhoeal diseases has been emphasised by studies from Gothenburg which showed that the secretory response to bacterial toxins and inflammatory mediators could be blocked by neural blockade *in vivo* [47,48]. For example, local anaesthetics, ganglion blockers, and tetrodotoxin each inhibited cholera toxin-induced secretion in anaesthetised cat's intestine. Detailed studies of the neural interconnections and neuropeptide localisation lend support to such a role for VIP-containing nerves [49]. These observations have been coupled with the finding of increased VIP release in response to cholera toxin administration [50]. The proposed mechanism for secretory diarrhoea therefore includes a neural reflex with a final common pathway through VIP to the epithelial cell. These observations may be important because they point to a possible line of therapeutic attack with nerve blockers. However, the studies remain to be confirmed and others have not been able to demonstrate a similar mechanism *in vitro* [51].

There is no doubt, however, that the Verner Morrison syndrome, in which an adenoma of the islets of Langerhans releases large amounts of VIP and PHI into the peripheral circulation, produces severe cholera-like diarrhoea induced by these peptides. Removal of the tumour lowers the VIP concentrations and stops the diarrhoea.

Other neuropeptides, listed in Table 1, provoke secretion through the adenylyl cyclase or phosphoinositol turnover messenger systems. A few of these peptides, including VIP, acetyl choline, substance P, and possibly alpha-2 adrenergic agonists, act directly on the epithelium. Other agents, including enkephalins and neurotensin, act indirectly through the local innervation.

Some neuropeptides inhibit secretion and promote absorption, suggesting that there is normally a balance between secretory and absorptive stimuli acting on the epithelium via the enteric nervous system [52].

Interrelationships between neural and inflammatory mediators

There is also evidence of a co-operative interaction between the enteric nervous system and the mediators of an inflammatory response. Experimental animals challenged with the nematode *Nippostrongylus brasiliensis* develop an inflammatory reaction associated with intestinal secretion. Several mediators, including histamine, are released from activated mast cells. In addition, changes in neurally mediated secretion were observed during the inflammatory phase, suggesting that there is a close interrelationship between these two processes [44].

In animals made tolerant to morphine, withdrawal of the opiate causes diarrhoea at least partly due to

Table 1. Neurotransmitters which influence intestinal absorption and secretion

Agonist	Site of action	2nd messenger	
Stimulators of secretion			
Acetyl choline	Epithelium	$IP_3/Ca^{2+}/DG$	
VIP ⎱ PHI ⎰	Epithelium	cAMP	
Substance P	Epithelium Nerves	Ca^{2+}	
Calcitonin gene-related peptide	?	?	
Galanin	?	?	
CCK	?	?	
'Enterochromaffin cells'			
5HT	Epithelium Nerves	$IP_3/Ca^{2+}/DG$	
Neurotensin	Nerves	–	
Stimulators of absorption/inhibitors of secretion			
Noradrenaline (α_2)	Nerves Epithelium	$?G_1$	cAMP inhibition
Somatostatin	Epithelium Nerves	G_1	cAMP inhibition Ca^{2+} inhibition
NPY	Epithelium	?	
Enkephalins	Nerves	– (delta receptors)	

inhibition of salt and water absorption [53]. This process is mediated through the nervous system and is associated with liberation of 5-hydroxytryptamine and prostaglandins in the gut wall, which may therefore be involved in this response [54].

Receptors for substance P on mucosal mast cells and for VIP, substance P, and somatostatin on lymphocytes are probably involved in controlling release of a number of inflammatory mediators including prostanoids, leukotrienes, histamine, kallikreins, and platelet-activating factor from these cells. Furthermore, receptors for histamine, prostaglandins, and platelet-activating factor have been demonstrated on neurones.

It seems likely, therefore, that not only is the epithelium directly influenced by neuropeptides and by inflammatory mediators, but also receptors on nerves and on mesenchymal cells for mediators and neuropeptides point to a close interrelationship between these two types of pathway which has yet to be fully elucidated.

Some substances which influence absorption and secretion are liberated from enterochromaffin or APUD cells (amine-precursor uptake and decarboxylation) sited in the mucosal layer: they include 5-hydroxytryptamine, neurotensin, and somatostatin. The stimulus for their release may be through neural or so-called 'inflammatory' mediator stimulation.

