PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroentero, 2000; 6(4):470-474
World Journal of Gastroenterology
 ISSN 1007-9327

New insights into the pathogenesis of intestinal dysfunction: secretory diarrhea and cystic fibrosis

Kim E. Barrett

Subject headings gatrointestinal motility; diarrhea/ etiology; cystic fibrosis; intestinal secret ions; chlorides; Crohn\s disease; intestinal absorption

Barrett KE. New insights into the pathogenesis of intestinal dysfunction: secretory diarrhea and cystic fibrosis. ***World J Gastroentero***, 2000;6(4):470-474

INTRODUCTION

A major function of the intestinal epithelium is to control the amount of fluid entering into and being absorbed from the lumen[1]. In healthy conditio ns, net fluid movement follows an absorptive vector, although significant secret ion also takes place to subserve digestive function. Thus, the secretion of flui d, driven by the active secretion of electrolytes, is important for maintaining the fluidity of intestinal contents during various stages of digestion and there by allowing for diffusion of enzymes and nutrients. In the setting of disease, dysregulation of intestinal transport mechanisms may alter the balance between ab sorptive and secretory processes such that secretion predominates, leading to th e clinical consequence of diarrhea. However, under conditions of both health and disease, fluid secretion is driven largely by the active secretion of chloride ions. Thus, there are both basic and clinical reasons for wishing to gain a full understanding of the basis and regulation of this transport process. The goal o f my article, therefore, will be to review our understanding of intestinal chlor ide secretion and the ways in which it is regulated. Recent insights in this are a enhancing our ability to intervene in diseases where chloride secretion is over-expressed, such as infectious and inflammatory diarrheal illnesses will also be discussed. This article will also cover the implications of intestinal secretory mechanisms for a genetic disease where chloride secretion is under-expressed, namely cystic fibrosis, where significant intestinal dysfunction, including obstruction and malabsorption, may also ensue.

Professor and Vice-Chair for Research, Department of Medicine, University of California, San Diego, School of Medicine, San Diego, CA 92103, USA
Professor Kim E. Barrett received her Ph.D. degree from University College London, England, in 1982, and postdoctoral training from the National Institutes of Health, USA. She is currently Professor and Vice-Chair for Research in the Department of Medicine, University of California, San Diego, School of Medicine, and directs an NIH-funded research program that focuses on the mechanisms and regul ation of intestinal electrolyte transport. She has published over 140 original papers, book chapters and review articles, and is the current Editor-in-Chief of American Journal of Physiology-Cell Physiology.
Correspondence to: Kim E. Barrett, Ph.D. UCSD Medical Center, 8414 200 West Arbor Drive San Diego, CA 92103-8414 USA
Tel. 619-5433726. Fax. 619-5436969
Email. kbarrett@ucsd.edu
Received 2000-05-25 **Accepted** 2000-06-15

MECHANISMS OF INTESTINAL CHLORIDE SECRETION

The secretory mechanism

The details of the ion transport pathways making up the intestinal chloride secretory mechanism have been quite well worked out at this point[1,2]. The mechanism is predominantly expressed, at least as a ssessed functionally, in epithelial cells lining the crypts of both the small in testine and colon, although there may be a small degree of secretion derived fro m villus or surface epithelial cells in addition[2]. Chloride is taken up from the bloodstream across the basolateral membrane of epithelial cells via a sodium/potassium/2 chloride cotransporter that has been cloned and designated as NKCC1[3]. This cotransporter is driven secondarily by the low intrace llular sodium concentration established by the active sodium/potassium ATPase, also localized to the basolateral membrane. This allows chloride to accumulate in the cell cytosol above its electrochemical equilibrium. When apical chloride channels are opened, this chloride is then free to flow out of the cell down this electrochemical gradient, resulting overall in net transepithelial transfer of the anion. Chloride exit occurs predominantly through a channel referred to as CF TR, which is the product of the gene that is defective in the setting of cystic fibrosis[4]. More recently, in addition, it has been recognized that the apical membrane of intestinal epithelial cells may also contain calcium-activa ted chloride channels of the CLCA family, and perhaps other channels for these a nd/or other anions[5,6]. The transport mechanism is also critically depe ndent on the presence of basolateral potassium channels which serve to recycle co-transported potassium back across the basolateral membrane, and thus prevent cell depolarization[7,8].

