Apical leptin induces chloride secretion by intestinal epithelial cells and in a rat model of acute chemotherapy-induced colitis

M. Raschid Hoda, Michael Scharl, Stephen J. Keely, Declan F. McCole, and Kim E. Barrett

Department of Medicine, University of California, San Diego, School of Medicine, La Jolla, California

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Hoda MR, Scharl M, Keely SJ, McCole DF, Barrett KE. Apical leptin induces chloride secretion by intestinal epithelial cells and in a rat model of acute chemotherapy-induced colitis. Am J Physiol Gastrointest Liver Physiol 298: G714–G721, 2010. First published March 4, 2010; doi:10.1152/ajpgi.00320.2009.—The purpose of this study was to investigate whether luminal leptin alters ion transport properties of the intestinal epithelium under acute inflammatory conditions. Monolayers of human intestinal T84 epithelial cells and a rat model of chemotherapy-induced enterocolitis were used. Cells were treated with leptin and mounted in Ussing chambers to measure basal and secretagogue-induced changes in transepithelial short-circuit current (Isc). Furthermore, the role of MAPK and phosphatidylinositol 3-kinase (PI3K) signaling pathways in mediating responses to leptin was investigated. Acute colitis in Sprague-Dawley rats was induced by intraperitoneal injection of 40 mg/kg methotrexate. Leptin (100 ng/ml) induced a time-dependent increase in basal Isc in T84 intestinal epithelial cells (P < 0.01). Moreover, pretreatment of T84 cells with leptin for up to 1 h significantly potentiated carbachol- and forskolin-induced increases in Isc. Pretreatment with an inhibitor of MAPK abolished the effect of leptin on basal, carbachol- and forskolin-induced chloride secretion (P < 0.05). However, the PI3K inhibitor, wortmannin, only blunted the effect of leptin on forskolin-induced increases in Isc. Furthermore, leptin treatment evoked both ERK1/2 and Akt1 phosphorylation in T84 cells. In the rat model, luminal leptin induced significant increases in Isc across segments of proximal and, to a lesser extent, distal colon (P < 0.05). We conclude that luminal leptin is likely an intestinal chloride secretagogue, particularly when present at elevated concentrations and/or in the setting of inflammation. Our findings may provide a mechanistic explanation, at least in part, for the clinical condition of secretory diarrhea both in hyperleptinemic obese patients and in patients with chemotherapy-induced intestinal inflammation.

intestinal ion transport

Obesity is increasingly recognized as a risk factor for a number of benign and malignant gastrointestinal conditions (28). Obesity is also associated with functional disorders of the intestine, manifesting clinically as a wide range of symptoms such as diarrhea, nausea, vomiting, and constipation (17, 31). In particular, a recent report from a general population sample of young adults revealed a significant univariate association between body mass index (BMI) and diarrhea that was characterized by a higher prevalence of diarrhea among obese and overweight individuals, compared with normal-weight participants (42). Furthermore, mesenteric adipose tissue has been implicated in a wide range of gastrointestinal disorders, including fatty liver, gastrointestinal cancers, acute pancreatitis, Crohn’s disease, and even functional bowel disorders (11, 28). Finally, although obesity does not seem to increase the risk of inflammatory bowel disease, it is associated with increased disease severity and anorectal complications (9).

Obesity is also associated with hyperleptinemia (33). Leptin, the obesity gene cloned in 1994, is produced mainly by adipocytes and is the key hormone involved in central regulation of body weight (23). In addition to adipocytes, several other organs such as the stomach, skeletal muscle, placenta, and brain are able to synthesize leptin (34). In particular, the gastric mucosa has been shown to secrete leptin in both an exocrine and endocrine fashion, implying a possible physiological role (2). Immunohistochemistry revealed that chief cells of the fundic mucosa contain leptin in their secretory granules (39). Upon stimulation by nervous or hormonal factors, leptin is released into the gastric juice linked to a protein of high molecular weight, and this complex is translocated in its intact form to the small intestine. Moreover, at least a portion of the stomach-derived leptin escapes proteolysis, suggesting that leptin could reach the distal part of the intestine in an active form. It could therefore initiate biological processes that control functions of the intestinal tract, such as absorption and secretion (35). In keeping with this, leptin receptors, including the functional long isoforms (Ob-Rb), are expressed on the brush borders and basolateral membranes of human enterocytes, rat colonocytes, and cultured colonic epithelial cells, such as the T84 cell line (1, 6, 18).

