Activation of PPARγ by rosiglitazone attenuates intestinal Cl− secretion

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Bajwa PJ, Lee JW, Straus DS, Lytle C. Activation of PPARγ by rosiglitazone attenuates intestinal Cl− secretion. Am J Physiol Gastrointest Liver Physiol 297: G82–G89, 2009. First published May 14, 2009; doi:10.1152/ajpgi.90640.2008.—The thiazolidinedione (TZD) drugs rosiglitazone (Ro) and pioglitazone (Po) are PPARγ agonists in widespread clinical use as insulin-sensitizing agents in Type 2 diabetes.

Although the principal function of the intestine is absorption, large quantities of electrolytes and fluid are normally secreted in support of digestion. These transport activities are regulated by a complex interplay of neurocrine, paracrine, endocrine, and inflammatory signals to provide for optimal luminal fluidity, convective mixing, and flushing in defense against pathogenic intruders (5). In general, intestinal fluid assimilation is osmotically driven by nutrient and Na+ absorption and fluid secretion by electrogenic Cl− (and HCO3−) secretion. Various modes of intestinal Cl− secretion can be distinguished on the basis of differences in their stimuli, mechanisms, rates, and longevity. Signals that elevate cAMP (VIP, PGE2, forskolin, cholera toxin) and cGMP (guanylin, STa toxin) within intestinal crypt cells evoke a sustained “flushing” secretory response by stimulating Cl− loss via apical CFTR channels, compensatory Cl− uptake via basolateral NKCC1, and regenerative basolateral K+ recycling through KCNQ1/KCNE3 K+ channel complexes (23, 28). A smaller, transient “cholinergic” secretory response is evoked by agonists that elevate intracellular Ca2+ (acetylcholine, carbachol, histamine), mainly by stimulating basolateral Kc3.1 (KCNN4) K+ channels that hyperpolarize the cell membrane and thereby amplify the driving force for apical Cl− exit (7, 12, 15, 23, 36). A third “synergistic” mode of intestinal Cl− secretion can be observed when crypt cells are exposed to a combination of agonists that act via cAMP and Ca2+; with this mode, maximal rates of sustained Cl− secretion are presumed to reflect costimulation of CFTR Cl− and Kc3.1 K+ channels (23).

The thiazolidinedione (TZD) drugs rosiglitazone (Ro) and pioglitazone (Po) are oral insulin-sensitizing agents widely utilized for glycemic control in patients with Type 2 diabetes (51). The same drugs are currently under investigation for their prospective benefits in combating other human diseases, including hypertension, atherosclerosis, inflammation, and cancer (48). The therapeutic effects of Ro and Po are generally attributed to their action as synthetic high-affinity ligands of peroxisome proliferator-activated receptor-γ (PPARγ), a nuclear receptor that, in association with the retinoid X receptor, regulates the expression of diverse genes involved in energy homeostasis, inflammation, cellular differentiation, and apoptosis (48).

Although PPARγ is prominently expressed in intestinal epithelial cells (14, 30, 33, 46), its influences on the organization and functions of the gut epithelium remain uncertain (47). It is apparent that PPARγ is an important modulator of epithelial differentiation and mucosal inflammation (10, 14, 24, 26, 33, 42, 45). The ability of PPARγ agonists to suppress proliferation and promote differentiation of intestinal epithelial cells (9, 10, 14, 30, 33, 42, 47), and to decrease the size of the proliferative zone of the intestinal crypts (33, 46), suggested that TZD drugs might attenuate the secretory function of the intestine, which depends on the number and differentiation state of crypt epithelial cells. To evaluate this possibility, we measured electrogenic anion secretion in intestinal segments isolated from mice that had been administered Ro (orally) for up to 28 days and in human HT29 intestinal cell monolayers that had been exposed to Ro in culture for up to 14 days. In both experimental models, treatment with Ro attenuated the secretory responses to forskolin (cAMP) persistently and to carbachol (Ca2+) transiently. Studies using HT29 cells revealed that Ro lessens cAMP-dependent Cl− secretory capacity by downregulating the expression of critical transport proteins, including CFTR, KCNQ1, and NKCC1.

