Sulfate secretion and chloride absorption are mediated by the anion exchanger DRA (Slc26a3) in the mouse cecum

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Whittamore JM, Freel RW, Hatch M. Sulfate secretion and chloride absorption are mediated by the anion exchanger DRA (Slc26a3) in the mouse cecum. Am J Physiol Gastrointest Liver Physiol 305: G172–G184, 2013. First published May 9, 2013; doi:10.1152/ajpgi.00084.2013.—Inorganic sulfate (SO_4^{2-}) is essential for a multitude of physiological processes. The specific molecular pathway that has been identified for uptake from the small intestine is virtually unknown for the large bowel, although there is evidence for absorption involving Na^+-independent anion exchange. A leading candidate is the apical chloride/bicarbonate (Cl^-/HCO_3^-) exchanger DRA (down-regulated in adenoma; Slc26a3), primarily linked to the Cl^- transporting defect in congenital chloride diarrhea. The present study set out to characterize transepithelial 35SO_4^- fluxes across the isolated, short-circuited cecum from wild-type (WT) and knockout (KO) mice and subsequently to define the contribution of DRA. The cecum demonstrated simultaneous net SO_4^- secretion (−8.39 ± 0.88 nmol·cm^{-2}·h^{-1}) and Cl^- absorption (10.85 ± 1.41 μmol·cm^{-2}·h^{-1}). In DRA-KO mice, SO_4^- secretion was reversed to net absorption via a 60% reduction in serosal to mucosal SO_4^- flux. Similarly, net Cl^- absorption was abolished and replaced by secretion, indicating that DRA represents a major pathway for transepithelial SO_4^- secretion and Cl^- absorption. Further experiments including the application of DIDS (500 μM), bumetanide (100 μM), and substitutions of extracellular Cl^- or HCO_3^-/CO_2 helped to identify specific ion dependencies and driving forces and suggested that additional anion exchangers were operating at both apical and basolateral membranes supporting SO_4^- transport. In conclusion, DRA contributes to SO_4^- secretion via DIDS-sensitive HCO_3^-/SO_4^- exchange, in addition to being the principal DIDS-resistant Cl^-/HCO_3^- exchanger. With DRA linked to the pathogenesis of other gastrointestinal diseases extending its functional characterization offers a more complete picture of its role in the intestine.

large intestine; epithelial ion transport; Slc26a6; PAT1

A DIVERSE NUMBER OF PHYSIOLOGICAL PROCESSES require inorganic sulfate (SO_4^{2-}) where it is a critical factor for growth and development, as well as the maintenance of normal structure and function in cells and tissues throughout the body (35, 37). The kidneys are primarily responsible for maintaining levels of extracellular SO_4^{2-} (typically ≤1 mmol/l), but the majority of available SO_4^{2-} is sourced from the diet, either directly or from the sulfur-containing amino acids methionine and cysteine (35, 37). The intestine therefore contributes to the acquisition of this vital anion, placing emphasis on understanding how SO_4^- is handled, the associated transport mechanisms, and identification of the transporters involved. Overall, rates of intestinal SO_4^- absorption are relatively small, although early surveys distinguished the ileum as the primary site of active uptake. Extensive in vitro studies focused on this segment described transcellular absorption by an apical, Na^+-dependent, DIDS-insensitive entry step and basolateral exit via anion exchange (reviewed by Refs. 35, 37). More recent work has gone on to identify members of two distinct gene families, the apical Na^+-SO_4^- cotransporter NaS1 (SLC13A1) and basolateral anion exchanger SAT1 (sulfate anion transporter 1; SLC26A1), as the specific molecular pathway responsible for SO_4^- absorption by the ileum (15, 17, 36). The generation of NaS1 and SAT1 knockout (KO) mice revealed prominent hyposulfatemia and hypersulfaturia in these animals due to impaired (re)absorption from the small intestine and proximal tubule (15, 17). Even though the clinical significance of these perturbations to SO_4^- homeostasis is not fully understood, they were shown to have far-reaching consequences for a variety of physiological functions and behaviors (reviewed in Ref. 36). For the intestine in particular, the undersulfation of secreted mucins in NaS1-KO mice was found to compromise normal epithelial function by increasing permeability and enhancing susceptibility to colitis and bacterial infection (16).

Beyond the ileum, however, virtually nothing is known about epithelial SO_4^- transport by other segments, particularly the large intestine, which can accumulate SO_4^- not absorbed by the small intestine. Initial studies in humans suggested absorption from the large bowel (19), consistent with the prior discovery of SO_4^- absorption following in vivo perfusion of the colon (41). Despite this, net SO_4^- fluxes across the rabbit distal colon in vitro were not significantly different from zero (21). In the apparent absence of NaS1, the identity of SO_4^- transporters in the large intestine remain unknown, but distinct apical anion exchange pathways for SO_4^- exist in the proximal colon (57) and Caco-2 cells (1, 22). One prospective candidate that has been suggested and speculated on is the SLC26 anion exchanger, DRA (down-regulated in adenoma; SLC26A3) (1, 2, 35, 37, 38, 49). However, with the exception of SAT1, the contribution of DRA, or indeed any other members of the SLC26 family, to intestinal SO_4^- absorption has not been determined.

