Inhibition of enterotoxin-induced porcine colonic secretion by diarylsulfonylureas in vitro

ERIN K. O’DONNELL,1 ROGER L. SEDLACEK,1 ASHVANI K. SINGH,2 AND BRUCE D. SCHULTZ1

1Department of Anatomy and Physiology, Kansas State University, Manhattan, Kansas 66506; and 2Department of Cell Biology and Physiology, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

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O’Donnell, Erin K., Roger L. Sedlacek, Ashvani K. Singh, and Bruce D. Schultz. Inhibition of enterotoxin-induced porcine colonic secretion by diarylsulfonylureas in vitro. Am J Physiol Gastrointest Liver Physiol 279: G1104–G1112, 2000.—Muscle-stripped piglet colon was used to evaluate changes in short-circuit current (Isc) as an indicator of anion secretion. Mucosal exposure to Escherichia coli heat-stable (STa) or heat-labile enterotoxins (LT) stimulated Isc by 32 ± 5 and 42 ± 7 μA/cm2, respectively. Enterotoxin-stimulated Isc was not significantly affected by either 4,4’-diaminostilbene-2,2’-disulfonic acid or CdCl2, inhibitors of Ca2+-activated Cl- channels and ClC-2 channels, respectively. Alternatively, N-(4-methylphenylsulfonyl)-N’-(4-trifluoromethylphenyl)urea (DASU-02), a compound that inhibits cystic fibrosis transmembrane conductance regulator (CFTR)-mediated Cl- secretion, reduced Isc by 29 ± 7 and 34 ± 11 μA/cm2, respectively. Two additional diarylsulfonylurea (DASU)-based compounds were evaluated for their effects on enterotoxin-stimulated secretion. The rank order of potency for inhibition by these three closely related DASU structures was identical to that observed for human CFTR. The degree of inhibition by each of these compounds was similar for both STa and LT. The structure- and concentration-dependent inhibition shown indicates that CFTR mediates both STa- and LT-stimulated colonic secretion. Similar structure-dependent inhibitory effects were observed in forskolin-stimulated rat colonic epithelium. Thus DASUs compose a family of inhibitors that may be of therapeutic value for the symptomatic treatment of diarrhea resulting from a broad spectrum of causative agents across species.

cystic fibrosis transmembrane conductance regulator; diarrhea; pharmacology; LY-295501

CONSIDERABLE SOCIOLOGICAL and economic cost is associated with intestinal hypersecretion. Secretory diarrhea ranks as one of the world’s top infectious killers, affecting mostly children in third world countries (1) and causing an estimated 5 million human deaths annually (33). In the United States, children under the age of 5 years experience 20–35 million bouts of diarrhea every year, resulting in 2–3.7 million doctor visits, >200,000 hospitalizations, and 500 deaths (12, 20). Although significant focus might be placed on neonatal and pediatric diarrhea, >50% of diarrhea-related deaths in the United States occur in persons over the age of 74, a growing portion of the population (19). Thus significant incentive is present to develop safe, effective, and broad-spectrum treatments for diarrhea.

Vibrio cholera and Escherichia coli are among the major agents responsible for infectious diarrhea in both humans and animals (14). Enterotoxins of E. coli are classified as either heat stable (STa and STb) or heat labile (LT) (13). Cholera toxin (CT) is virtually identical to LT in both structure and function (29, 34). These toxins are not cytotoxic, but they interact directly with intestinal epithelial cells to stimulate profound fluid and electrolyte secretion into the lumen of the gut. Severe dehydration compromises multiple physiological systems in affected individuals and can ultimately lead to death or can set the individual up for opportunistic infection by other organisms.

The biochemistry of enterotoxins and the cellular pathways that contribute to enterotoxin stimulation of electrolyte transport are relatively well characterized (see Refs. 5, 13, 15, 29, and 34 for review). A key player in the response is thought to be the cystic fibrosis transmembrane conductance regulator (CFTR), an apical membrane anion channel. Treatments for diarrhea aimed at blocking this ion channel have been theoretically proposed (7). Indeed, attempts have been made to reduce toxin-stimulated secretion by blocking anion channels (10), although the compounds available proved not to be effective. The widespread and successful use of oral rehydration therapy in humans indicates that simply circumventing or preventing dehydration will greatly reduce morbidity and mortality associated with enterotoxigenic infections. Thus an intervention that acutely reduces enterotoxin-stimulated intestinal fluid loss will be of great value in both human and veterinary medicine.