METHODS

Cell culture. Human intestinal cell lines (HT29/cl.19A and T84) were obtained from Dr. Kim Barrett (University of California, San Diego). HT29 cells (passages 25–42) were cultured in McCoy’s 5A medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 µg/ml streptomycin, in 5% CO2-95% air. Cells were maintained in T-75 tissue culture flasks until 80–90% confluent, then subcultured onto 12 mm Snapwell inserts (Corning, Corning, NY) at a density of 2 × 104 cells/well. After the monolayers achieved a

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resistance of $>300$ $\Omega$-cm$^2$ (EVOM, WPI, Sarasota, FL), the cells were treated with either 10 $\mu$M Ro or vehicle (0.2% DMSO) for 3 days. Medium was replaced every 24 h. T84 cells were cultured in DMEM and Ham’s F-12 media (1:1) supplemented with 5% newborn calf serum (Biomedia), 100 U/ml penicillin, and 100 $\mu$g/ml streptomycin, in 5% CO$_2$-95% air.

**Mice.** Female CD1 mice (Charles River) were fed ad libitum a standard powdered diet (Purina LabDiet, St. Louis, MO) or the same diet supplemented with Ro to provide a dose of 10–20 mg Ro-kg body wt$^{-1}$-day$^{-1}$, for up to 28 days. Ro was mixed with powdered mouse chow by the method of geometric dilution (38). In some experiments, mice were administered this dose of Ro or vehicle alone (2.5% methylcellulose) by oral gavage for 8 days, with similar results. All animal protocols were approved by the University of California, Riverside, Institutional Animal Care and Use Committee. Ro was provided by GlaxoSmith Kline or was purchased from Cayman Chemical (Ann Arbor, MI).

**Mouse model of secretory diarrhea.** Secretory responses to cholera toxin (Sigma) were measured by using the adult mouse enteropooling technique as described previously (25). Food was available ad libitum except for a 12-h period preceding gavage, and water was available continuously. Mice that had been fed a control diet or Ro-supplemented diet for 8 days were gavaged with cholera toxin (30 $\mu$g) or vehicle alone (100 $\mu$l 7% Na-HCO$_3$) via an orogastric feeding needle. Five hours later, the mice were euthanized and the entire small intestine was isolated. After removal of the mesentery and connective tissue, the weight of the tissue was determined. The intestine was cut along its entire length and the luminal fluid was removed by blotting. The tissue was dried in an oven (60°C for 1 day) and then reweighed. Fluid accumulation was measured as the ratio of luminal fluid weight to tissue dry weight.

**Electrical measurements.** The short-circuit current ($I_{sc}$) and electrical resistance ($R_e$) across isolated intestinal mucosa were measured by a conventional Ussing chamber technique (19). Segments of proximal jejunum (7–15 cm from pylorus), terminal ileum (3–5 cm from cecum), proximal colon (0–2 cm from the cecum), or “early” distal colon (0–2 cm proximal from the peritoneal border) were isolated from control or Ro-treated mice. The intestinal wall was cut open along the mesentery and partially stripped of serosal and muscle layers to obtain a conventional mucosa-submucosa preparation. Tissues were mounted on small pins across an oblong aperture (2.8 $\times$ 11 mm, exposed surface area 0.33 cm$^2$), Physiologic Instruments P2304) and incubated in an Ussing chamber (Physiologic Instruments EM-CSYS-2). Colonic segments were incubated a Tyrode’s solution containing (in mM) 140 NaCl, 4 KCl, 1 MgCl$_2$, 1.25 CaCl$_2$, 12 glucose, and 10 Na-HEPES (pH 7.4) and mixed continuously by gas (100% O$_2$) lift. Segments of small intestine were incubated in a standard Parson’s solution composed of (in mM) 110 NaCl, 24 NaHCO$_3$, 4 KCl, 2 H$_3$PO$_4$, 0.5 MgSO$_4$, 1.25 CaCl$_2$, 12 glucose (serosal chamber), or 12 mmol (mucosal chamber), and mixed by gas (95% O$_2$-5% CO$_2$) lift. Chambers were maintained at 37°C by heated water jackets. Indomethacin (1 $\mu$M) was routinely included in the serosal bath to suppress endogenous prostanoid generation and basal Cl$^-$ secretion. The transmucosal potential difference ($V_{tr}$) was measured through 3 M KCl agar bridges connected to a pair of calomel electrodes and monitored with a voltage clamp amplifier (Physiologic Instruments VCC-MC2). $V_{tr}$ was maintained at a command-voltage of 0 V, with compensation for solution resistance, by applying an $I_{sc}$ through a pair of Ag-AgCl electrodes kept in contact with the mucosal and serosal solutions through 3 M KCl agar bridges. Transmucosal conductance ($G_t$) was calculated using Ohm’s law from the change in current evoked by a 2-mV bipolar pulse every 20 s. Output from the voltage clamp was recorded on an Apple computer through an 8-bit analog-to-digital converter (Biopac Systems MP30).