Originally characterized as a SO_4^- transporter (10, 12, 38, 49), DRA is targeted to the apical membrane of enterocytes from the duodenum, distal small intestine, and colon (5, 9, 27, 46, 55, 60, 61). The link between defective chloride (Cl^-) and fluid absorption by the ileum and large intestine and mutations in the DRA gene have led to its association with the disease congenital chloride diarrhea (CCD) (27, 38). More recent, functional studies have suggested that DRA is a poor transporter of SO_4^- and principally operates as a Cl^-/HCO_3^- exchanger (13, 32, 54). In fact, SO_4^- uptake by the mouse cecum, where DRA is most intensely expressed, was reported...
to be undetectable (51), casting further doubt on any contribution to intestinal SO₄²⁻ absorption. Another anion exchanger of interest is PAT1 (putative anion transporter 1, SLC26A6); like DRA it is present in the apical membrane but appears less interest is PAT1 (putative anion transporter 1, SLC26A6); like DRA it is present in the apical membrane but appears less abundant in the large intestine (62, 63). Perhaps the most versatile of the SLC26 family, PAT1 is primarily a Cl⁻/HCO₃⁻ exchanger, but it demonstrates affinity for a range of anions including SO₄²⁻ (14, 28, 54, 62, 64). In the duodenum, PAT1 can function as a SO₄²⁻/HCO₃⁻ exchanger (50, 51) and may potentially contribute to SO₄²⁻ absorption.

Compared with SO₄²⁻ uptake demonstrated by the small intestine, there is scant information available for the large intestine, where DRA is the primary Cl⁻/HCO₃⁻ exchanger. With questions over the ability of DRA to effectively transport SO₄²⁻ there is uncertainty as to what contribution (if any) DRA will make to SO₄²⁻ handling. The present study therefore set out to characterize SO₄²⁻ transport by the mouse cecum and to specifically clarify the function of DRA. Experiments were performed comparing transepithelial fluxes of ³⁵SO₄²⁻ and ³⁶Cl⁻ measured simultaneously across the cecum from wild-type (WT), DRA knockout (DRA-KO), and PAT1 knockout (PAT1-KO) mice in vitro. The cecum was chosen because it possesses some of the highest levels of functional DRA expression in the entire intestine (4, 9, 55), but the mechanism of epithelial SO₄²⁻ transport by this portion of the large intestine has not previously been evaluated.

MATERIALS AND METHODS

Experimental animals. WT, DRA-KO (Slc26a3−/−), and PAT1-KO (Slc26a6−/−) mice were obtained from breeding pairs at the University of Florida that had been bred onto a C57BL/6 background. Information on the targeting vector construction and subsequent generation of the PAT1 and DRA knockout mice have been described elsewhere (46, 63). Genotype analysis of the offspring was performed by PCR of DNA isolated from tail snips as detailed previously (46, 63). All mice were housed in the Association for Assessment and Accreditation of Laboratory Animal Care-accredited animal facility in the Biomedical Sciences Building at the University of Florida, where they were given free access to standard mouse chow (diet 20185; Harlan Teklad) and drinking water. Pedialyte was added to the drinking water (50% vol/vol) of DRA-KO mice to help offset the persistent electrolyte and volume losses associated with chronic diarrhea (60). In the following experiments both male and female mice were used, ranging in age from 2 to 5 mo with a mean body mass of 28.3 ± 1.1 g, n = 6 (PAT1-KO) and 28.3 ± 1.1 g, n = 30 (DRA-KO). Mice were rapidly euthanized by inhalation of 100% CO₂ followed by exsanguination via cardiac puncture, after which the entire large intestine was removed to ice-cold buffer. All animal experimentation was approved by the University of Florida Institutional Animal Care and Use Committee and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Transepithelial flux experiments. Unidirectional fluxes of SO₄²⁻ and Cl⁻ were measured across intact isolated segments of cecum under short-circuit conditions as detailed in previous studies (25). In brief, immediately following removal of the intestine a pair of tissues was prepared from the cecum for mounting as a flat sheet in modified low resistance, high-permeability chambers for recording of electrical and osmotic fluxes (60). Each tissue pair was placed in each chamber with the mucosal side facing the opposing chamber. The serosal side was designated as the “hot” side. At 10-min intervals for up to 70 min, the appearance of these tracers were then subsequently detected in 1-ml samples taken from the opposing “cold” side. In addition, transepithelial potential difference (mV) and short-circuit current, Iₛₑ (μA/cm²) were also recorded at each of these sampling intervals. This total measurement time was often divided into two periods, an initial control period, representing the average of the first three 10-min intervals (0–30 min), and a second period extending from 50 to 70 min. At the beginning and end of the experiment 50-μl samples from the hot side were collected to determine the specific activity of each isotope. The activity of ³⁵SO₄²⁻ and ³⁶Cl⁻ in collected samples was determined by liquid scintillation spectrophotometry (Beckman LS6500, Beckman-Coulter) at constant quench or with quench correction (in the case of experiments with DIDS). By using a series of external standards, the validity of counting dual-labeled samples was independently established, this allowed the individual activities of ³⁵S and ³⁶Cl to be calculated on the basis of their relative counting efficiencies after minimizing the overlap of their differing energy spectra. These data were then used to calculate the unidirectional (mucosal to serosal (Jₘₖ) and serosal to mucosal (Jₘₛ) fluxes of each of these ions, which, in turn, permitted the estimation and magnitude of the net flux to be determined (where Jₘₖ = Jₘₛ − Jₛₑ).