Positive regulation of chloride secretion

Chloride secretion is stimulate d in the intestine by a broad array of substances derived from local and more di stant endocrine cells, enteric nerves, neighbouring cell types such as immune cells and subepithelial myofibroblasts, and exogenous factors such as bacterial enterotoxins[2]. These regulatory pathways provide for minute-to-minute physiological increases in the extent of secretion (such as in response to inge stion of a meal) but also contribute to the marked upregulation of secretion that can occur in the setting of a disease[1]. Despite many hormones, neur otransmitters and other mediators that have been identified and charac terized to date as stimuli of chloride

secretion, however, appear to exert their effects t hrough two main signaling pathways[1]. The first of these is mediated by changes in intracellular cyclic nucleotides, and results in large magnitude, sustained secretory responses. At an intracellular level, the primary locus for regulation appears to be the cyclic-nucleotide mediated phosphorylation and open ing of the CFTR chloride channel, particularly via cAMP and the cAMP-dependent protein kinase, PKA[9,10]. It is increasingly recognized that at least o ne type of PKA is "scaffolded" in the proximity of CFTR via various interactin g proteins, which likely contributes to the efficiency of signal transduction in response to cAMP-mobilizing agonists[11,12]. Chloride secretion can a lso be evoked by agonists that increase intracellular levels of cGMP, the best physiological example of this pathway being the stimulation by the peptide agonis t guanylin, which binds to an apical receptor also shared by the heat-stable to xin of *E.coli*[13]. cGMP-dependent secretion involves a cGMP-depend ent protein kinase and/or cross-activation of PKA, and is very similar to that induced by increases in cAMP in terms of its magnitude, kinetics and dependence on CFTR activation. Cyclic nucleotide-dependent secretion, in general, may also involve regulatory events at additional levels within the transport machinery.For example, there is evidence to suggest that sustained secretion requires the insertion of additional NKCC1 molecules into the basolateral membrane (or at least an alteration in their rate of endocytosis), and cAMP-activated potassium channels have also been postulated on functional grounds[3,8,14].

Chloride secretion can also be evoked by agonists that are capable of causing an increase in cytosolic calcium concentrations. In contrast to the cyclic-nucleo tide dependent chloride secretory responses discussed above, calcium-dependent responses are distinctive in that they are smaller and also considerably more tr ansient[2]. However, calcium-dependent secretion may nevertheless play a physiological role, particularly in the setting where only a brief, self-lim ited secretory response is called for. Moreover, synergism occurs between the se cretory effects of cAMP- and calcium-dependent agents, allowing the host, to call on markedly upregulated rates of secretory function at times of threat (such as invasion by pathogenic microorganisms). Finally, calcium-dependent secretor y responses may assume far greater significance in the setting of cystic fibrosi s, where the cAMP- dependent chloride channel, CFTR, is lost or dysfunctional[15]. At an intracellular level, at least part of the chloride secretory e ffect occurring in response to an increase in cytosolic calcium is secondary to the opening of basolateral, calcium-sensitive potassium channels. This, in turn , increases the driving force for chloride to exit across the apical membrane th rough the small proportion of CFTR channels that are hypothetically open even in resting cells[16]. However, evidence is also accumulating to suggest th at elevations in calcium, acting in concert with the calmodulin-dependent prote in kinase CaMKII, open additional apical chloride channels of the CLCA family [17-20]. This view lends additional credence by the observation that calci um-dependent agonists can cause chloride secretion in at least some epithelial cells even in the absence of any measurable CFTR function[17,21].

Negative regulation of chloride secretion

Work from our laboratory has al so indicated that certain intracellular signaling pathways may lead to the inhib ition of ongoing chloride secretion, and/or may result in its termination[2]. Such negative signals appear to be particularly pertinent in the case of c alcium-stimulated chloride secretion, with the transience of this secretory res ponse implying that endogenous factors may serve to limit its extent. We have id entified two main pathways whereby calcium-dependent chloride secretion is inhi bited. The first of these, interestingly, is activated by substances that alsos erve to initiate calcium-dependent chloride secretion. An example is the muscar inic agonist carbachol, which evokes a transient chloride secretory response tha t renders intestinal epithelial cells refractory to re-stimulation by another calcium-dependent secretagogue[22]. Both the stimulatory and inhibitory effects of carbachol are dependent on cytosolic calcium [23]. The inhib itory pathway involves the generation of a novel inositol phosphate mediator, in ositol 3,4,5,6 tetrakisphosphate [Ins (3,4,5,6) P_4], which reduces the open pr obability of calcium-activated chloride channels [22,24]. The upstream pathways leading to generation of this messenger involve tyrosine-kinase depend ent events, and the transactivation of the receptor for epidermal growth factor (EGF) and recruitment of the MAP kinase signaling cascade[25]. Inhibitors of both the EGF receptor and MAP kinase pathway potentiate and prolong secret ory responses to carbachol[25].