The same setup was used for measuring electrogenic Cl$^-$ secretion by HT29 cells grown on permeable supports (Costar Snapwell 3407) with Parson’s solution in both chambers. Pilot experiments indicated that vehicle DMSO at its maximal concentration (0.2%) was without effect.

**Permeabilized HT29 cell monolayers.** Basolateral membrane K$^+$ conductance was measured by permeabilizing the apical membrane of HT29 cells with the pore-forming antibiotic amphotericin B (20 $\mu$M; Sigma) while imposing a serosal to mucosal K$^+$ gradient, as described previously (27, 41). Cells grown to confluence on Snapwell supports were mounted in an Ussing chamber with a mucosal solution containing predominantly K$^+$ (143 mM K-glucanate) and a serosal solution containing predominantly N-methyl-d-glucamine$^+$ (137 NMDG-glucanate and 5 mM K-glucanate). Both solutions also contained (in mM) 1.25 CaCl$_2$, 0.4 MgSO$_4$, 0.43 KH$_2$PO$_4$, 0.35 K$_2$HPO$_4$, 5.6 glucose, and 10 HEPES at pH 7.4 and were continuously mixed by gas (100% O$_2$) lift. Under these conditions, $I_{sc}$ and $G_t$ largely reflect K$^+$ electrodiffusion through basolateral channels (27). The changes in $I_{sc}$ and $G_t$ evoked by sequential addition of cAMP (20 $\mu$M forskolin) and Ca$^{2+}$ (100 $\mu$M carbacol) were recorded 30 min after apical membrane permeabilization.

**Apical membrane K$^+$ conductance** was measured by permeabilizing the basolateral membrane of HT29 cells with nystatin while imposing a mucosal to serosal Cl$^-$ gradient, as described previously (43). Monolayers were mounted in Ussing chambers with a mucosal solution containing (in mM) 120 NaCl, 25 NaHCO$_3$, 1.2 CaCl$_2$, 1.2 MgCl$_2$, 3.3 K$_2$HPO$_4$, 0.8 KH$_2$PO$_4$, 10 glucose, and bubbled with 5% CO$_2$-95% O$_2$ (pH 7.4). In the serosal solution, sodium gluconate replaced NaCl, and CaCl$_2$ was increased to 4 mM to compensate for buffering by gluconate. Nystatin (180 $\mu$g/ml Sigma) was added to the serosal solution from a freshly prepared 90 mg/ml DMSO stock solution. Under these conditions, the recorded $I_{sc}$ mainly reflects apical membrane Cl$^-$ channel activity (43). Twenty minutes after basolateral membrane permeabilization, the effects of cAMP and Ca$^{2+}$ stimulation were measured by sequentially adding 20 $\mu$M forskolin and 100 $\mu$M carbacol to the serosal bath.