Buffer solutions and reagents. The standard bicarbonate buffer used in these experiments contained the following concentrations of ions (mM): 139.4 Na⁺, 122.2 Cl⁻, 21 HCO₃⁻, 5.4 K⁺, 2.4 HPO₄²⁻, 1.2 Ca²⁺, 1.2 Mg²⁺, 0.6 H₂PO₄⁻, 0.5 SO₄²⁻, with 10 glucose included in the serosal buffer and 10 mannitol added to the mucosal buffer. Chloride-free buffer was prepared by replacing NaCl with an equimolar amount of isethionic acid sodium salt, whereas CaCl₂ and MgCl₂ were substituted for their respective gluconate salts. For the HCO₃⁻/CO₂-free buffer, NaHCO₃ was replaced with equimolar HEPES and gassed with 100% O₂ (pH 7.4). The contribution of endogenous CO₂ production to the supply of HCO₃⁻ was limited by including the carbonic anhydrase inhibitor ethoxzolamide (Sigma, St. Louis, MO) in the HCO₃⁻/CO₂-free buffer at a final concentration of 100 μM. To inhibit spontaneous prostaglandin production during experiments all buffers contained 5 μM indomethacin (Sigma). The radioisotope ³⁵S was purchased as Na₂SO₄ (specific activity 1.49 mCi/mmol) from Perkin Elmer (Billerica, MA) and ³⁶Cl as HCl (specific activity >0.11 mCi/mmol) from Amersham Biosciences (Piscataway, NJ). Concentrated stock solutions of DIDS ( Molecular Probes, Eugene, OR) and bumetanide (Sigma) were made fresh on the day of each experiment in DMSO and applied to a final concentration of 500 and 100 μM, respectively. The concentration of DMSO presented to the tissues in experiments with DIDS never exceeded 0.25%.

Statistical analyses. The following data are presented as means ± SE. Comparisons between WT and KO mice were made by unpaired Student’s t-test. Similarly, the effects of ion substitution/replacement were directly compared with values obtained in standard bicarbonate buffer by unpaired t-test. When evaluating the effect of pharmacological treatments, a repeated-measures, one-way ANOVA was used to test for significant changes to unidirectional fluxes for each subsequent time point following the application of DIDS and bumetanide compared with the prior control period (0–30 min). Multiple, pairwise comparisons were made by using Holm-Sidak post hoc tests. For data failing to meet the assumptions of approximate normality and equality of variance, the equivalent nonparametric tests were performed. The results of all tests were accepted as significant at P < 0.05. Statistical analysis was carried out with SigmaStat v3.5 and figures were drawn in SigmaPlot v10.0.

RESULTS

Sulfate and chloride transport by the cecum. Comparing unidirectional and net fluxes of SO₄²⁻ and Cl⁻ by the cecum from WT, DRA-KO, and PAT1-KO mice revealed significant differences in the handling of these ions. Table 1 shows that

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WT mice display a net secretion of SO$_4^{2-}$ in conjunction with a simultaneous, robust net Cl$^-$/SO$_4^{2-}$ absorption. In the absence of DRA, this net SO$_4^{2-}$ secretion was eliminated entirely, through a 60% reduction in serosal-to-mucosal SO$_4^\text{2-}$ flux ($J_{\text{SO4}}$), with no change to mucosal-to-serosal SO$_4^\text{2-}$ flux ($J_{\text{SO4}}^{\text{ms}}$). Yet even more dramatic was the abolition of net Cl$^-$ absorption, which has also been recently documented in DRA-KO mice for this particular segment (4). The decrease in mucosal-to-serosal chloride flux ($J_{\text{Cl}}^{\text{ms}}$) revealed that DRA was responsible for >90% of this flux, and interestingly serosal-to-mucosal chloride flux ($J_{\text{Cl}}$) was also reduced by 63%. Accompanying these fluxes was a striking change in $I_{\text{sc}}$ from 0.00 ± 0.15 to −4.29 ± 0.26 μeq·cm$^{-2}$·h$^{-1}$ that was similar in magnitude to net Cl$^-$ secretion (−5.58 ± 0.68 μeq·cm$^{-2}$·h$^{-1}$). Given that DRA is localized exclusively to the apical membrane, these changes suggest that it is simultaneously mediating the absorption of Cl$^-$ and secretion of SO$_4^{2-}$ manifested through $J_{\text{Cl}}^{\text{ms}}$ and $J_{\text{SO4}}$, respectively. Net SO$_4^{2-}$ secretion was sustained in PAT1-KO mice, albeit at approximately half the rate recorded in WT animals owing to a significantly diminished $J_{\text{SO4}}^{\text{ms}}$. Since PAT1 is also expressed at the apical membrane (62, 63), this too supports its contribution to SO$_4^{2-}$ secretion via $J_{\text{SO4}}$, similar to the indication for DRA. On the basis of their respective contributions to net SO$_4^{2-}$ secretion shown in Table 1, PAT1 and DRA together can potentially account for almost all (96%) of the $J_{\text{SO4}}^{\text{ms}}$ across the apical membrane in WT cecum. Unlike DRA, however, the absence of PAT1 did not have any deleterious impact on Cl$^-$ transport, but it did reveal a significant reduction in tissue conductance.

Sensitivity of cecal $J_{\text{SO4}}$ and $J_{\text{Cl}}$ to inhibition by DIDS. Application of the disulfonic stilbene inhibitor DIDS (500 μM) to the mucosal bath produced dramatic changes to both unidirectional fluxes of SO$_4^{2-}$. Mucosal DIDS greatly decreased net SO$_4^{2-}$ secretion in WT mice (Fig. 1A) but had no effect on the corresponding fluxes of Cl$^-$ (Fig. 1C). For DRA-KO mice, $J_{\text{SO4}}^{\text{ms}}$ displayed near-identical sensitivity to mucosal DIDS (Fig. 1B), and, although a similar trend was apparent for $J_{\text{Cl}}^{\text{ms}}$, this was more variable than previously observed and did not attain statistical significance. For PAT1-KO cecum, $J_{\text{SO4}}^{\text{ms}}$ was also acutely inhibited by DIDS (Fig. 2A). This persistent sensitivity of $J_{\text{SO4}}^{\text{ms}}$ to mucosal DIDS supports DRA in mediating SO$_4^{2-}$ secretion across the apical membrane, but surprisingly this was not accompanied by an associated inhibition of $J_{\text{SO4}}$ (Fig. 1C), for which DRA was also primarily responsible (Table 1). No significant alterations to $I_{\text{sc}}$ or $G_{\text{i}}$ were apparent for any genotype in the presence of mucosal DIDS (Figs. 1E and 2C).