In the normal colon, luminal leptin is in the low nanomolar range. However, in inflammatory states, leptin staining in colonic epithelial cells was detected and luminal leptin concentrations increased significantly (~10 times greater than in noninflamed samples) (13). In addition, recent studies have demonstrated that the leptin concentration in colonic lavage fluids from patients with mild to severe inflammatory bowel disease (IBD) is >15-fold higher than that seen in normal subjects (38). Although it is likely that plasma leptin could more readily cross a leaky epithelium and thus be present in the intestinal lumen, it is thought that inflamed colonic epithelial cells additionally secrete leptin into the intestinal lumen, which in turn may act on these cells in an autocrine fashion (38).

The results outlined above form part of a growing body of evidence for a pathophysiological role of intraluminal leptin during states of obesity and intestinal inflammation. In obese individuals (BMI >30 kg/m2) plasma leptin levels can be as high as 100 ng/ml, which is more than 20-fold higher than plasma levels in individuals of normal weight (10). Several studies have additionally indicated that leptin may be involved in the acute stress response to severe illness and surgery (26, 45). Of note, other neurohormonal and inflammatory mediators and cytokines associated with states of intestinal inflammation...
and systemic stress have been shown to alter critical hallmark functions of the intestinal epithelium, such as ion transport (24). However, studies of the effect of luminal leptin on intestinal ion transport are limited. Therefore, the aim of the present study was to determine whether luminal leptin affects the ion transport properties of the intestinal epithelium under physiological, hyperleptinemic, and/or acute intestinal inflammatory conditions.

**MATERIALS AND METHODS**

**Materials.** Human and rat recombinant leptin were purchased from Sigma (St. Louis, MO). Upstate Biotechnology blocking reagent for Western blotting (skim milk), rainbow-colored protein molecular weight markers ECL Plus (Amersham Pharmacia Biotech, Piscataway, NJ), phospho-p44/42 MAPK antibody (ERK1/2) (rabbit polyclonal IgG; Cell Signaling Technology, Beverly, MA), total ERK and GAPDH antibodies, as well as phospho-Akt 1/PKB-α and total Akt antibodies (rabbit polyclonal or monoclonal IgG; New England Biolabatories, Frankfurt, Germany), carbachol (CCh) and forskolin (FSK) (Sigma), the MEK inhibitor PD98059 (PD), and the phospho-tidylinositol 3-kinase (PI3K) inhibitor wortmannin (EMD Biosciences, San Diego, CA) were obtained from the sources indicated.