Results from the present study document that LT and STa functionally share a common anion conductive component in porcine colon. Pharmacological evidence indicates that the channel most likely responsible for enterotoxin-induced Cl- secretion in neonatal porcine...
colon is the CFTR Cl− channel, which is blocked by diarylsulfonylureas (DASUs). Comparative results are presented for the inhibition of rat colonic secretion. Thus pharmacological intervention to selectively inhibit CFTR is a means by which a broad spectrum of secretory diarrheas might be managed across species.

MATERIALS AND METHODS

Tissue acquisition. Twenty-seven mixed-breed suckling 7- to 11-day-old pigs were purchased from reputable local sources for use as tissue donors for in vitro studies. Pigs were euthanized by an overdose of pentobarbital sodium in accordance with protocols approved by the Kansas State University Institutional Animal Care and Use Committee. Female Sprague-Dawley rats were anesthetized and then euthanized by cervical dislocation. The spiral colon was removed from each pig, linearized, and flushed with ice-cold Ringer’s solution of the following composition (in mM): 120 NaCl, 25 NaHCO3, 1.2 MgCl2, 1.2 CaCl2, 3.3 KH2PO4, and 0.8 K2HPO4. Indomethacin (50 μM; Sigma Chemical, St. Louis, MO) was included in all solutions to preclude prostaglandin synthesis. Distal rat colon was prepared by the same method. In all cases, the colon was split along the mesenteric margin and the muscularis was carefully separated from the epithelial mucosa.

Apparatus. Mucosal epithelium was mounted in modified Ussing chambers (model DCV9, Naviyete, San Diego, CA) with 0.64 cm2 of exposed surface area. Mucosal and serosal compartments contained 5 ml of Ringer solution with 10 mM mannitol and glucose, respectively. Chambers were maintained at 39°C and continually mixed with a bubble lift system (95% O2-5% CO2) that maintained pH at 7.4. Tissues were clamped to zero transepithelial voltage (model 558C, Department of Bioengineering, University of Iowa, Iowa City, IA), and the short-circuit current (Isc), which represents the algebraic sum of active ion-transport processes of the tissues, was recorded. Electrical conductance was determined using Ohm’s law by exposing the tissues to a 1-mV bipolar pulse (5-s duration) at 100-s intervals and recording the current deflections. Isc was digitally acquired using an MP100A-CE interface and AcqKnowledge software (version 3.2.6; BIOPAC Systems, Santa Barbara, CA) on a Macintosh computer (Apple Computer, Cupertino, CA).

General protocol. After mounting, tissues were allowed to acclimate for 5–10 min before the transepithelial voltage was clamped. Once a stable baseline was observed, TTX (1 μM; Sigma Chemical) was added to the mucosal chambers to eliminate the residual activity of any nerves remaining associated with the mucosa. Amiloride (10 μM; Sigma Chemical) was then added to the apical compartment to reduce variation between tissues caused by electrogenic Na+ absorption. Tissues were then exposed to either STa (200 ng/ml; Sigma Chemical) or LT (2.5 μg/ml; Sigma Chemical) via the apical solution and allowed adequate time to develop a secretory response. For LT, tissues from 10 pigs were used with the period of exposure ranging from 99 to 259 min (186 ± 16 min) before the addition of putative antagonists. The response to STa was much more rapid, reaching a stable plateau in <20 min. Forskolin, vasoactive intestinal polypeptide (VIP), and serotonin were used as stimulators of Isc in a limited number of experiments. Various selective pharmacological agents were then used to identify cellular components responsible for anion secretion.