In a separate series of experiments, DIDS was applied to the serosal bath. Across the WT cecum $J_{\text{SO4}}^{\text{sm}}$ demonstrated a more sustained, albeit less rapid, inhibition by serosal DIDS than $J_{\text{SO4}}$ (Fig. 3A). In contrast, where DRA was absent, $J_{\text{SO4}}^{\text{sm}}$ also displayed a delayed response, whereas inhibition of $J_{\text{SO4}}$ was more immediate showing a progressive decline over time (Fig. 3B). Both unidirectional fluxes of Cl$^-$ were steeply inhibited in parallel following the application of serosal DIDS in WT (Fig. 3C), but not DRA-KO cecum (Fig. 3D). These findings therefore suggest that the DRA-dependent transport of Cl$^-$ and SO$_4^{2-}$ are reliant on a DIDS-sensitive basolateral transport pathway(s), despite their differential responses to mucosal DIDS. Associated with these observations, the $I_{\text{sc}}$ from WT mice rapidly changed in the presence of serosal DIDS from −0.77 ± 0.40 to −3.51 ± 0.68 μeq·cm$^{-2}$·h$^{-1}$, together with an increase in conductance. By comparison, the $I_{\text{sc}}$ from DRA-KO cecum displayed a much smaller, transient response that was followed by a gradual decline in $G_{\text{i}}$ upon the return of $I_{\text{sc}}$ to baseline (Fig. 3, E and F).

Dependence of cecal sulfate fluxes on extracellular chloride. The results so far indicate that DRA significantly contributes to net SO$_4^{2-}$ and thus net SO$_4^{2-}$ secretion while also mediating Cl$^-$ absorption. Experiments were performed in bilateral, Cl$^-$-free buffer to examine whether net SO$_4^{2-}$ secretion was Cl$^-$ (as well as DRA)-dependent (Fig. 4). Removing extracellular Cl$^-$ in WT cecum did indeed eliminate net SO$_4^{2-}$ secretion but through a 2.2-fold increase in $J_{\text{Cl}}^{\text{ms}}$ (Fig. 4A). This finding does not support the interpretation that DRA was operating as a simple apical Cl$^-/SO_4^{2-}$ exchanger since $J_{\text{Cl}}^{\text{ms}}$ should have exhibited some dependence on extracellular Cl$^-$. Repeating these experiments with DRA-KO cecum revealed that $J_{\text{Cl}}^{\text{ms}}$ increased twofold when Cl$^-$ was absent, again indicating that SO$_4^{2-}$ and Cl$^-$ may be competing for transport by a DRA-independent pathway (Fig. 4B). Removal of DRA and Cl$^-$ therefore unmasked an additional interaction between Cl$^-$ and SO$_4^{2-}$, leading to an increase in $J_{\text{SO4}}^{\text{sm}}$. The electrical characteristics of the cecum were significantly altered by the absence of Cl$^-$. The substantial $I_{\text{sc}}$ associated with DRA-KO tissues (−4.29 ± 0.26 μeq·cm$^{-2}$·h$^{-1}$) was significantly reduced following Cl$^-$ removal (−2.56 ± 0.10 μeq·cm$^{-2}$·h$^{-1}$), suggesting that part of this $I_{\text{sc}}$ may be related to the net movement of Cl$^-$ across DRA-KO cecum. Tissue conductance was also significantly reduced in the absence of extracellular Cl$^-$ for both WT and DRA-KO genotypes, which was probably due to reduced flow of the replacement isethionate ion through the paracellular pathway.

Role of extracellular bicarbonate in $J_{\text{SO4}}$ and $J_{\text{Cl}}$ by the cecum. On the basis of the findings so far, the dual function of DRA as a SO$_4^{2-}$/Cl$^-$ transporter in the cecum cannot be explained in terms of simple Cl$^-$/SO$_4^{2-}$ exchange. The bilateral
substitution of HCO$_3$/$\text{CO}_2$ therefore examined the involvement of HCO$_3$ on SO$_4^{2-}$ transport by DRA. For WT cecum, removal of HCO$_3$/$\text{CO}_2$ abolished net SO$_4^{2-}$ secretion (Fig. 5A). $J_{\text{sm}}^\text{SO}_4$ was reduced by 42%, alongside a 33% increase in $J_{\text{ms}}^\text{SO}_4$ compared with standard buffer (Fig. 5A). When this series was repeated for DRA-KO cecum neither unidirectional SO$_4^{2-}$ flux was significantly affected (Fig. 5B), and notably the magnitude of $J_{\text{sm}}^\text{SO}_4$ was almost identical to the corresponding flux measured for WT cecum under HCO$_3$/$\text{CO}_2$-free conditions (Fig. 5B). This demonstrates evidence of a role for HCO$_3$ in DRA-mediated SO$_4^{2-}$ transport. Establishing a dependence of $J_{\text{sm}}^\text{SO}_4$ on extracellular HCO$_3$ opens up the possibility that mucosal HCO$_3$ (rather than Cl$^-$) is the counter-ion for exchange with intracellular SO$_4^{2-}$ on DRA. Importantly, without extracellular HCO$_3$ there was a 42% reduction in $J_{\text{ms}}^\text{Cl}$, abolishing net Cl$^-$ absorption in WT cecum (Fig. 5C). A substantial fraction of Cl$^-$ being absorbed by DRA therefore requires HCO$_3$ and thus supports its wide characterization as a prominent Cl$^-$/HCO$_3$ exchanger. The large $I_{\text{sc}}$ characteristic of the DRA-KO cecum was still present but was 30% smaller following the omission of HCO$_3$ ($3.02 \pm 0.16 \text{ eq cm}^{-2} \text{ h}^{-1}$ compared with $4.29 \pm 0.26 \text{ eq cm}^{-2} \text{ h}^{-1}$ in standard buffer), indicating that this current was partly HCO$_3$ dependent. For WT cecum, transepithelial conductance was significantly reduced, which coincided with the observed reduction in $J_{\text{sm}}^\text{SO}_4$, yet for DRA-KO tissue $G_{\text{t}}$ was similarly affected but with no alteration to $J_{\text{ms}}^\text{SO}_4$. The decrease in passive permeability in HCO$_3$/$\text{CO}_2$-free condi-
DIDS (M) displays the responses of preceding control period (0–30 min). J sensitivity of reduction in tions was therefore unlikely to be related to the associated reduction in Jsm.