A second inhibitory mechanism also centers around the EGF receptor, but the mess engers and targets involved in the inhibiti on of chloride secretion are differen t[26-28]. Thus, when the EGF receptor is activated by its cognate liga nds, it heterodimerizes with another member of the ErbB receptor family, ErbB2, and thereby recruits alternative signaling events than those resulting from EGF receptor transactivation in response to carbachol[29]. This signaling d iversification, in turn, appears to activate phosphatidylinositol 3-kinase, an ovel isoform of protein kinase C (PKC-), and ultimately leads to th e inhibition of a basolateral potassium channel, thereby inhibiting the overall process of chloride secretion by preventing potassium recycling[27,28,30]. It is of interest to note that EGF, and related growth factors, inhibit chloride secreti on without themselves serving as agonists in the process. Since such growth factors and their receptors are known to be upregulated in the setting of mucosal in jury, their ability to inhibit chloride secretory responses may represent an ada ptive response that would limit diarrhea under these conditions.

ALTERATIONS IN CHLORIDE SECRETION IN THE SETTING OF DISEASE

Secretory diarrhea

An excess of chloride secretion into the intestine, a bove that which can be compensated for by the reserve capacity of intestinal abso rptive mechanisms, manifests clinically as diarrhea[1]. The classical example of this response is seen in cholera, where up to 20 liters of stool fluid can be lost per day[1]. Cholera, in common with a number of other intes tinal pathogens, elaborates enterotoxin that interact with epithelial cell signal transduction machinery to elicit profound and sustained chloride secretion due to an irreversible increase in intracellular cyclic nucleotide concentrations. Cholera toxin is internalized and induces a massive increase in intracellular cAMP, whereas the heat stable enterotoxin of *E.coli* bind to an apical recepto r that contains an integral guanylyl cyclase activity, and thereby stimulates ch loride secretion secondary to an increase in intracellular cGMP. The direct effects of enterotoxins on secretory epithelial cells are also amplified by a number of additional actions. For example, cholera toxin also activates enteric nerve endings, enterochromaffin cells and mast cells, all of which are capable of rele asing neurotransmitters and other agonists that themselves can stimulate chloride secretion either directly or indirectly[2].

Secretory diarrheal illness is also seen in the setting of infections with patho gens that are not known to elaborate enterotoxins activities. For example, Sal monella Dublin, an invasive bacterium, appears to activate a program of gene e xpression within the epithelial cell that predisposes to excessive fluid secreti on. Thus, there is upregulation of cyclooxygenase-2 and nitric oxide synthase e xpression, and in cell culture models, both of these enzymes appear to contribute to an enhanced capacity of the epithelial cell to secrete chloride in response to a range of stimuli[31]. Similarly, over-expression of cyclooxygenase-2 leads to an increased production of prostaglandins, particularly those of the E series that are known to be potent chloride secretagogues[31]. Sa lmonella invasion also evokes the synthesis of a wide range of chemokines and other cytokines by intestinal epithelial cells, and the resulting inflammatory influx that is targeted to infected cells almost certainly further amplifies secr etory responses in the intact tissue setting[32]. Furthermore, some work ers have shown that *Salmonella* infection is associated with the de novo expression of receptors for the neuropeptide galanin, a known secretory agonist[33,34]. Antibodies that interrupt galanin signaling can significantly dampen intestinal fluid secretion in a murine model of salmonellosis[33]. Thus, the host defense response of diarrhea can be generated even by pathogenic microorganisms that do not elaborate any known enterotoxins. Indeed, upregulati on of the secretory capacity of intestinal epithelial cells can even occur in the setting of infection with a non-invasive parasite also associated with diarrh eal illness, *Giardia lamblia*. These parasites adhere to, but do not penetrate, the intestinal epithelium. Co-culture of intestinal epithelial cells with G. *lamblia* trophozoites results in enhanced chloride secretory responses, and a pparent upregulation of expression of membrane transport proteins involved in th e chloride secretory mechanism such as CFTR and NKCC1 (Resta-Lenert *et al*, submitted). However, paradoxically, Giardia co-incubation appears to signif icantly reduce the ability of certain agonists to mobilize intracellular calcium. These data, in addition to being potentially significant for our understanding of infectious diarrhea, are also of interest because they underscore the concept that calcium-dependent secretagogues may exert negative as well as positive effects on secretion.