Chemicals. 4,4’-Diaminostilbene-2,2’-disulfonic acid (DNDS) was purchased from Acros Organics (Fairlawn, NJ). Forskolin (Coleus forskohlii) was purchased from Calbiochem (La Jolla, CA). E. coli STa and LT, TTX, cadmium chloride, bumetanide, and carbamylcholine were purchased from Sigma Chemical. The DASUs were generously provided by Lilly Research Laboratories (Indianapolis, IN) or were synthesized de novo. All other chemicals were reagent or USP grade. Stock solutions were prepared as follows. Indomethacin (50 mM) and forskolin (10 mM) were dissolved in ethanol; DNDS (5 mM), STa (50 μg/ml), and LT (500 μg/ml) were suspended in Ringer solution; CdCl2 (300 mM) was in water; and DASUs (300 mM) were dissolved in DMSO. For the experiments described here, amiloride, LT, STa, and CdCl2 were added only to the apical compartment, TTX and bumetanide were added only to the basolateral compartment, and all other compounds were added to both compartments.

Data analysis. Numerical results are reported as means ± SE with the tissue in a single Ussing chamber as the experimental unit with the following exceptions. Summary data for basal tissue resistance, basal Isc, and the effects of TTX and amiloride are reported using the pig as the experimental unit. Statistical analysis, including paired t-tests, was completed with Microsoft Excel (version 8.0; Microsoft, Redmond, WA). Treatment effects were considered to be statistically significant if P ≤ 0.05 for a type I error. Sigma Plot 2000 (version 6.0; SPSS, Chicago, IL) was used for graphical presentation of the data.

RESULTS

In nonstimulating (i.e., basal) conditions, porcine spiral colon epithelium exhibited an Isc of 48.8 ± 6.2 μA/cm2 (mean ± SE; 27 pigs, 110 tissues), which is consistent with either net anion secretion or cation absorption. Basal resistance in these tissues was 117 ± 8 Ω·cm2. Treatment of the tissues with TTX to inhibit the activity of any adherent enteric nerves resulted in a modest but significant reduction in Isc (−3.6 ± 0.9 μA/cm2; 24 pigs, 98 tissues). The average change in Isc caused by the addition of amiloride was −16.8 ± 5.1 μA/cm2 (n = 24 pigs, 98 tissues), indicating that a portion of the basal current could be attributed to electrogenic Na+ absorption. Three anion transport inhibitors were used throughout the study [CdCl2, DNDS, and N-(4-methylphenylsulfonfonyl)-N’-(4-trifluoromethylphenyl)urea (DASU-02)]. CdCl2 and DNDS were consistently without effect on basal Isc. DASU-02 was sometimes associated with a reduction in basal Isc (see, e.g., Fig. 4), although statistical significance was not achieved (P > 0.06) with observations on four tissues.

LT-induced secretion is selectively inhibited by DASUs. Depicted in Fig. 1 are results from experiments demonstrating that LT stimulates porcine colonic Isc in a manner consistent with anion secretion and that this secretion is selectively sensitive to anion conductance blockers. As expected, TTX and amiloride reduced Isc. LT was added to the apical compartment and, after a delay of 1 h, caused a slowly mounting increase in Isc. An increase in current to 95 μA/cm2 was observed after an additional 80 min. Anion channel blockers were then evaluated for their effects on LT-stimulated Isc. CdCl2, an inhibitor of CIC-2 channels, and DNDS, an inhibitor of outwardly rectifying and Ca2+-activated Cl− channels, were virtually without effect. Alternatively, DASU-02, a DASU that was previously reported...
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Fig. 1. Escherichia coli heat-labile enterotoxin (LT)-induced intestinal anion secretion is selectively inhibited by a diarylsulfonylurea, N-(4-methylphenylsulfonyl)-N’-(4-trifluoromethylphenyl)urea (DASU-02). A: representative current trace showing the stimulatory effect of LT on porcine spiral colon pre-treated with TTX and amiloride. Effects of various selective anion transport inhibitors (CdCl2, 4,4’-diaminostilbene-2,2’-disulfonic acid (DNDS), DASU-02, and bumetanide) are shown, with DASU-02 having the most profound inhibitory effect on short-circuit current (Isc). The structure of DASU-02 is included in Fig. 5. B: summary of the data from A and 11 additional tissues from a total of 5 pigs. *P<0.05, statistically significant difference compared with the previous condition.