**Western blotting.** The effects of Ro on transport protein expression by measured by Western blot analysis. Briefly, HT29 cells were grown on permeable supports until the monolayer developed a stable resistance (~5 days). The cells were then exposed to either 10 $\mu$M Ro or vehicle alone (DMSO) for an additional 5 days. To isolate cell proteins, the supports were cut from their frames, transferred to an ice-cold extraction buffer (1% Na-deoxycholate, 0.5% Triton X-100, 0.1% SDS, 150 mM NaCl, 2.5 mM EGTA, 20 mM Tris·HCl, pH 7.4, protease inhibitors), sonicated for 10 s, and incubated on ice for 10 min. After removal of insoluble material by centrifugation (12,000 g, 5 min), the protein concentration was determined using a Micro BCA protein kit (Pierce, Rockford, IL). Western blot analysis was performed by standard techniques, as described previously (34), with the following antibodies: CTRF, rabbit 24-1 from R&D Systems (13); KCNQ1, mouse N37/A10 from NeuroMab (UC Davis/NIH); KCNN4, rabbit aKc3.1 from Cell Applications; NKKC1, rabbit affinity-purified NT and TEFS2 (37).

**Statistics.** Data are presented as means ± SE. Statistical significance was calculated by t-test. $P < 0.05$ was considered significant.

**RESULTS**

The effects of Ro on cAMP- and Ca$^{2+}$-dependent Cl$^-$ secretory responses were first investigated through measurements of $I_{sc}$ across mucosal-submucosal sheets isolated from various intestinal segments. Mice were allowed free access to a standard diet or to the same diet supplemented with Ro, formulated to deliver 10–20 mg-kg body wt$^{-1}$-day$^{-1}$, for up to 28 days. This dose of Ro is comparable to those used in previous studies of PPARγ signaling in mice (1, 33) and rats (29, 44). Mice treated with Ro exhibited no external signs of pathology. Mucosal sheets isolated from the proximal colon, distal colon, and terminal ileum were mounted in Ussing...
chambers and preincubated for 1 h to achieve a stable baseline $I_{sc}$ and to wash out residual Ro. In intestinal segments from mice treated with Ro for 8 days, the Cl– secretory responses to both forskolin (cAMP) and carbachol (Ca$^{2+}$) were all markedly attenuated (Fig. 1). As expected, the secretory currents were accompanied by increased transmucosal conductance, and these changes in $G_t$ were significantly reduced in tissue from Ro-treated mice; for example, in the control proximal colon, forskolin and carbachol increased $G_t$ by 34 ± 6 and 19 ± 6%, respectively, whereas by only 7 ± 4 and 0.1 ± 5% in the Ro-treated proximal colon ($n = 3–5$).

Systemic administration of Ro affected intestinal Cl– secretion in three distinct phases. In the proximal colon (Fig. 2A), an initial period (3 days) of increased reactivity to carbachol preceded a period (8 days) of complete insensitivity to secretory stimuli. After 12 days, the response to carbachol recovered fully, whereas the response to forskolin remained attenuated (Fig. 2A). Three similar phases were likewise apparent in the proximal jejunum, although over a longer time course (Fig. 2B). Initially (5 days), Ro treatment rendered the jejunum more responsive to carbachol and forskolin, although only the augmented response to forskolin reached statistical significance. With continued treatment (13 days), the effect of Ro instead became antisecretory, with $I_{sc}$ responses to both carbachol and forskolin reduced by more than 65%. After 28 days of Ro treatment, the carbachol-induced mode of Cl– secretion exhibited a significant degree of recovery, whereas the forskolin-induced mode remained attenuated.

The antisecretory effect of Ro, once established, was long lasting. When proximal colon from Ro-treated (8 day) mice was incubated in Ussing chambers lacking Ro, it remained unresponsive to stimulation for at least 2 h (data not shown). The slow onset of the Ro effect and its persistence after Ro washout are consistent with a genomic mechanism involving PPARγ activation, gene regulation, and de novo protein synthesis.

We next tested whether Ro protects mice from cholera toxin-induced secretory diarrhea using a conventional unsealed intestine model (17, 31, 35, 40). Under basal conditions, the small intestine of fasted mice contained 1.05 ± 0.15 g of fluid per gram of dry tissue. Gastric infusion of cholera toxin (30 μg) promoted a substantial increase in luminal fluid content (to 6.2 ± 0.35 g/g dry) after 5 h (Fig. 3). Significantly less enteropooling (2.85 ± 0.6 g/g dry) was observed in mice that had received dietary Ro for 8 days. Treatment with Ro reduced the cholera toxin-induced component of enteropooling by ~65% (Fig. 3).