Diarrhea is also a common sequella of non-infectious, inflammatory conditions o f the intestine, such as ulcerative colitis and Crohn\s disease[1]. A substantial body of evidence now suggests that cells of the immune system that p resumably are activated in the course of such conditions can release a wide range of products capable of evoking chloride secretion. Conversely, inflammation an d tissue injury is also often accompanied by upregulation of the expression of g rowth factors, such as EGF, and their receptors, which may serve ultimately to c ounteract excessive secretion associated with inflammatory conditions of the gut[35]. Such mechanisms may underlie the efficacy of growth factors such as EGF and insulin-like growth factor that has been demonstrated in diarrheal conditions in various models of inflammation[35].

Cystic fibrosis

The disease states discussed above are characterized by e xcessive chloride secretion. However, inadequate chloride secretion, as occurs in the setting of cystic fibrosis, can be equally or perhaps even more disadvanta geous for the patient. Cystic fibrosis is characterized functionally by the abse nce of a cAMP-regulated chloride secretory pathway[15]. This is due to a series of more than 800 known mutations although about 70% of cases are relat ed to a single mutation, of the CFTR gene, $\triangle$F508, that results in a channel protein that fails to traffic normally to the apical plasma membrane of secretory epithelial cells. Much of the morbidity and mortality in cystic fibrosis relates to pulmonary manifestations[36]. However, with advances in diagnosis a nd treatment, the median survival age for patients with cystic fibrosis has incr eased dramatically over the last several years. This means that disease manifest ations in organs other than the lungs are becoming more recognized, and may carry a substantial burden in terms of morbidity.

There are a number of gastrointestinal manifestations of cystic fibrosis, as wel l as complications related to dysfunction of the biliary and pancreatic systems. A large proportion of newborns with the disease are born with a form of intest inal obstruction known as meconium ileus, assumed to result from inspissated int estinal secretions that are poorly hydrated and thus difficult to clear from the lumen. In some cases, prompt surgical intervention is necessary, and evidence suggests that children presenting

with this complication will go on to have poorer nutritional status and growth, even if treated successfully, than cystic fibr osis patients without this manifestation[37,38]. Similarly, intestinal obstruction associated with cystic fibrosis may also be seen in older children and also in the increasing number of adults living with this disease[39]. The role of CFTR in promoting biliary and pancreatic ductular secretion also me ans that the majority of patients suffer from pancreatic insufficiency, up to ha lf experience biliary disorders, and approximately 5% may display Frank liver disease[39]. In addition, it has recently been postulated that unsuspected cystic fibrosis (of genotypes other than those associated with pancreatic insuf ficiency, such as △F508) may be the cause of at least some cases of idiopathic pancreatitis[40]. In each of these cases of gastrointestinal complicat ions of cystic fibrosis, the pathology suggests that any alternate chloride secr etory mechanism that are present in intestinal, pancreatic or biliary epithelial cells are insufficient to compensate for the loss of cAMP-regulated chloride secretion. In this respect, the knowledge that intestinal epithelial cells, at least, express intrinsic negative signaling pathways that limit the extent of calc ium-dependent chloride secretion may offer both an explanation for this situati on, as well as a possible therapeutic target if such negative signaling events can be countermanded.