(26, 27) to inhibit CFTR Cl− channels, caused an immediate and significant reduction in Isc. It should be noted that the order of addition had no impact on the outcomes; CdCl2 and DNDS were without effect and DASU-02 caused profound inhibition. Bumetanide, a loop diuretic that inhibits intestinal Cl− secretion by blocking the loading step at the basolateral membrane, was then added to determine the magnitude of anion secretion remaining. A modest, although statistically significant, inhibition was observed. Data from a total of 12 similarly treated tissues are summarized in Fig. 1B. The data show that, compared with the previous conditions, amiloride (P ≤ 0.02), LT (P ≤ 0.001), DASU-02 (P ≤ 0.005), and bumetanide (P ≤ 0.003) significantly altered Isc, whereas TTX, CdCl2, and DNDS were without effect.

Anion conductance modulators had similar effects on forskolin-stimulated Isc. Shown in Fig. 2A are results recorded in the presence of TTX and amiloride. In this tissue, forskolin caused a 118 μA/cm² increase in Isc that then modestly decreased over time. Neither the addition of CdCl2 nor DNDS caused any inflexion in the Isc recording. Alternatively, DASU-02 caused an immediate and marked decrease in Isc after which bumetanide was associated with a modest increment of inhibition. The results presented in Fig. 2A are representative of results from 11 tissues (5 pigs), which are summarized in Fig. 2B. Because the forskolin-induced increase in Isc tended to decline slowly, there was a modest but statistically insignificant reduction in Isc that occurred while CdCl2 and DNDS were being evaluated. In every case, however, dramatic inhibition of Isc accompanied exposure to DASU-02. In two additional tissues, substantial inhibition of forskolin-stimulated Isc was observed with a second recognized inhibitor of CFTR, diphenylamine-2-carboxilic acid (1 mM; data not shown).

A third agonist that putatively acts by stimulation of adenylyl cyclase, VIP, was also used as a stimulant of Isc. The effects of anion channel blockers on VIP-stimulated Isc were virtually identical to those observed in the presence of LT or forskolin. It is obvious from inspection that neither DNDS nor CdCl2 was associated with any change in Isc. However, because the VIP-induced increase in Isc tended to decline slowly, there was a statistically significant reduction in Isc that occurred while CdCl2 and DNDS were being evaluated. More importantly, DASU-02 caused rapid and nearly complete inhibition of VIP-stimulated Isc (Fig. 2).

STa-induced secretion is selectively inhibited by DASUs. Depicted in Fig. 3 are results from experiments demonstrating that, like LT, STa stimulates colonic Isc in a manner consistent with anion secretion and that this secretion is selectively sensitive to anion conductance blockers. Again, TTX and amiloride reduced basal Isc. STa was added to the apical compartment and, without delay, caused an increase in Isc. Anion channel blockers were then evaluated for their effects on STa-stimulated Isc. DNDS and CdCl2 were without effect. Alternatively, DASU-02 caused an immediate and significant reduction in Isc. It should again be noted that the order of addition had no impact on the outcomes; CdCl2 and DNDS were without effect and DASU-02 caused profound inhibition. Bumetanide again had only a modest effect on Isc after inhibition by DASU-02, indicating that little Cl− secretion remained. Data from a total of 14 tissues (7 pigs) are summarized in Fig. 3B. The data show that, compared with the previous conditions, TTX (P ≤ 0.003), amiloride (P ≤ 0.001), STa (P ≤ 0.001), and DASU-02 (P ≤ 0.001), significantly altered Isc, whereas CdCl2 and DNDS were without effect.