A similar antisecretory effect of Ro was observed in vitro with human colonic HT29 adenocarcinoma cells cultured on permeable supports (Fig. 4). To circumvent possible effects of Ro on the initial growth and polarization of the cells in culture, treatment with Ro was initiated after the epithelial monolayers had reached confluency and had acquired a stable high resistance. Treatment with 10 μM Ro for 5 days reduced the Cl– secretory responses to both cAMP (vasoactive intestinal peptide and forskolin) and Ca$^{2+}$ (carbachol) agonists by more than 50% (Fig. 4A). Ro treatment likewise inhibited the response to the cell-permeant cAMP analog 8-cpt-cAMP (Fig. 4A), indicating that the inhibitory step is distal to the generation of cAMP by adenylate cyclase. As expected, stimulation of electrogenic Cl– secretion was associated with a decrease in $G_t$, and this change was significantly smaller in Ro-treated cells; for example, stimulation with vasoactive intestinal peptide reduced $R_t$ by 53 ± 8% in control cells, whereas by 17 ± 7% in Ro-treated cells ($n = 3$, $P = 0.005$).

The inhibitory effect of Ro in HT29 cells became evident after 1 day and peaked after 4–5 days (Fig. 4B). In contrast to

![Fig. 1. Dietary rosiglitazone (Ro) attenuates intestinal Cl– secretion in mice.](#) Short-circuit current ($I_{sc}$) responses to forskolin (A) and carbachol (B) stimuli by intestinal segments isolated from mice maintained on a control diet ($n = 6–9$) (open bars) or a Ro-supplemented diet ($n = 3–6$) for 8 days (shaded bars); *$P < 0.05$. The inhibitory effect of Ro in HT29 cells became evident after 1 day and peaked after 4–5 days (Fig. 4B). In contrast to
Together these findings suggest that synthetic activators of PPARγ exert powerful (and possibly transient) antisecretory effects on the intestinal epithelium.

Intestinal Cl− secretion requires the concerted operation of four transport processes: the controlled exit of Cl− via apical CFTR channels, the compensatory entry of Cl− via basolateral Na-K-2Cl cotransporter-1, the regenerative recycling of K+ via basolateral K+ channels (KCNQ1 or KCa3.1), and primary active transport via basolateral Na+/K+-ATPase. The antisecretory effect of TZD drugs likely involves a PPARγ-mediated downregulation of one or more of these four processes. To investigate this possibility, we first examined the effect of Ro on apical membrane Cl− channel activity in HT29 cells (Fig. 5). Apical Cl− conductance was measured by selectively permeabilizing the basolateral membrane with nystatin while imposing a steep mucosal-to-serosal Cl− gradient. Under these conditions, the transepithelial Isc mainly reflects CFTR Cl− channel activity (2, 43). As expected, addition of the cAMP agonist forskolin evoked a prominent increase in Isc that was almost fully inhibited by the CFTR blocker CFTRinh172. This Cl− channel activity was largely absent in cells that had been treated with 10 μM Ro for 3 days. As expected, apical addition of CFTRinh172 increased apical membrane resistance to a significantly greater extent in control cells (108 ± 26%) than in Ro-treated cells (37 ± 8%). Thus the antisecretory effect of Ro appears to involve a diminution of CFTR Cl− channel activity.

In HT29 cells, acute bilateral exposure to Ro had no effect for at least 2 h, and in cells pretreated with Ro for 1 day the secretory dysfunction persisted for at least an hour after Ro washout (not shown), again consistent with a slow and protracted mode of inhibition. We observed a similar degree of inhibition with other TZD activators of PPARγ, including Po (control: 155 ± 6.8 μA·cm−2; 10 μM Po: 101 ± 11.3 μA·cm−2; n = 3), substantiating the supposition that the antisecretory response is a glitazone class effect. It has been reported that Ro acutely stimulates AMP-activated kinase (AMPK) (16) and that AMPK inhibits intestinal Cl− secretion by suppressing CFTR Cl− channel activity (22, 50). However, we detected no acute effect of Ro on HT29 cell Cl− secretion, and, moreover, the inhibition seen after prolonged exposure to Ro was not relieved by pretreatment with 25 μM Compound C, a cell-permeant inhibitor of AMPK (53). Together these findings support the idea that Ro lessens cholera toxin-induced luminal fluid accumulation in mice. Mice were maintained on control (open bars) or Ro-supplemented (shaded bar) diets for 8 days and then gavaged with vehicle alone (100 μl 7% Na-HCO3; “control”) or with 30 μg cholera toxin (“cholera toxin”) to provoke secretory diarrhea. Data indicate the ratio of luminal fluid weight to tissue dry weight after gavage (n = 5); *P < 0.05.