Response of sulfate and chloride to serosal bumetanide. The sensitivity of Jsm to serosal DIDS led to consideration of whether secreted SO$_4^{2-}$ was being imported into the cell through basolateral Cl$^-$/SO$_4^{2-}$ exchange, driven by the outwardly directed gradient for Cl$^-$. We hypothesized that the bumetanide-sensitive basolateral Na$^+$/K$^+$-2Cl$^-$ cotransporter, NKCC1 (Slc12a2), could supply intracellular Cl$^-$ for exchange with serosal SO$_4^{2-}$ to support Jsm. Application of 100 μM serosal bumetanide to WT cecum, however, did not influence Jsm, indicating that NKCC1 was not integral to net SO$_4^{2-}$ secretion (Fig. 6A). An additional prompt for utilizing this loop diuretic was to probe the contribution of NKCC1 to Jsm and Isc, since Jsm was linked to DRA (Table 1) and the large current exhibited by the DRA-KO cecum was associated with Cl$^-$ (Fig. 4). However, serosal bumetanide was unable to interrupt basal Jsm by WT (Fig. 6C) or DRA-KO cecum (Fig. 6D), with no dramatic effects on Isc (Fig. 6E).

DISCUSSION

The present study set out to establish the mechanisms of transepithelial SO$_4^{2-}$ and Cl$^-$ transport by the mouse cecum, with particular focus on evaluating the contribution of the Cl$^-$/HCO$_3^-$ exchanger DRA (Slc26a3). In contrast to the absorptive capacity of the adjacent ileum, we have shown that the isolated, short-circuited cecum actually supports a large net secretion of SO$_4^{2-}$. This facilitated secretory flux was almost exclusively transepithelial involving both DRA and PAT1, was DIDS sensitive, and was dependent on HCO$_3^-$ but not Cl$^-$. Importantly, these observations definitively reveal that DRA does indeed function as a SO$_4^{2-}$ transporter, but it does not contribute to absorption as previously thought. Furthermore, the simultaneous net absorption of Cl$^-$ was also entirely DRA-mediated operating via DIDS-resistant Cl$^-$/HCO$_3^-$ exchange and only displayed sensitivity to DIDS when applied at the serosal side.

Characteristics of sulfate transport by the cecum. One of the novel findings by this study was active net SO$_4^{2-}$ secretion, which is contrary to the perceived notion of absorption by the large intestine and challenges a previous report that the mouse cecum does not detectably transport SO$_4^{2-}$ (51). In the small intestine and renal proximal tubule, the secretory flux of SO$_4^{2-}$ is overwhelmed by a central, regulated (re)absorptive flux (35, 53). The Na$^+$/SO$_4^{2-}$ cotransporter NaS1 (Slc13a1) is responsible for this dominant absorption (15, 36). Although NaS1 mRNA has been detected in the cecum (at levels ~5-fold less than the ileum and proximal tubule; Ref. 8), such an emphatic Jsm flux was conspicuously absent (Table 1). Rather, both unidirectional SO$_4^{2-}$ fluxes bore the traits of an anion exchange mechanism. The apical anion exchanger largely responsible for SO$_4^{2-}$ secretion was DRA (60%), followed by PAT1 (36%) (Table 1). Together their individual contributions account for almost all (96%) of the Jsm measured across the apical membrane of the WT cecum. This indicates a minimal paracellular contribution as noted previously for rabbit ileum (53) and distal colon (21), and Caco-2 cell monolayers (22).

The lesser involvement of PAT1 in net Jsm (although offering no support to Jsc), was not surprising given that it is far less abundant in the large intestine, displaying a pattern of expression opposite that of DRA (62). Contrary to the contribution of PAT1 to SO$_4^{2-}$ secretion by the cecum, for the small intestine PAT1 performs efficient SO$_4^{2-}$ absorption via SO$_4^{2-}$/HCO$_3^-$ exchange in the upper villous of the duodenum (50, 51). In the ileum, too, PAT1 may similarly be responsible for a fraction of HCO$_3^-$ secreted by the ileum when exposed to high
and more than 3-fold in DRA-KO cecum (Fig. 4B), indicating a competitive or inhibitory interaction with Cl\(^{-}\). Previous studies have noted an inhibition of DIDS-sensitive \(\text{SO}_4^{2-}/\text{H}_2\text{SO}_4\) uptake with increasing extracellular [Cl\(^{-}\)] for Caco-2 cells (1) and brush-border membrane vesicles (BBMVs) from the proximal colon (57) with the \(k_i\) reported to be 5.9 and 9.3 mM Cl\(^{-}\), respectively. One candidate is DTDST (Slc26a2), a DIDS-sensitive, \(\text{SO}_4^{2-}/\text{H}_2\text{SO}_4\)/anion exchanger (43) localized to the apical membrane of epithelial cells in the upper colonic crypts (24). When expressed in Xenopus oocytes, DTDST functions increasingly as a \(\text{SO}_4^{2-}/\text{Cl}^{-}\) exchanger at low extracellular Cl\(^{-}\) concentration ([Cl\(^{-}\)]) (39), demonstrating a \(k_i\) of 18.4 mM Cl\(^{-}\).
Fig. 4. Unidirectional and net $J^{\text{SO}_4}$ measured across isolated, short-circuited segments of cecum taken from WT (A) and DRA-KO (B) mice following bilateral replacement of chloride in the buffer. Values represent means ± SE from $n = 6$ mice for each genotype. *Statistically significant difference from the corresponding flux measured in standard buffer. For comparison, the accompanying flux data in standard buffer (solid bars) has been reproduced from Table 1. In Cl- free buffer (shaded bars), $I_s$ and $G_s$ were $-1.16 ± 0.11 \mu$A·cm$^{-2}$·h$^{-1}$ and $8.98 ± 0.23$ mS/cm$^2$ for WT mice ($n = 12$), and $-2.56 ± 0.10 \mu$A·cm$^{-2}$·h$^{-1}$ and $7.17 ± 0.37$ mS/cm$^2$ for DRA-KO mice ($n = 12$), respectively.