DASU-02 precludes enterotoxin-induced anion secretion. Experiments were conducted to test the hypothesis that DASU-02 could be effective as a prophylactic treatment for enterotoxin-induced secretion. Data presented in Fig. 4 show that DASU-02, but not DNDS and CdCl2, can reduce basal anion secretion. It should be noted, however, that such a reduction in Isc was not consistently observed. Subsequent exposure to STa re-
sulted in an increase in $I_{sc}$, but the maximal change in $I_{sc}$ in the presence of DASU-02 was $<40\%$ of that observed in control tissues ($n = 2$ pairs of tissues from 2 pigs). There was no difference in response to STa between control tissues and tissues pretreated with DNDS and CdCl$_2$ (not shown). Virtually identical results were obtained when VIP was used as the stimulant; maximal response in the presence of DASU-02 was $<20\%$ of that observed in control tissues or tissues pretreated with DNDS and CdCl$_2$ ($n = 2$ pairs of tissues from 2 pigs).

The results presented in Fig. 4 also demonstrate that inhibition of intestinal ion transport by DASU-02 is not caused by a nonselective, toxic effect of the compound. In the presence of DASU-02, colonic epithelial cells retain their ability respond to receptor-mediated stimuli from both the apical (STa) and basolateral (VIP) membranes, albeit at a much reduced magnitude. Further evidence of this conclusion is provided by the observation that the mucosa displays a prototypical response to carbamylcholine that is characterized by a transient increase in $I_{sc}$.

Structure dependence of DASU inhibition. Data presented in Fig. 5 demonstrate that inhibition of enterotoxin-stimulated ion transport by DASUs is structure dependent. All tissues were pretreated with TTX and amiloride before stimulation with either LT (Fig. 5, A, C, E, and G) or STa (Fig. 5, B, D, F, and H). Data from three to five similar experiments for each set of conditions are summarized for LT- and STa-stimulated tissues in Fig. 5, I and J, respectively. Concentration dependence of $I_{sc}$ inhibition for the benchmark compound DASU-02 is presented in Fig. 5, A and B. It should be noted that the secretory effects of both LT and STa are significantly inhibited by 30 $\mu$M DASU-02, the lowest concentration tested ($P \leq 0.053$ for LT; $P \leq 0.048$ for STa; $n = 5$ each); the concentration of 300 $\mu$M is maximally effective, as evidenced by the fact that increasing the concentration from 300 to 600 $\mu$M had virtually no effect. These data demonstrate a similar concentration dependence for in-
Fig. 3. E. coli heat-stable enterotoxin (STa)-induced intestinal anion secretion is selectively inhibited by a diaryl-
sulfonylurea, DASU-02. A: representative current trace showing the stimulatory effect of STa on porcine spiral colon pretreated with TTX and amiloride. Effects of various selective anion transport inhibitors (CdCl₂, DNDS, DASU-02, and bumetanide) are shown, with DASU-02 having the most profound inhibitory effect on I_{sc}. B: summary of the data in A and 13 additional tissues from a total of 7 pigs. Bumetanide was not included in the summary because it was not used in all experiments. *P ≤ 0.05, statistically significant difference compared with the previous condition.

Fig. 4. Reduction in E. coli STa-induced intestinal anion secretion by pretreatment with a diarylsulfonylurea, DASU-02. Paired tissues from porcine spiral colon were exposed to either DASU-02 (300 μM; A) or DNDS and CdCl₂ (500 and 300 μM, respectively; B). DASU-02 caused a modest decrease in basal I_{sc} that was not consistently observed. Exposure to STa resulted in a rapid and sustained response that was reduced >60% by the presence of DASU-02 compared with DNDS/CdCl₂ or untreated controls (not shown). Carbamylcholine (Carb; 100 μM) was used to further demonstrate that tissues retained responsiveness in the presence of DASU-02. Results are typical of 2 sets of paired tissues.