**Cell culture.** The human colonic epithelial cell line, T84, was cultured in DMEM/Ham’s F-12 medium (50:50 mix) (Mediatech-Cellgro, Herndon, VA), supplemented with 5% (vol/vol) newborn calf serum (HyClone, Logan, UT). For Ussing chamber/voltage clamp studies, ~5 × 10⁶ cells were seeded onto 12-mm Millicell-HA Transwells (Millipore, Bedford, MA). For experiments involving Western blotting, ~10⁶ cells were seeded onto 30-mm Millicell-HA Transwells. Cells seeded onto Millicell filters were cultured for 10–15 days at 37°C in an atmosphere of 95% O₂-5% CO₂ before use. In cells pretreated with leptin, the hormone was added in regular T84 medium for 15 min equilibration period, chloride secretion was elicited using 140 Na⁺, 5.2 K⁺, 1.2 Ca²⁺, 0.8 Mg²⁺, 119.8 Cl⁻, 25 HCO₃⁻, 2.4 H₂PO₄⁻, and 10 glucose. The medium was warmed to 37°C by a circulating water jacket and gently mixed and oxygenated with 95%O₂-5% CO₂. Spontaneous tissue potential difference was short circuit continuously via an automatic voltage clamp (WP Instrument, New Haven, CT) and Ag-AgCl electrodes, except for brief (2–5 s) intervals at each time point when the open circuit potential difference was recorded. Short-circuit current and transepithelial resistance were determined at 20-s intervals by application of rectangular current (I) pulses during continuous voltage monitoring. Instrument calibration was performed before each experiment using a filter/ring unit without cells. All comparative studies used matched pairs of monolayers seeded at the same time and studied concurrently. Previous studies have shown that short-circuit current (Isc) values in this system are wholly reflective of net chloride transport (16). After a 15-min equilibration period, chloride secretion was elicited using well-characterized secretagogues. cAMP-dependent chloride secretion was induced by FSK (10 μM to mucosal and basolateral sides); Ca²⁺-dependent secretion was induced by the muscarinic agonist, CCh (100 μM added to the basolateral side).

**Animal model of acute chemotherapy-induced intestinal inflammation.** In vivo studies were performed with male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing ~230–280 g. They received standard pellet chow and water ad libitum. All studies received the approval of the UCSD Committee on Investigations using Animal Subjects. Acute colitis was induced by intraperitoneal injection of 40 mg/kg methotrexate (MTX) essentially as described by Ermens et al. (21). Animals were assessed for clinical signs of colitis, i.e., body weight, stool consistency, and fecal blood using Hemoccult testing as described previously by Cooper et al. (15). No weight loss was counted as 0 points, weight loss of 1–5% as 1 point, 5–10% as 2 points, 10–20% as 3 points, and 20% or more as 4 points. For stool consistency, 0 points were given for well-formed pellets, 2 points for pasty and semifornmed stools that did not stick to the anus, and 4 points for liquid stools that did stick to the anus. Bleeding was scored as 0 points for no blood by hemoccult testing, 2 points for positive hemoccult, and 4 points for gross bleeding. These scores were added and divided by 3, forming a total clinical score that ranged from 0.0 (healthy) to 4.0 (maximal activity of colitis). A control group of animals received an intraperitoneal injection of an equal volume of PBS. On day 4 of MTX-induced colitis, rats were euthanized by halothane anesthesia, followed by cervical dislocation. The colon was removed and opened along the mesenteric border. Five-centimeter segments of proximal colon (starting ~1 cm distal to the ileocecal junction) and distal colon (starting ~1 cm distal to the left colic flexure) were stripped of their smooth muscle layers and cut into smaller sections that were then mounted on specially designed Ussing chamber inserts with a window area of 0.5 cm². Both sides of the tissue segments were bathed with 10 ml of Ringer’s solution as described above. The Ringer’s solution was maintained at 37°C, pH 7.4, and was gassed with 95% O₂-5% CO₂. Tissues were allowed to equilibrate for a period of 20 min, at which point baseline PD, short-circuit current (Isc), and tissue conductance were measured. Thereafter, recombinant rat leptin (100 ng/ml) or an equal volume of vehicle (PBS) was added to the apical side, and changes in Isc were recorded. The studies were performed in a paired fashion so that leptin-treated tissues could be compared with control tissues from the same animal.