for $\text{SO}_4^{2-}$ uptake (26). With an average stool [Cl$^-\rangle$] of 15–20 mM in the rat cecum (55), DTDST (if present) could enhance $\text{SO}_4^{2-}$ absorption in vivo at low luminal [Cl$^-\rangle$]. Absence of the DTDST gene is embryonically lethal in mice, which has precluded specific studies in a KO model. Although a “knock-in” mutant mouse has been developed that displays impaired $\text{SO}_4^{2-}$ transport by chondrocytes (20), a contribution of DTDST to intestinal $\text{SO}_4^{2-}$ absorption, although intriguing, has not been investigated so far. The absorptive $\text{SO}_4^{2-}$ flux was also sensitive to serosal DIDS, and this was particularly apparent in the absence of DRA (Fig. 3). The basolateral exit step completing transcellular $\text{SO}_4^{2-}$ absorption by the ileum has been identified as SAT1, which is also present in the cecum and proximal colon (17). Interestingly, the cecal content of SAT1-KO mice contained 50% more $\text{SO}_4^{2-}$ than WT counterparts (17); however, this might well be attributed to impaired absorption from the ileum, rather than the cecum. Although $\text{SO}_4^{2-}$ transport by SAT1 demonstrates a requirement for extracellular Cl$^-\rangle$ when expressed in oocytes (34, 43), as well as HCO$_3$- (34), here in native tissue $J^{\text{SO}_4}$ was significantly enhanced in Cl$^-\rangle$-free (Fig. 4), and to a lesser extent in HCO$_3$-free (Fig. 5) buffers. There

Fig. 5. Unidirectional and net $J^{\text{SO}_4}$ (A and B) and $J^{\text{Cl}}$ (C and D) measured simultaneously across isolated, short-circuited segments of cecum taken from WT and DRA-KO mice following bilateral replacement of HCO$_3$/CO$_2$- in the buffer. Values represent means ± SE from $n = 6$ mice for each genotype. *Statistically significant difference from the corresponding flux measured in standard buffer. For comparison, the accompanying flux data in standard buffer (solid bars) has been reproduced from Table 1. In HCO$_3$/CO$_2$-free buffer (shaded bars), $I_s$ and $G_s$ were $-1.09 ± 0.08 \mu$A·cm$^{-2}$·h$^{-1}$ and $14.90 ± 0.47$ mS/cm$^2$ for WT mice ($n = 12$), and $-3.02 ± 0.16 \mu$A·cm$^{-2}$·h$^{-1}$ and $12.08 ± 0.53$ mS/cm$^2$ for DRA-KO mice ($n = 12$), respectively.
could also be contributions from other additional anion ex-
changers on the basolateral membrane explaining the sensitiv-
ity of $J_{SO4}$ (and $J_{Cl}$) to serosal DIDS (Fig. 3). Potential
candidates might be AE2 (SLC4A2) and AE3 (SLC4A3),
which are localized exclusively to the basolateral membrane
and expressed throughout the large intestine (2, 3), functioning
as DIDS-sensitive Cl$^-$/HCO$_3$- exchangers (6).

DIDS sensitivity of DRA-mediated sulfate and chloride transport. Another striking feature of these results was the
steep inhibition of $J_{sm}SO4$ (Fig. 1A), but the complete resistance of
$J_{ms}Cl$ to mucosal DIDS (Fig. 1C), despite DRA being primarily
responsible for both. Of note, this contradiction could not be
accounted for by the sensitivity of PAT1 to DIDS since $J_{sm}SO4$
(and not $J_{ms}Cl$) was also inhibited to the same degree in
PAT1-KO cecum (Fig. 2). DIDS has long been used as an
effective inhibitor for targeting members of the SLC26 and
SLC4 transporter families, although some studies have re-
ported variable sensitivity of DRA to this drug (7, 13, 54).