Fig. 3. Dietary Ro lessens cholera toxin-induced luminal fluid accumulation in mice. Mice were maintained on control (open bars) or Ro-supplemented (shaded bar) diets for 8 days and then gavaged with vehicle alone (100 μl 7% Na-HCO3; “control”) or with 30 μg cholera toxin (“cholera toxin”) to provoke secretory diarrhea. Data indicate the ratio of luminal fluid weight to tissue dry weight after gavage (n = 5); *P < 0.05.

Fig. 4. Antisecretory effects of Ro in human HT29 intestinal cells. A: after growth to confluence, HT29 cell monolayers were cultured for 5 additional days ± 10 μM Ro. Data indicate average Isc responses to 50 nM VIP, 10 μM forskolin, 75 μM 8-chlorophenyl-thio-cAMP (8-cpt-cAMP) and 100 μM carbachol (n = 3–10); *P < 0.05. B: time course of Ro effect. After growth to confluence, HT29 cell monolayers were cultured for up to 14 days with 10 μM Ro or vehicle alone. Data represent the Isc responses to forskolin (•) and carbachol (○) relative to those observed in control monolayers (n = 3). All values are significantly different from those recorded at day 0.
Additional experiments revealed that the antisecretory effect of Ro also involves a suppression of conductive basolateral K\(^+\) recycling (Fig. 6). To monitor changes in basolateral membrane K\(^+\) conductance, the apical membrane of confluent HT29 cell monolayers was selectively permeabilized with the cationophore amphotericin-B, a steep luminal-to-serosal K\(^+\) gradient was imposed, and the transepithelial voltage was clamped at 0 mV. Under these conditions, the measured \(I_{sc}\) mainly reflects electrodiffusive K\(^+\) movement through basolateral membrane channels (2, 27, 41). Stimulation of the monolayer with forskolin elicited an abrupt increase in \(I_{sc}\) (Fig. 6) that presumably reflects the activation of cAMP-stimulated KCNQ1/KCNE3 K\(^+\) channel complexes. In Ro-treated cells, this component of K\(^+\) channel activity amounted to only 24 ± 4% \((n = 5)\) that in paired control monolayers. Subsequent addition of the permeant cAMP agonist 8-cpt-cAMP evoked an additional small increase in \(I_{sc}\) in control cells but not in Ro-treated cells, confirming that Ro inhibits at a step distal to cAMP generation. Serosal application of 25 \(\mu\)M chromanol-293B, a KCNQ1 channel inhibitor (32), eliminated this K\(^+\) channel activity in control cells yet had little or no further effect in Ro-treated cells. Finally, addition of the Ca\(^{2+}\) agonist carbachol evoked another K\(^+\) current that in other intestinal epithelial cells has been attributed to the K\(_{Ca}\).3.1 K\(^+\) channel (23). As expected, this current was inhibited by agents known to block K\(_{Ca}\).3.1, including charybdotoxin (control: 16.2 ± 2.8 \(\mu\)A·cm\(^{-2}\), +100 \(\mu\)M charybdotoxin: −1.8 ± 1.1 \(\mu\)A·cm\(^{-2}\), \(n = 3\)). In Ro-treated cells, this Ca\(^{2+}\)-induced K\(^+\) current was only 36 ± 8% \((n = 5)\) that in control cells.