**Immunoblotting.** Approximately 10⁶ T84 cells were seeded onto 30-mm Millicell-HA Transwells for these studies and treated with recombinant human leptin (100 ng/ml) for various times. All incubations were stopped by washing (×3) with ice-cold PBS. Ice-cold lysis buffer was added (consisting of 1% Triton X-100, 1 mM NaVO₄, 1 μg/ml leupeptin, 1 mg/ml pepstatin, 1 mM NaF, 1 mM EDTA, and 100 mg/ml PMSF in PBS), and the cells were incubated at 4°C for 30 min. Cells were then scraped into microcentrifuge tubes, centrifuged at 10,000 rpm for 10 min, and the supernatant retained. Aliquots were assayed from each sample to determine protein content, and samples were adjusted so that they contained equal amounts of protein. Samples were then mixed with gel loading buffer (50 mM Tris, pH 6.8, 2% SDS, 100 mM DTT, 0.2% bromophenol blue, 20% glycercol). The samples were boiled for 5 min and proteins separated by SDS-PAGE. Separated proteins were transferred onto a PVDF membrane (DuPont NEN, Boston, MA). The membrane was washed in 1% blocking buffer for 30 min, followed by incubation of the membrane with an appropriate dilution of primary antibody against pERK1/2 or pAkt in blocking buffer for 60 min. This was followed by washing (×3) in Tris-buffered saline with 1% Tween (TBST). Following washes, a horseradish peroxidase-conjugated secondary antibody was added to the membrane in 1% blocking buffer and allowed to incubate for an additional 30 min. This was followed by further washing (×3) in TBST. Immunoreactive proteins were detected using an enhanced chemiluminescence detection kit and exposure of the membrane to X-ray film. Quantification of protein phosphorylation was determined by densitometry using NIH Image software.

**Statistical analysis.** Data are presented as the means ± SE. Statistical analysis was performed using SigmaPlot software v8.0 (SPSS, Chicago, IL). One-way ANOVA or, where appropriate, repeated-measures ANOVA with a Student-Newman-Keuls post hoc test, were performed on all data. A P value <0.05 was considered to be statistically significant.
RESULTS

Effect of apical leptin on basal chloride secretion in T84 cells. We first examined whether leptin alone could stimulate chloride secretion in T84 cells. Monolayers of T84 intestinal epithelial cells were treated with apically added leptin (100 ng/ml) and mounted in modified Ussing chambers, and any difference in basal $I_{sc}$ compared with untreated cells was noted. As shown in Fig. 1A, pretreatment with leptin more than doubled basal $I_{sc}$ when monolayers were studied after treatment with the peptide for 5 min ($I_{sc}$: 2.5 μA/cm² vs. control 1.1 μA/cm²; $P < 0.01$). The stimulatory effect of leptin pretreatment declined thereafter but persisted for at least 60 min. Furthermore, as shown in Fig. 1B, pretreatment of cells with increasing doses of leptin (5–100 ng/ml) for 5 min showed that the effects of leptin were concentration dependent.

Effect of leptin on agonist-induced chloride secretion. We next tested whether leptin was able to potentiate responses to known chloride secretagogues. T84 cell monolayers were treated with 100 ng/ml apical leptin for various times then mounted in modified Ussing chambers. After a 10-min period of equilibration, CCh was added basolaterally at a concentration of 100 μM. As shown in Fig. 2, A and B, pretreatment of T84 cells with leptin for up to 1 h significantly potentiated the subsequent CCh-induced increase in $I_{sc}$, without altering the kinetics of the response ($P < 0.01$). Next, we tested the effect of leptin on chloride secretory responses to the cAMP-dependent agonist, FSK. T84 cell monolayers were treated with 100 ng/ml apical leptin for various times and then mounted in Ussing chambers. After a 10-min period of equilibration, FSK was added at a concentration of 100 μM to both the apical and basolateral sides. As shown in Fig. 3, A and B, pretreatment of T84 cells with leptin significantly increased FSK-induced $I_{sc}$, again without altering the kinetics of the response ($P < 0.05$).