The prevalence of cystic fibrosis in, especially, Caucasian populations, where estimates suggest that more than 3% of the population are heterozygous carriers o f the disease, raises questions about the evolutionary pressures that have drive n the retention of mutant CFTR alleles in the gene pool. Hypothetically, suchi ndividuals might have thereby gained a heterozygote advantage in resistance to enterotoxin-mediated diarrheal illnesses, such as cholera, although this may be inconsistent with the relative prevalence of cystic fibrosis in Caucasian compared with other ethnic groups. Moreover, studies in mouse models of cystic fibrosi salso fail to suggest that heterozygotes show any diminished intestinal secretory responses to cholera toxin[41,42]. However, more recent studies sugg est that tissues obtained from mice with one mutant CFTR allele do display an in creased resistance to invasion by bacteria such as *Salmonella*, although the mechanism of this effect is unknown[43]. This does provide an attractive hypothesis for cystic fibrosis prevalence, especially given that typhoid fever was common in Western Europe until relatively recent times.

CONCLUSION

Advances in our understanding of the molecular basis of intestinal chloride secr etion, as well as its regulation, may offer significant insights into disease states where this transport mechanism is either over or under-expressed in the intestine, such as secretory diarrhea and cystic fibrosis. Ultimately, it is hoped that such insights will spawn new therapies for these conditions. Secretoryd iarrheal illness, in particular, still constitutes a major burden of morbidity and mortality, particularly among children in developing countries. Moreover, in creased international travel, and the emergence of more highly pathogenic strains of certain bacteria, such as *E.coli*, will also increase the prevalence of diarrhea. Thus, improved therapies are urgently needed, in concert with the public health measures that will reduce exposure to the infectious agents that cause such disease.

ACKNOWLEDGEMENTS I thank Glenda Wheeler-Loessel for assistance with manuscript preparation. I am also grateful to the following former and present members of my laboratory who have contributed to some of the studies described herein: Lone Bertelsen, Ph.D. Sean Calandrella, Jimmy Yip Chuen Chow, Ph.D., Cornelia Gelbmann, M.D., Stephen Keely, Ph.D., Declan McCole, Ph.D., Silvia Resta-Lenert, M.D., Ph.D., Jrgen Ri es, M. D., Jane Smitham, Jrgen Stein, M.D., Ph.D., Jorge Uribe, Ph.D., M.D., and Mana Vajanaphanich, M.D. Studies from the author's laboratory have been support ed by grants from the National Institutes of Health (USA): DK28305, DK53480 and DK35108 Unit 5.

REFERENCES

1 Montrose MH, Keely SJ, Barrett KE. Secretion and a bsorption: small intestine and colon. In: Yamada T, Alpers DH, Laine L, Ow yang C, Powell DW, eds.Textbook of Gastroenterology.Lippincott: Willi ams and Wilkins, Philadelphia, 1999:320-355
2 Barrett KE, Keely SJ. Chloride secretion by the intestinal epithelium: molecular basis and regulatory aspects. *Annu Rev Physiol*, 2000; 62:535-572
3 D'Andrea L, Lytle C, Matthews JB, Hofman P, Forbush B III, Madara JL. Na:K:2Cl cotransporter (NKCC) of human intestinal epithelial cells. Surface expression in response to cAMP. *J Biol Chem*, 1996;271:28969- 28976
4 Fuller CM, Benos DJ. *CFTR Am J Physiol*, 1992;263:C 267-C286
5 Gruber AD, Elble RC, Ji HL, Schreur KD, Fuller CM, Pauli BU. Geno mic cloning, molecular characterization, and functional analysis of human CLCA1, the first human member of the family of Ca 2+ activated Cl - channel proteins. *Genomics*, 1998;54:200-214
6 Gruber AD, Schreur KD, Ji HL, Fuller CM, Pauli BU. M olecular cloning and transmembrane structure of hCLCA2 from human lung, trachea, and mammary gland. *Am J Physiol*, 1999;276:C1261-C1270
7 Mandel KG, McRoberts JA, Beuerlein G, Foster ES, Dharmsathaphorn K. Ba^{2+} inhibition of VIP and A23187 stimulated Cl- secreti on by T 84 cell monolayers. *Am J Physiol*, 1986;250:C486-C494
8 Mandel KG, McRoberts JA, Beuerlein G, Foster ES. Ba^{2+} inhibition of VIP-and A23187 stimulated Cl- secretion by T-84 cell monolayers. *Am J Physiol*, 1986;250:C486
9 Jia Y, Mathews CG, Hanrahan JW. Phosphorylation by protein kinase C is required for acute activation of cystic fibrosis transmembrane con ductance regulator by protein kinase A. *J Biol Chem*, 1997;272:4978-4984
10 Luo J, Pato MD, Riordan JR, Hanrahan JW. Differential regula tion of single CFTR channels by PP2C, PP2A, and other phosphatases. *Am J Physi ol*, 1998;274:C1397-C1410
11 Singh AK, Tasken K, Walker W, Frizzell RA, Watkins SC, Bri dges RJ, Bradbury NA.Characterization of PKA isoforms and kinase depende nt activation of chloride secretion in T 84 cells. *Am J Physiol*, 1998;2 75:C562-C570
12 Short DB, Trotter KW, Reczek D, Kreda SM, Bretscher A, Bou cher RC, Stutts MJ, Milgram SL. An apical PDZ protein anchors the cystic fibr osis transmembrane conductance regulator to the cuytoskeleton. *J Biol Chem*, 1998;273:19797-19801
13 Forte LR, Hamra FK. Guanylin and uroguanylin: intestinal peptid e hormones that regulate epithelial transport. *News in Physiol Sci*, 1996;11: 17-24
14 Matthews JB, Smith JA, Hrnjez BJ. Effects of F-actin stabili zat ion or disassembly on epithelial Cl^- secretion and Na-K_2Cl cotransport. *Am J Physiol*, 1997;272:C254-C262
15 Frizzell RA. The molecular physiology of cystic fibrosis. *News in P hysiol*