To distinguish whether the channel dysfunction observed in Ro-treated cells reflects a reduction in the number or the activity of channel units in the apical membrane, we measured the abundance of CFTR, KCNQ1, and K\(_{Ca}\).3.1 proteins in HT29 cells by Western blot analysis. Treatment of confluent polarized monolayers with 10 \(\mu\)M Ro for 3 days resulted in a marked reduction in the abundance of both CFTR and KCNQ1 proteins (Fig. 7). We also observed a 47 ± 4% reduction in the abundance of Na-K-2Cl cotransporter-1 (NKCC1) protein in Ro-treated cells. By contrast, Ro had no significant affect on K\(_{Ca}\).3.1 or Na-K-ATPase α-subunit protein abundance.

**DISCUSSION**

TZD drugs like Ro and Po have long served as a cornerstone therapy for type-II diabetes through their ability to selectively activate the nuclear receptor PPAR\(\gamma\) and increase insulin sensitivity. Our results indicate that these drugs also exert a powerful antisecretory effect on the small intestine and colon. In mice, oral administration of TZD drugs at doses known to activate PPAR\(\gamma\) resulted in marked reductions in both cAMP- and Ca\(^{2+}\)-induced modes of Cl\(^-\) secretion in the jejunum, ileum, and colon. The inhibitory effect developed gradually and became maximal after ~8 days in mice and after 5 days in cultured human intestinal HT29 cells. With continued Ro treatment, the secretory response to forskolin remained substantially inhibited, whereas the response to carbachol recovered significantly.

The loss of cAMP-dependent Cl\(^-\) secretion was attributable, at least in part, to a diminished expression of key transport proteins. Treatment of HT29 cells with Ro for 5 days caused pronounced reductions in CFTR Cl\(^-\) channel, KCNQ1 K\(^+\) conductance, and Ca\(^{2+}\)-induced CFTR Cl\(^-\) secretion when these cells were activated with forskolin. These findings are consistent with previous reports showing a downregulation of CFTR protein expression in HT29 cells treated with Ro (33).

**Fig. 5.** Ro attenuates apical CFTR Cl\(^-\) channel activity. Confluent HT29 cell monolayers were cultured for 5 additional days with 10 \(\mu\)M Ro or vehicle. Apical membrane Cl\(^-\) conductance was measured after selective permeabilization of the basolateral membrane with nystatin. Data indicate steady \(I_{sc}\) values recorded before and after sequential addition of 10 \(\mu\)M forskolin and apical 25 \(\mu\)M CFTRinh-172 \((n = 9)\); *\(P < 0.05\).

**Fig. 6.** Ro attenuates basolateral K\(^+\) channel activity. Confluent HT29 cell monolayers were cultured for 5 additional days with 10 \(\mu\)M Ro or vehicle. Basolateral membrane K\(^+\) conductance was measured after selective permeabilization of the apical membrane with amphotericin-B. Data indicate steady \(I_{sc}\) values recorded after sequential basolateral addition of 10 \(\mu\)M forskolin, 75 \(\mu\)M 8-cpt-cAMP, 25 \(\mu\)M chromanol-293B, and 100 \(\mu\)M carbachol \((n = 5)\); *\(P < 0.05\).
channel, and Na-K-2Cl cotransporter-1 proteins, each of which is required for cAMP-induced Cl⁻ secretion (2, 5, 8, 12, 19, 23, 25, 28, 36). The mechanistic basis for the effects of Ro on Ca²⁺-dependent Cl⁻ secretion remains less certain. Since this mode recovers while the cAMP-induced mode remains attenuated, some factor other than CFTR, KCNQ1, and Na-K-2Cl cotransporter-1 would appear to be involved. Our data implicate transient changes in basolateral K⁺ channel activity: in HT29 cells treated with Ro for 5 days, the Ca²⁺-activated, charybdotoxin-sensitive component of basolateral K⁺ conductance, which is believed to involve the K⁺ channel KCa3.1 (15, 38), was strongly attenuated, although no reduction in KCa3.1 protein abundance was detected. It remains possible that Ro, through PPARγ, acts indirectly through other proteins that influence the functional properties of individual KCa3.1 channels, the proportion of these channels at the cell surface, or both.