Without the benefit of comparison to KO tissues the responses
of the WT cecum alone would have supported the proposal of
$J_{ms}Cl$ and $J_{sm}SO4$ being mediated by two different transporters.
Indeed, differential sensitivity to DIDS has been cited as one of
the criteria supporting the residence of two or more distinct
anion exchange mechanisms in the same membrane (1, 7, 22,
26, 32).
One conceivable explanation might be the nature of the DIDS interaction with DRA and the relative abundance of its transported substrates, Cl\(^-\) and SO\(_4\)\(^2-\). DIDS is a competitive inhibitor and kinetic studies with the Band 3 anion exchanger, AE1 (SLC4A1), have suggested that Cl\(^-\) and DIDS do not bind to the same transport site. Rather the competitive interaction is based on an allosteric modulation of the DIDS affinity for the transporter that is inversely related to [Cl\(^-\)] (42). Interestingly, studies utilizing heterologous expression systems in which assays of DRA activity were based on SO\(_4\)\(^2-\) uptake all reported consistent high sensitivity up to 1 mM DIDS (10, 12, 38, 49). Comparable studies relying on 36Cl efflux found that 0.5 mM DIDS was only a weak inhibitor of DRA in media containing >100 mM Cl\(^-\) (13, 54), whereas 36Cl uptake by DRA from buffer containing just 1 mM Cl\(^-\) was almost 100% inhibited by 1 mM DIDS (38). This distinction is not entirely clear since 36Cl uptake into HEK (human embryonic kidney) cells transfected with DRA were insensitive to 1 mM DIDS in Cl\(^-\)-free buffer (7). Despite this, we suggest the observed differential sensitivity of DRA-mediated Cl\(^-\) absorption and SO\(_4\)\(^2-\) secretion to 0.5 mM DIDS may be a consequence of the extracellular concentrations of Cl\(^-\) (122 mM) and SO\(_4\)\(^2-\) (0.5 mM).

**Mechanism of sulfate secretion by DRA.** With DRA mediating simultaneous Cl\(^-\) absorption and SO\(_4\)\(^2-\) secretion, a simple explanation of the transport mechanism would be Cl\(^-\)/HCO\(_3\)\(^-\)/SO\(_4\)\(^2-\) exchange. However, substitution of Cl\(^-\) revealed that the DRA-mediated fraction of J\(_{\text{smSO}_4}\) was not Cl\(^-\) dependent (Fig. 4), counting against Cl\(^-\)/SO\(_4\)\(^2-\) exchange. Thermodynamic considerations also indicated this was unlikely since the driving force for Cl\(^-\)/SO\(_4\)\(^2-\) exchange by DRA would be intracellular SO\(_4\)\(^2-\) (Table 2). The sustained J\(_{\text{smSO}_4}\) in WT cecum in the absence of Cl\(^-\) (Fig. 4A) could therefore be the result of HCO\(_3\)/SO\(_4\)\(^2-\) exchange. Indeed, removal of HCO\(_3\)/CO\(_2\) produced a significant reduction in J\(_{\text{smSO}_4}\), an effect that disappeared in DRA-KO cecum (Fig. 5). Also, the availability of mucosal Cl\(^-\) in these experiments could not substitute for the absence of HCO\(_3\) to maintain J\(_{\text{smSO}_4}\) by Cl\(^-\)/SO\(_4\)\(^2-\) exchange. This finding may also explain why exchange of SO\(_4\)\(^2-\) with Cl\(^-\) was found to be either very low or undetectable by DRA-expressing oocytes in the absence of HCO\(_3\)/CO\(_2\) (13, 38, 49, 54).

The model presented in Fig. 7 explains the pathways for DRA-mediated SO\(_4\)\(^2-\) and Cl\(^-\) transport. We propose that DRA contributes to SO\(_4\)\(^2-\) secretion via a distinct HCO\(_3\)\(_{in}\)/SO\(_4\)\(_{out}\) mode in the mouse cecum (Fig. 7A), in addition to operating concurrently as the primary Cl\(^-\)/HCO\(_3\)\(^-\) exchanger (Fig. 7B). This is contrary to reports that both human and mouse DRA do not efficiently exchange HCO\(_3\) and SO\(_4\)\(^2-\) (32, 51). However, these studies were only set up to detect HCO\(_3\) efflux in exchange for extracellular SO\(_4\)\(^2-\), thus explaining this inconsistency. At least two separate apical anion exchange mechanisms for Cl\(^-\) and SO\(_4\)\(^2-\) transport have been described for Caco-2 cells (1, 22), as well as BBMVs from human proximal colon (57), rabbit ileum (31, 44), and isolated sheets of mouse ileum (58). None of these studies suggested these actions were performed by the same carrier. But the likelihood that DRA was operating as a Cl\(^-\)/HCO\(_3\) exchange partner to a separate HCO\(_3\)/SO\(_4\)\(^2-\) exchanger can also be ruled out. If this were the case then only J\(_{\text{smCl}}\) and not J\(_{\text{smSO}_4}\) would have been affected in DRA-KO cecum (Table 1). Interestingly, both apical Cl\(^-\)/HCO\(_3\) and SO\(_4\)\(^2-\)/HCO\(_3\) exchange has been ascribed to PAT1 residing in the upper villus of the mouse duodenum (50, 51). A somewhat similar localization of DRA-mediated Cl\(^-\) absorption and SO\(_4\)\(^2-\) secretion may be present along the surface-crypt axis of the cecum, with DRA expression in surface cells and the upper third of crypts (55). With the proposal that DRA is exclusively transporting SO\(_4\)\(^2-\) separate to Cl\(^-\)/HCO\(_3\) exchange, such an intriguing possibility could contribute to the differential sensitivities of J\(_{\text{smCl}}\) and J\(_{\text{smSO}_4}\) to mucosal DIDS discussed earlier.