Sci, 1993;8:117-120
16 Cartwright CA, McRoberts JA, Mandel KG, Dharmsathaphorn K. S yn ergistic action of cyclic adenosine monophosphate and calcium mediated secret ion in a colonic epithelial cell line. *J Clin Invest,* 1985;76:1837-1842
17 Wagner JA, CozensAL, Schulman H, Gruenert DC, Stryer L, Gardner P. Acti vation of chloride channels in normal and cystic fibrosis airway cel ls by multifunctional calcium/calmodulin protein kinase. *Nature,* 1991;349:79 3-796
18 Fuller CM, Ismailov II, Keeton DA, Benos DJ. Phosphorylatio n and activation of a bovine tracheal ion channel by Ca 2 +/ calmodulin depe ndent protein kinase II.J Biol Chem,1994;269: 26642-26650
19 Barrett KE, Dharmsathaphorn K. Secretion and absorption: small i ntestine and colon. In: Yamada T, ed. Textbook of Gastroenterology. Philadelphi a: JB Lippincott Company; 1991: 265-294
20 Luo H, Lindeman RP, Chase Jr HS. Participation of protein kin ase C in desensitization to bradykinin and to carbachol in MDCK cells. *Am J Ph ysiol,* 1992;262:F499-F506
21 Wagner JA, McDonald TV, Nghiem PT, Lowe AW, Schulman H, Gruenert DC, Str yer L, Gardner P. Antisense oligodeoxynu cleotides to the cystic fibrosis transmembrane conductance regulator inhibit cAMP-activated but not cal cium activated chloride currents. *Proc Natl Acad Sci USA,* 1992;89:6785-6789
22 Vajanaphanich M, Schultz C, Rudolf MT, Wasserman M, Enyedi P, Craxton A, Shears SB, Tsien RY, Barrett KE, Traynor- Kaplan A. Long-te rm uncoupling of chloride secretion from intracellular calcium levels by Ins (3, 4,5,6) P_4. *Nature,* 1994;371:711-714
23 Keely SJ, Calandrella SO, Barrett KE. Carbachol stimulated transactivation of epidermal growth factor receptor and MAP kinase in T 84 c ells is mediated by intracellular Ca 2+, PYK-2, and p60 src. *J Biol Chem,* 2000; in press
24 Ismailov II, Fuller CM, Berdiev BK, Shlyonsky VG, Benos DJ, Barrett KE. A biologic function for an "orphan" messenger: D myo inosit o l (3,4,5,6) tetrakisphosphate selectively blocks epithelial calcium activated chloride channels. *Proc Natl Acad Sci USA,* 1996;93:10505-10509
25 Keely SJ, Uribe JM, Barrett KE. Carbachol stimulates transactiv ation of epidermal growth factor receptor and MAP kinase in T-84 cells: imp lications for carbachol stimulated chloride secretion. *J Biol Chem,* 1998;27 3:27111-27117
26 Uribe JM, Gelbmann CM, Traynor-Kaplan AE, Barrett KE. Epiderm al growth factor inhibits calcium-dependent chloride secretion in T-84 hum an colonic epithelial cells. *Am J Physiol,* 1996; 271:C914-C922
27 Uribe JM, Keely SJ, Traynor-Kaplan AE, Barrett KE. Phosphatidylinositol 3kinase mediates the inhibitory effect of epidermal growth factor o n calcium-dependent chloride secretion. *J Biol Chem,* 1996;271:26588-26595
28 Barrett KE, Smitham J, Traynor-Kaplan AE, Uribe JM. Inhibition of Ca2+ dependent Cl- secretion in T-84 cells: membrane target (s) of inhibition are agonist-specific. *Am J Physiol,* 1998;274:C958-C965