Inhibition of both LT- and STa-stimulated secretion, further documenting that a common target is being affected in tissues stimulated by the two enterotoxins. Typical control data are presented in Fig. 5, G and H. The solvent vehicle DMSO had no effect on enterotoxin-stimulated I_{sc}, although nearly complete inhibition of enterotoxin-stimulated secretion was achieved in these tissues by the addition of 300 μM DASU-02. LY-295501 is a structurally related compound that is reported to be oncolytic and is in Phase II clinical trials. Like DASU-02, LY-295501 reduced I_{sc} in enterotoxin-stimulated tissues, although statistically significant inhibition (P < 0.05) was observed only at the highest concentration used (Fig. 5, C and D). The maximal magnitude of inhibition was similar to that observed with DASU-02, and little inhibition was observed when tissues were exposed to DASU-02 in the presence of LY-295501. In contrast, exposure to CH₃-DASU-H, which differs from DASU-02 only by the para-substituent of the urea phenyl (H vs. CF₃), resulted in virtually no inhibition of enterotoxin-stimulated I_{sc} even at the highest concentration tested (300 μM; Fig. 5, E and F). Subsequent exposure to DASU-02 resulted in profound and immediate reversal of the enterotoxin-stimulated increase in I_{sc}, ruling out the possibility that the tissues had become unresponsive to DASUs. The rank order of potency (DASU-02 > LY-295501 > CH₃-DASU-H) and the relative proportion of inhibition were identical, regardless of whether the stimulant was LT or STa. DASU-02 was shown to be the most potent inhibitor of LT- and STa-stimulated colonic secretion, with effects consistently observed at the lowest concentration tested.

Structure-dependent inhibition of rat colonic secretion. Results from experiments using rat colonic epithelium are presented in Fig. 6. It should be noted that these results closely parallel the observations presented in Fig. 5. A total of 10 closely related DASU-based structures were evaluated, and results from the three most instructive compounds are presented here. In the presence of indomethacin, TTX, and amiloride, basal I_{sc} was 50 ± 7 μA/cm² (n = 32 tissues). Forskolin caused an increase in
Isc of 192 ± 10 μA/cm² that was subsequently reversed by selected DASUs. Once again, DASU-02 was the most potent of the compounds tested, with inhibitory effects observed at the lowest concentration tested, 10 μM. More than 80% of the forskolin-stimulated increment in Isc was inhibited by 100 μM DASU-02. Inhibitory effects were likewise observed when LY-295501 was used as the antagonist. Modest inhibition was observed in the presence of 30 μM, whereas exposure to 100 μM yielded significant reduction in Isc (P ≤ 0.001). Neither CH3-DASU-H nor the carrier vehicle (DMSO) had any observable effect on forskolin-stimulated ion secretion in rat colon. Additional experiments indicated that LY-295501 had no effect on basal Isc but reduced the subsequent effects of VIP, serotonin, and forskolin (data not shown). These results further document that DASUs are effective inhibitors of intestinal secretion across a broad spectrum of stimulants and across species.

**DISCUSSION**

The results of this study have two major implications. First, a lead compound for the symptomatic treatment of secretory diarrhea is identified. Second, the results confirm previous indications that STα and LT affect a common final pathway of electrolyte secretion, CFTR.
Enterotoxigenic diarrhea depends on the activity of endogenous host mechanisms, which culminates in activation of an apical anion conductance. Thus interruption of the host response would limit fluid loss resultant from colonization and toxin production. Any required epithelial component would be a logical target for intervention. Zhang et al. (38) reported that inhibition of the guanylyl cyclase cascade precluded the effect of STa on intestinal epithelial cells. Such an approach provides proof of the concept, but specificity might be an issue in a clinical setting; either multiple cascades must be blocked or a common element in all cascades must be identified. Alternatively, it has been suggested (7, 21) that blockage of apical anion channels would be a logical therapeutic target. In support of this proposal are numerous studies (6, 11, 30) that showed that CFTR−/− mice were insensitive to enterotoxins, indicating that a CFTR channel blocker would render the gut resistant to enterotoxin stimulation. Such an approach was previously taken by Forsyth and Gabriel (10), although the compounds available at the time of their studies did not prove to be effective when used in vivo.