Signals involved in ion transport responses to leptin. As shown in Fig. 4, the MEK inhibitor PD (40 μM) abolished the stimulatory effect of pretreatment with leptin (5 min) on basal $I_{sc}$ ($P < 0.05$). In contrast, the PI3K inhibitor wortmannin (100 nM) failed to reverse the effect of leptin pretreatment on basal $I_{sc}$ in T84 cells. Similarly, PD abolished the ability of leptin to potentiate CCh-induced increases in $I_{sc}$ ($P < 0.01$), whereas the PI3K inhibitor was without a significant effect (Fig. 4). In contrast, the ability of leptin to potentiate FSK-induced increases in chloride secretion was lost in the presence of either

![Fig. 1. Effect of leptin on basal short-circuit current ($I_{sc}$) in T84 cells. A: T84 cell monolayers ($n = 20$/group) were pretreated with apically added leptin (100 ng/ml) for different times and were then mounted in modified Ussing chambers for measurement of basal $I_{sc}$. B: T84 cell monolayers ($n = 15$/group) were pretreated with increasing doses of apically added leptin for 5 min and then mounted in modified Ussing chambers. Control cells (CO) received no treatment. Data are presented as means ± SE. Asterisks denote values that are statistically significantly different from the control (*$P < 0.05$; **$P < 0.01$ by ANOVA).](http://ajpgi.physiology.org/10.1152/ajpgi.00070.2009)

![Fig. 2. Effect of leptin on carbachol (CCh)-induced chloride secretion across T84 cells. T84 cell monolayers ($n = 15$/group) were pretreated with apically added leptin (100 ng/ml) for different times and mounted in modified Ussing chambers, after which CCh (100 μM, basolateral) was added. Control cells received no pretreatment. A: chloride secretion was measured as changes in short-circuit current ($\Delta I_{sc}$). B: time course of changes in $I_{sc}$ after stimulation with CCh in T84 cells pretreated with leptin for different times. Data are presented as means ± SE. Asterisks denote values that are statistically significantly different from the control (*$P < 0.05$; **$P < 0.01$ by ANOVA).](http://ajpgi.physiology.org/10.1152/ajpgi.00070.2009)
fetal blood using Hemoccult testing, and loss of body weight (Fig. 6). At the time of maximal colitis (4 days), segments of the distal and proximal colon were mounted in Ussing chambers as described. Baseline $I_{sc}$ values were increased in all MTX-treated groups compared with control animals, consistent with active inflammation and diarrhea (Fig. 7, A–C). After a 10-min period of equilibration, leptin (100 ng/ml or vehicle) was added to the luminal side, and changes in $I_{sc}$ were recorded. As shown in Fig. 7, A–C, luminal leptin (L) induced a robust increase in $I_{sc}$ ($P < 0.05$) across proximal colon segments of MTX-treated rats ($R_{MTX} + L$; maximal $I_{sc}$ 78 $\mu$A/cm$^2$), compared with vehicle-treated (V) noncolitic rats ($R_v + L$; maximal $I_{sc}$ 37 $\mu$A/cm$^2$) and with MTX-treated tissues exposed only to PBS as vehicle ($R_{MTX} + V$; maximal $I_{sc}$ 21 $\mu$A/cm$^2$) or with vehicle-treated tissues exposed only to PBS vehicle ($R_v + V$; maximal $I_{sc}$ 16 $\mu$A/cm$^2$). This effect of leptin was comparable in magnitude to the stimulatory effect of FSK, which was used here as a positive control ($R_{MTX} + FSK$; maximal $I_{sc}$ 88 $\mu$A/cm$^2$). A qualitatively similar response was seen in the distal colon, albeit of lower magnitude (Fig. 7, A–C). Moreover, the fact that only a weak response to leptin was observed in tissues from animals not receiving MTX ($R_v + L$) indicates that the effect of leptin on net intestinal ion transport is strikingly potentiated in the setting of inflammation.