29 Keely SJ, Barrett KE. ErbB2 and ErbB3 recptors mediate inhibiti on of calciumdependent chloride secretion in colonic epithelial cells. *J Bio l Chem,* 1999;274:33449-33454
30 Chow JYC, Uribe JM, Barrett KE. A role for protein kinase C in the i nhibitory effect of EGF on calcium stimulated chloride secretion in human colonic epithelial cells. *J Biol Chem,* 2000; in press
31 Eckmann L, Stenson WF, Savidge TC, Lowe DC, Barrett KE, Fierer J, Smith JR, Kagnoff MF. Role of intestinal epithelial cells in the host secretory response to infection by invasive bacteria. Bacterial entry induces epithelial prostaglandin hsynthase 2 expression and prostaglandin E2 and F2alph a production. *J Clin Invest,* 1997;100:296-309
32 Eckmann L, Kagnoff MF, Fierer J. Epithelial cells secrete the c he mokine interleukin 8 in response to bacterial entry. *Infect Immun,* 1993;61: 4569-4574
33 Marrero JA, Matkowskyi KA, Danilovich A, Koutsouris A, Hecht G, Benya RV. Salmonella infection causes increased fluid secretion in mouse col on by activating NF kB and increasing galanin 1 receptor (GAL1R) expression. *Gastroenterology,* 1999;116:A860
34 Benya RV, Marrero JA, Ostrovskiy DA, Koutsouris A, Hecht G. H uman colonic epithelial cells express galanin-1 receptors, which when activated cause Cl-secretion. *Am J Physiol,* 1999;276:G64-G72
35 Barrett KE. Non mitogenic effects of growth factors and their recept ors in the gastrointestinal tract. In: Andus T, ed. Cytokines and cell hom eostasis in the gastrointestinal tract. Lancaster, UK: Kluwer Academic Publishers, in press
36 Boucher R. Status of gene therapy for cystic fibrosis lung disease. *J Clin Invest,* 1999;103:441-445
37 Kerem E, Corey M, Kerem B, Durie P, Tsui L-C, Levinson H. Clin ical and genetic comparisons of patients with cystic fibrosis, with or without m econium ileus. *J Pediatr,* 1989;114:767-773
38 Durie PR. Clues to the pathogenesis of meconium ileus. *Ped Pulmonol S,* 1996;13:193
39 Liddle R. Hereditary diseases of the pancreas. In: Yamada T, ed, T extbook of Gastroenterology. Philadelphia: JB Lippincott Company; 1995:2161-2173
40 Cohn JA, Friedman KJ, Noone PG, Knowles MR, Silverman LM, Jowell PS. Re lation between mutations of the cystic fibrosis gene and idiopathi cpancreatitis. *N Engl J Med,* 1998;339:653-658
41 Cuthbert AW. The biochemical defect in cystic fibrosis. *J Roy Soc M ed,* 1992;85:2-5
42 Valverde MA, O'Brien JA, Sepulveda FU, Ratcliff R, Evans MJ, Coll edge WH. Inactivation of the murine cftr gene abolishes cAMP-mediated bu t not Ca^{2+} mediated secretagogue induced volume decrease in small intesti nal crypts. *Pflugers Arch,* 1993; 425:434-438
43 Grout M, Zaidi T, Meluleni G, Mueschenborn SS, Banting G, Ratc liff R, Evans MJ, Colledge WH, Pier GB. Salmonella typhi uses CFTR to enter intestinal epithelial cells. *Nature,* 1998;393:79-82

Edited by Lu J
proofread by Mittra S