PPARγ has been recognized as an important determinant of the organization and function of the intestinal epithelium (6, 10, 14, 42, 47). Although the receptor is found throughout the intestinal epithelium, it is expressed at especially high levels in the differentiated enterocytes that inhabit the villus and surface (30, 46). The intestinal epithelium is perpetually renewed through the controlled proliferation and orderly migration of its principal cell lineages, and the position of each cell along the crypt axis corresponds to its stage of maturation (18). Newly formed cells in the proliferative compartment of the crypt begin life configured for rapid Cl⁻ secretion and coexpress high levels of proliferation markers (Ki67) and specialized transport proteins (NKCC1, CFTR, KCNQ1) until they migrate into the top third of the crypt, where they abruptly undergo a program of differentiation that includes a conversion of their principal function from Cl⁻ secretion to Na⁺ and nutrient absorption. As a result, the secretory and absorptive functions of the intestinal epithelium are largely segregated between crypt and surface or villus cell compartments. Thus signals or disease processes that modify the spatial and temporal patterns of gene expression along the crypt axis can have profound effects on the secretory and absorptive capacities of the intestine.

PPARγ is one such signal. Since its initial characterization as a master regulator of adipocyte differentiation (48), PPARγ has been implicated as a key regulator of proliferation and differentiation in a variety of other cell lineages (4, 47, 49), including those that comprise the intestinal epithelium (10, 14, 21, 42). PPARγ has been shown to operate in conjunction with tissue-specific coactivator proteins to retard proliferation and promote differentiation of intestinal epithelial cells (14, 21). This concept is consistent with evidence that mice lacking one allele of the PPARγ gene manifest lower levels of intestinal PPARγ and epithelial differentiation markers (14) and that mice treated with TZD drugs like Ro exhibit a smaller crypt proliferative zone (33, 46) along with a reduced abundance of secretory transport proteins (NKCC1, CFTR, KCNQ1) in the colonic mucosa (this study). The reduced number or secretory capacity of crypt cells in Ro-treated mice might also occur in conjunction with an increased number or absorptive capacity of differentiated surface enterocytes. In support of this possibility, treatment of mice with TZD drugs has been shown to result in an augmented expression of colonic differentiation markers (l-FABP, CD36, AQP-8) (33, 46); this appears to include an increased expression of colonic absorptive transport proteins, including Na/H exchanger-3 and SLC26A3 (unpublished studies). This could reflect an intestinal manifestation of the proabsorptive effects of Ro on the renal collecting duct (11, 20, 52) and could contribute to the progressive retention of Na⁺ and fluid observed in patients on Ro therapy (39). Experiments to assess whether the antisecretory effect of Ro reflects an anatomical contraction of the proliferative (crypt secretory cell) compartment and whether Ro also exerts proabsorptive effects on the colon are currently underway.

Our finding that Ro attenuates intestinal Cl⁻ secretory capacity raises the prospect that PPARγ agonists could lessen intestinal fluid losses in secretory diarrhea diseases. In mice, pretreatment with Ro provides substantial protection from cholera toxin-induced fluid accumulation throughout the small intestine. This substantiates our finding that the antisecretory effect of Ro extends beyond the colon and ileum to the jejunum, which in humans is the major site of intestinal Cl⁻ and fluid secretion and the primary target of cholera toxin (3). In addition to providing protection against acute secretory diarrhea, Ro might mitigate fluid losses in chronic diarrheal diseases. Indeed, the suppressive effect of Ro on the cAMP-induced mode of intestinal Cl⁻ secretion is at least partially maintained long term and might be accompanied by proabsorptive effects. Fortuitously, constipation is not a common side effect of TZD drugs, perhaps because the Ca²⁺-induced mode of intestinal Cl⁻ secretion, which is necessary for proper stool hydration (15), recovers toward normal levels with continued Ro treatment.

An interesting possibility is that systemic activation of PPARγ by TZD drugs also attenuates Cl⁻ secretion by other
exocrine glands outside the intestinal tract. Epithelial cells comprising the acini or secretory end pieces in the pancreas, salivary gland, exorbital lacrimal gland, airway submucosal gland, mammary gland, and sweat gland are known to utilize a cognate mechanism for electrogenic Cl− secretion (28) and therefore may be affected similarly by PPARγ signaling. To our knowledge, the effect of TZD drugs on electrolyte transport in these glands has not been explored.

REFERENCES