**DISCUSSION**

Leptin, the product of the ob gene, is an adipose tissue-secreted peptide that signals the magnitude of fat stores to the brain and regulates energy homeostasis. Recent data suggest that leptin could also be considered a gastrointestinal hormone (14). Leptin mRNA and protein, as well as its receptor, have

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**Fig. 3.** Effect of leptin on forskolin (FSK)-induced chloride secretion across T$_{84}$ cells. T$_{84}$ cell monolayers ($n = 15$/group) were pretreated with apically added leptin (100 ng/ml) for different times and mounted in modified Ussing chambers, after which FSK (10 $\mu$M, apical and basolateral) was added. Control cells received no pretreatment. A: chloride secretion was measured as $\Delta I_{sc}$. B: time course of changes in $I_{sc}$ after stimulation with FSK in T$_{84}$ cells pretreated with leptin for different times. Data are presented as means ± SE. Asterisks denote values that are statistically significantly different from the control ($^*P < 0.05$; **$P < 0.01$ by ANOVA).

PD or wortmannin (Fig. 4). This could suggest a role for PI3K in leptin-induced chloride secretion that is specific for the cAMP pathway although we must also acknowledge the possibility of an independent effect of wortmannin on the response to FSK as we have reported previously for other cAMP-dependent secretagogues (16).

The previous experiments suggested a role for MAPK and, perhaps to a lesser extent, PI3K in the effects of leptin on basal and stimulated intestinal chloride secretion. To explore this further, T$_{84}$ cells were treated with leptin, and phosphorylation of p42/44 MAPK (ERK1/2) and the downstream target of PI3K, Akt1, were assessed by Western blotting. As shown in Fig. 5, A–D, and as we have reported previously (8), leptin treatment evoked both ERK1/2 and Akt1 phosphorylation in T$_{84}$ cells in a time- and dose-dependent manner. Responses were maximal after 5 min of leptin treatment and persisted for at least 1 and up to 15 h in the case of Akt1 or ERK phosphorylation, respectively.

**Leptin increases chloride secretion in chemotherapy-induced colitis.** To evaluate further whether luminal leptin might participate in stimulating chloride secretion in the setting of acute intestinal inflammation, an in vivo model of MTX-induced colitis was used. MTX-induced colitis 3–5 days after treatment as measured by a graded score of stool consistency, include:

- **Fig. 4.** Effects of inhibitors of MAPK and phosphatidylinositol 3-kinase (PI3K) pathways on leptin-induced increases in basal and stimulated chloride secretion. T$_{84}$ cell monolayers ($n = 10$/group) were pretreated with either leptin alone (100 ng/ml, 5 min, apical addition) or with leptin plus the MEK inhibitor, PD98059 (PD, 40 $\mu$M) or the PI3K inhibitor, wortmannin (100 nM) and were mounted in modified Ussing chambers. After recording basal $I_{sc}$ values, chloride secretion was stimulated by CCh (100 $\mu$M, basolateral) or FSK (10 $\mu$M, apical and basolateral). Control cells received no pretreatment. Secretagogue-induced chloride secretion was measured as $\Delta I_{sc}$. Data are presented as means ± SE. Asterisks denote values that are statistically significantly different from the control ($**P < 0.01$ by ANOVA); pound signs denote values that are statistically significantly different from corresponding values obtained with leptin alone ($#P < 0.05$; $$P < 0.01$ by ANOVA).
Fig. 5. A–B: leptin activates ERK1/2 isoforms of MAPK. T84 cell monolayers were cultured in serum-free media (DMEM/F12) for 24 h followed by exposure to recombinant human leptin. Leptin was added at 100 ng/ml for the times indicated (A) or at different doses for 5 min (B). Cellular extracts were fractioned by 12% SDS-PAGE, and Western immunoblotting was performed with a rabbit polyclonal anti-phospho-p44/42 MAPK and anti-total ERK as described in MATERIALS AND METHODS.