Results presented in this study suggest that a newly identified family of compounds, DASUs, could prove to be effective in the treatment of enterotoxigenic diarrhea. The results demonstrate a similar pharmacological profile of ion-transport inhibition for LT, CT, forskolin, VIP, and STa. The possibility that receptor antagonism, G protein inhibition, or inhibition of adenyl cyclase could account for the inhibition is ruled out by the spectrum of stimulants used. It remains possible that DASUs could mediate their effects by modulation of endogenous kinases or phosphatases. However, it was previously reported (25, 28) that DASUs reversibly block CFTR Cl− channels in excised membrane patches. A reversible effect in an excised membrane patch strongly suggests a direct interaction with the ion channel rather than a regulatory protein. Thus the results presented indicate that each of these agonists stimulates a cascade that culminates in the activation of CFTR.

The possibility of other anion conductances contributing to the enterotoxin-induced secretion was evaluated with the experimental paradigms used here. CIC Cl− channels are reported to be present in a variety of epithelial cells including those of the gastrointestinal tract (17). At least some members of this gene family of Cl− channels are blocked by Cd2+ in the micromolar range (3, 9, 23). Additionally, CIC-2g channels expressed in Xenopus oocytes are reversibly inhibited by 300 μM Cd2+ (B. D. Schultz, unpublished observations). Ca2+-activated Cl− channels (CaCC) and outwardly rectifying Cl− channels (ORCC) are likewise reported to be present in a variety of epithelia, including those of the gastrointestinal tract. Although these channels are not yet fully described at the molecular level, both classes of Cl− channels are widely reported to be inhibited by disulfonic stilbenes (2, 4, 18, 22, 32, 36, 37). Thus the complete lack of inhibition by both Cd2+ and DNDS indicates that CIC, CaCC, and ORCC

![Fig. 6. Structure dependence for inhibition of forskolin-stimulated anion secretion in rat colon by diarylsulfonylureas. Representative current traces showing concentration-dependent inhibition of forskolin-stimulated Isc are shown. All tissues were isolated and evaluated in the presence of indomethacin (50 μM) and exposed to TTX and amiloride before forskolin exposure. Chemical structures of diarylsulfonylureas are as shown in Fig. 5. Records are representative of 2–5 observations for each analog.](http://ajpgi.physiology.org/)
channels do not participate in enterotoxin-stimulated anion secretion in the colon, thus eliminating them as possible targets for therapeutic intervention.

DASUs show promise in the symptomatic treatment of enterotoxigenic diarrhea across species. Because oral rehydration therapy is effective in the treatment of enterotoxigenic diarrhea, it is obvious that antibiotic intervention is not required to treat the disease. Rather, if adequate hydration can be maintained, normal physiological processes can bring about a complete cure. We (25) previously found that DASUs inhibited ion transport in epithelial cells of human colonic origin (T84 cells) and here report their effectiveness in pig and rat colon. Thus DASUs hold therapeutic promise for the treatment of secretory diarrhea across a variety of species.

Sheppard and Welsh (31) first reported that sulfonylureas could inhibit CFTR-mediated anion transport. The compounds that they (31) identified, glibenclamide and tolbutamide, were subsequently shown (24, 35) to directly modulate CFTR channel activity in excised membrane patches. However, these compounds are not viable candidates for the therapeutic treatment of diarrhea because they are widely used as antidiabetic agents and are known to induce hypoglycemia. Alternatively, DASUs do not universally cause hypoglycemia (16) and were thus investigated for effects on CFTR. Preliminary evidence has shown that this family of compounds exhibit structure-dependent inhibition of CFTR channel gating (25). The structure and concentration dependence of CFTR inhibition in excised membrane patches was similar to the inhibition of intestinal secretion demonstrated in the present study. Furthermore, the compounds that were most effective in reducing enterotoxin-stimulated anion secretion are known to have modest (DASU-02) or no (LY-295501) effect on insulin secretion or blood glucose levels (16, 25). More importantly, LY-295501 is currently in Phase II clinical trials as an oncologic, which indicates that it can be safely administered to humans. This compound can be delivered orally and is expected to exhibit a relatively long half-life of elimination based on observations in other species (8). Because CFTR exhibits little variation in structure across species, it is reasonable to predict that similar therapeutic effects would be observed in all species. Certainly, additional studies will be required to determine the in vivo efficacy. At the very least, a lead structure has been identified that is not antimicrobial and appears to be therapeutic for treatment of diarrhea in a variety of species.

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