Interrelationships between the enteric, autonomic, and central nervous systems in intestinal secretion

Evidence has accumulated in support of the concept that the autonomic nervous system exerts some control over intestinal absorption and secretion [55]. Parasympathetic stimulation causes secretion both *in vivo* and *in vitro*. There is a basal parasympathetic drive to secretion which is revealed as increased absorption when the parasympathetic is blocked by atropine [56,57]. There is analogous evidence for an adrenergic drive to absorption [58]. It seems likely that these effects of the autonomic are mediated through the local enteric nervous system. There is good evidence for a cholinergic and VIPergic stimulus to the enterocyte but, although there are noradrenergic receptors on enterocytes, few adrenergic nerves reach them [49]. The pro-absorptive action may therefore be mediated by inhibition of cholinergic or VIPergic nerves. These influences are modulated by other neuropeptides liberated in the enteric nervous system.

Much better appreciated is the influence of the autonomic and enteric nervous systems on intestinal motility. In circumstances where short-term stimulation of the autonomic results in gastrointestinal symptoms, it is usually assumed that this is due to its influence on the motor system. That view is likely to be too simplistic, and it is reasonable to assume that there is a mixed response including changes in epithelial salt and water transport.

A series of experiments has been performed in which several different neuropeptides have been injected, in minute amounts, into the cerebral ventricles of experimental animals. The results suggest that intestinal absorption and secretion are markedly influenced by such manoeuvres. Thus, injection of an enkephalin analogue into the cerebral ventricles of anaesthetised rats enhanced absorption in tied-off loops of jejunum and ileum and inhibited secretion provoked by cholera toxin in similar loops [59]. Injection of GABA-like compounds and GABA antagonists into the cerebral ventricles of rats caused secretion and absorption respectively in the intestine [60]. These and similar findings point to the influence of the central nervous system on intestinal transport.

It is rather more difficult to obtain this type of evidence in man, but several non-invasive and indirect experiments support this conclusion in man. Sham feeding, in which normal human volunteers are allowed to chew a meal but not to swallow it, stimulates gastric-acid secretion (the well-known 'cerebral' phase of gastric secretion) and at the same time inhibits salt and water absorption in the perfused jejunum [61]. Stress, induced by placing the hand repeatedly in ice-cold water and thus causing controlled pain, also inhibits salt and water absorption in the perfused human jejunum [62]. Blockade of cold pain-induced changes in intestinal absorption were inhibited by intravenous atropine infusions, suggesting that the effects were mediated via a cholinergic, probably vagal, mechanism.

Psychological stress induced by the dichotomous listening test induced a similar response [63]. In these experiments, normal volunteers were asked to listen through a pair of head-phones to two different novels, one played into each ear. The subjects were asked, at the same time, to answer questions relevant to one or other of the novels. The stress induced by this manoeuvre caused a rise in pulse rate, blood pressure, and respiratory rate, and also induced secretion of salt and water into the jejunum.

These observations could be relevant to stress-induced diarrhoea as, for example, in subjects suffering from examination nerves.

Summary

A wide range of different stimuli is perceived by the intestinal epithelium. They include luminal factors, especially bacterial toxins, and agonists such as inflammatory mediators and neuro peptides, acting from the interstitial fluid surrounding the epithelial cells. It is likely that in any individual patient with diarrhoea there is a range of stimuli acting upon the epithelium. Specific receptors on the apical and basolateral membrane, activated by these stimuli, transduce the perceived signals to stimulate a series of membrane-bound enzyme systems. They in turn generate second messengers which are liberated into the cytoplasm. These include cyclic adenosine monophosphate, cyclic

guanosine monophosphate, inositol triphosphate (which goes on to liberate free calcium), and diacyl glycerol. Each of these second messengers activates a different protein kinase, each of which then induces the phosphorylation of a series of cytoplasmic and membrane-bound proteins. Each of the protein kinases is likely to influence the activity of the others so that their effects are closely integrated. The final common pathways through which intestinal secretory stimuli pass involve the opening of an anion channel in the apical membrane, together with the stimulated uptake of chloride at the basolateral membrane. Anions, especially chloride and possibly bicarbonate, are then secreted into the lumen, and sodium and water passing between the cells accompany them. The net result is secretion of salt and water, which lies at the centre of a number of diarrhoeal diseases.

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Address for correspondnece: Professor L. Turnberg, Department of Medicine, Hope Hospital, Eccles Old Road, Salford M6 8HD.