C–D: leptin activates Akt in T84 cells. Monolayers were cultured in serum-free media (DMEM/F12) for 24 h followed by exposure to human recombinant leptin at a concentration of 100 ng/ml for up to 15 h (C) or at different doses for 5 min (D). Cellular extracts were analyzed by Western immunoblotting with anti-phospho-Akt 1/PKB-α and anti-total Akt as described in MATERIALS AND METHODS. Equal loading was also assessed using a rabbit monoclonal antibody to GAPDH. In all panels, representative blots are shown above with summary data from at least 3 similar experiments, analyzed by densitometry, below. These latter values are means ± SE, and those that differ significantly from controls are designated with asterisks (*P < 0.05; **P < 0.01 by ANOVA).
been found in the gastric epithelium in rats and humans (43). However, relatively little is known about the possible actions of leptin in the intestine. A regulatory role for leptin in the absorption of nutrients has been postulated by some authors (12, 19, 32, 35, 40). This led us to study the possible effect of leptin on intestinal ion transport, another important epithelial function.

Chloride secretion is the predominant driving force for fluid secretion in the intestine (7). To examine the effect of leptin on intestinal chloride transport, we mounted T84 human colonic epithelial cells in Ussing chambers and measured changes in short-circuit current (Isc). We observed that apical pretreatment of T84 cells with leptin at supraphysiological concentrations, which have been detected in obese patients, increased basal Isc, although lower, physiological concentrations of leptin had no appreciable effects (38). Supraphysiological concentrations of leptin also potentiated responses to other known chloride secretagogues, such as the calcium-dependent muscarinic agonist, CCh, and the cAMP-dependent agonist, FSK. Our data imply that levels of leptin observed in obese patients, as well as in the setting of intestinal inflammation, have at least the potential to trigger excessive fluid and electrolyte secretion under such conditions.

We hypothesized that specific signal transduction proteins might be involved in the prosecretory effects of leptin. Leptin receptors have the ability to activate components of intracellular signaling cascades including JAK/STAT, PI3K, and MAPK pathways (25, 41). Pretreatment of cells with the MEK inhibitor, PD, abolished the effect of leptin on both basal Isc and secretagogue-induced secretory responses in T84 cells. Similarly, although the PI3K inhibitor, wortmannin, had no significant effect on leptin-induced increases in basal Isc or responses to CCh, it apparently reversed the effect of leptin on FSK-stimulated secretory responses. These data were consistent with those from Western blot studies, which showed that leptin treatment evoked p42/44 MAPK (ERK1/2) and Akt1 phosphorylation in T84 cells with kinetics compatible with its activation by leptin (12). Pretreatment with the Akt1 inhibitor, GSK, abolished the effect of leptin on both basal MAPK pathways (25). Pretreatment of cells with the MEK inhibitor, PD, abolished the effect of leptin on both basal MAPK pathways (25). Pretreatment with the PI3K inhibitor, wortmannin, had no appreciable effect, but pretreatment with the Akt1 inhibitor, GSK, abolished the effect of leptin on both basal MAPK pathways (25, 41). Pretreatment of cells with the MEK inhibitor, PD, abolished the effect of leptin on both basal MAPK pathways (25, 41). Pretreatment of cells with the PI3K inhibitor, wortmannin, had no appreciable effect, but pretreatment with the Akt1 inhibitor, GSK, abolished the effect of leptin on both basal MAPK pathways (25, 41). Pretreatment of cells with the MEK inhibitor, PD, abolished the effect of leptin on both basal MAPK pathways (25). Pretreatment with the PI3K inhibitor, wortmannin, had no appreciable effect, but pretreatment with the Akt1 inhibitor, GSK, abolished the effect of leptin on both basal MAPK pathways (25, 41). Pretreatment of cells with the MEK inhibitor, PD, abolished the effect of leptin on both basal MAPK pathways (25, 41). Pretreatment with the PI3K inhibitor, wortmannin, had no appreciable effect, but pretreatment with the Akt1 inhibitor, GSK, abolished the effect of leptin on both basal MAPK pathways (25, 41). Pretreatment of cells with the MEK inhibitor, PD, abolished the effect of leptin on both basal MAPK pathways (25, 41). Pretreatment with the PI3K inhibitor, wortmannin, had no appreciable effect, but pretreatment with the Akt1 inhibitor, GSK, abolished the effect of leptin on both basal MAPK pathways (25, 41).

Fig. 6. Acute colitis was induced by intraperitoneal injection of 40 mg/kg methotrexate (MTX) in rats as measured by a graded score that combined measures for stool consistency, the presence of fecal blood and loss of body weight, as described in MATERIALS AND METHODS. Data are presented as means ± SE for 35 animals. Asterisks denote values that are statistically significantly different from those on treatment day 1 (**P < 0.01 by ANOVA).
effects on secretion. The differential involvement of PI3K, moreover, may relate to the fact that CCh is known not to activate this enzyme in T84 cells, whereas the secretory response to cAMP-dependent agonists appears to rely, at least in part, on PI3K activity (8, 20).

Several studies suggest that leptin and its receptors may be upregulated in inflammatory conditions (29, 30). We therefore hypothesized that leptin might additionally be involved in disrupting intestinal ion transport in the setting of acute intestinal inflammation. This hypothesis was tested in an in vivo model of chemotherapy-induced colitis, a condition accompanied by acute clinical, functional, and inflammatory changes in the intestinal epithelium. After induction of colitis in rats by MTX administration, apical leptin was able to induce a significant increase in $I_{sc}$ in proximal colonic segments, and to a lesser extent in the distal colon. Indeed, the secretagogue action of leptin in this setting was comparable to that of the positive control, FSK, and much greater than seen in T84 cells. Because luminal leptin is increased in the setting of acute colitis, the actions described may contribute to the pathogenesis of inflammatory diarrhea. Indeed, blood levels of leptin were elevated transiently during the early stages of trinitrobenzene sulfonic acid-mediated colitis in rats, with the increase correlated with the degree of inflammation and anorexia (4). Similarly, Sitaraman et al. (38) showed that leptin participates in the pathogenesis of enterotoxin-mediated intestinal secretion and inflammation, with inflamed colonic cells serving themselves as a source of luminal leptin. These authors also examined colonic lavage fluids from normal subjects and patients with ulcerative colitis and demonstrated that colonic lavage fluid contained more than 15-fold higher concentrations of leptin in mild to severe IBD than seen in controls (38). Others have shown that mice deficient in leptin (ob/ob mice) or the long isoform of its receptor (db/db mice) are resistant in a variety of experimental models of inflammation/autoimmunity (37), including intestinal inflammation induced by administration of dextran sulfate sodium or trinitrobenzene sulfonic acid (37). Resistance to Clostridium difficile toxin A-induced enteritis has also been demonstrated in ob/ob mice (36). On the other hand, supporting a role for leptin in chronic IBD in humans are sparse and sometimes conflicting (3, 4, 22, 27, 44). Overall, further investigations will be needed to clarify fully the multiple possible roles of leptin in IBD.

In conclusion, we demonstrate that increased apical leptin concentrations can induce an ion transport response consistent with chloride, and thus fluid, secretion. It is conceivable that this corresponds to the clinical condition of diarrhea seen often in extremely obese patients or in patients with intestinal inflammation. The effects of leptin may be mediated, at least in part, by the activation of components of MAPK and PI3K signal transduction pathways. Our observations, together with recent studies showing participation of leptin in animal models of IBD, suggest that the use of specific leptin receptor antagonists could represent a novel therapeutic approach to the treatment of diarrheal symptoms seen in intestinal injury, IBD, or obesity, or following the administration of chemotherapeutic drugs during cancer therapy, particularly because leptin levels are positively correlated with colon cancer risk and may account for the association of such malignancy with obesity (25).

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DISCLOSURES

The authors are not aware of financial conflicts with the subject matter or materials discussed in this article with any of the authors or any of their academic institutions or employers.

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