In support of SO\(_4\)\(^2-\) secretion, the DIDS-sensitive basolateral pathway responsible for bringing serosal SO\(_4\)\(^2-\) into the cell could be exchanged with intracellular Cl\(^-\) or HCO\(_3\) driven by their respective outward gradients (Fig. 7A). Contrary to the mouse cecum, appreciable net SO\(_4\)\(^2-\) secretion by the rabbit distal colon could only be elicited via stimulation of cAMP (21). Serosal SO\(_4\)\(^2-\) was supplied to the cell by a distinct DIDS-inhibitable basolateral pathway that was coupled to bumetanide-sensitive NKCC1, suggesting exchange with intracellular Cl\(^-\) (21). If Cl\(^-\) were the exchange partner driving SO\(_4\)\(^2-\) into the cell across the basolateral membrane, this was not being supplied by NKCC1, given the failure of J\(_{\text{smSO}_4}\) to respond to serosal bumetanide (Fig. 6A). This is consistent with the lack of effect of another loop diuretic, furosemide, on SO\(_4\)\(^2-\) transport across basolateral membrane vesicles from rabbit ileum (33). An alternate pathway for Cl\(^-\) uptake in (cAMP-stimulated) mouse duodenum and colon takes place via basolateral Cl\(^-\)/HCO\(_3\) exchange, coupled to Na\(^+\)/HCO\(_3\) cotransport (47, 59). Support for this mechanism shown in Fig. 7A was revealed by the effects of serosal DIDS, which would have targeted these basolateral HCO\(_3\) transporters, and as such could explain the significant reduction of basal J\(_{\text{smSO}_4}\) (Fig. 3A) secondary to a steep inhibition of J\(_{\text{smCl}}\) (Fig. 3C).

The role of DRA in chloride transport by the cecum. The present study has shown that the Cl\(^-\)/HCO\(_3\) exchanger, DRA

### Table 2. To evaluate the thermodynamic constraints on potential DRA-mediated anion exchange mechanisms, δμ was calculated across the apical cell membrane

<table>
<thead>
<tr>
<th>Ion</th>
<th>[Mucosal], mmol/l</th>
<th>[Cytoplasm], mmol/l</th>
<th>δμ, kJ/mol</th>
<th>Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+)</td>
<td>139.4</td>
<td>15.0</td>
<td>−11.54</td>
<td>m−∞</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>122.2</td>
<td>20.0</td>
<td>1.12</td>
<td>c−m</td>
</tr>
<tr>
<td>HCO(_3)</td>
<td>21.0</td>
<td>8.0</td>
<td>3.30</td>
<td>c−m</td>
</tr>
<tr>
<td>SO(_4)(^2-)</td>
<td>0.5</td>
<td>0.2(^+)</td>
<td>9.22</td>
<td>c−m</td>
</tr>
</tbody>
</table>

Calculations were based on standard buffer in the mucosal bath. The direction of the driving force acting upon each respective ion is indicated (m = mucosal; c = cytoplasm). The electrochemical potential (δμ) for Na\(^+\) was included for comparison. +δμ = RT / nF(ΣC/c) (F/δFE), where C\(_2\) and C\(_1\) represent the intracellular and extracellular ion concentrations, respectively (in mmol/l). δF is the apical membrane potential difference (−60 mV). R is the universal gas constant, T is absolute temperature (K), z is valence of the ion species, and F is the Faraday constant. Published measurements of SO\(_4\)\(^2-\) within intestinal epithelial cells have reported [SO\(_4\)\(^2-\) \(_i\)] to be a linear function of [SO\(_4\)\(^2-\) \(_o\)] (45). Based on these relationships and the [SO\(_4\)\(^2-\) \(_i\)] used by the present study (0.5 mM), [SO\(_4\)\(^2-\) \(_o\)] would have been between 0.93 and 1.98 mM. At a medium concentration of 2.4 mM, [SO\(_4\)\(^2-\) \(_o\)] was determined to be 1.1 mM (53), equivalent to 0.23 mM [SO\(_4\)\(^2-\) \(_i\)], at 0.5 mM [SO\(_4\)\(^2-\) \(_i\)], if similarly linear. This most conservative estimate (0.2 mM) was therefore chosen to represent [SO\(_4\)\(^2-\) \(_i\)].
not only secretes $\text{SO}_4^{2-}$ but also confers the enormous capacity
for $\text{Cl}^-$ absorption by the mouse cecum (Table 1). This net flux
of $\text{Cl}^-$ was dependent on a supply of extracellular $\text{HCO}_3^-$ (Fig. 5),
emphasizing DRA-mediated $\text{Cl}^-$ absorption via apical
$\text{Cl}^-/\text{HCO}_3^-$ exchange, and likewise its important contribution
to $\text{HCO}_3^-$ secretion (52, 61), where intracellular $\text{HCO}_3^-$
($\text{HCO}_3^-$) will be the predicted driving force (Table 2). With
inclusion of a carbonic anhydrase inhibitor in the $\text{HCO}_3^-/\text{CO}_2$
free buffer, the precise source of $\text{HCO}_3^-$ could not be resolved
here. Previously, $\text{Cl}^-$-dependent $\text{HCO}_3^-$ secretion by the
mouse cecum was shown to be exclusively reliant on serosal
$\text{HCO}_3^-$ and unresponsive to carbonic anhydrase inhibition (30),
indicating a limited role for intracellular $\text{CO}_2$ hydration in
supplying $\text{HCO}_3^-$. At the basolateral membrane, $\text{Na}^+-\text{HCO}_3^-$
cotransport (NBC) is recognized as transporting $\text{HCO}_3^-$ into
intestinal epithelial cells in support of $\text{HCO}_3^-$ secretion (6),
and when coupled to basolateral $\text{Cl}^-/\text{HCO}_3^-$ exchange it can also
support net $\text{Cl}^-$ secretion (47, 59). Here, $\text{HCO}_3^-$ import via
NBC is proposed to contribute to net $\text{Cl}^-$ absorption (Fig. 7B),
as well as net $\text{SO}_4^{2-}$ secretion (Fig. 7A) by the cecum. To
complete $\text{Cl}^-$ absorption, $\text{K}^+-\text{Cl}^-$ cotransport, $\text{Cl}^-$ conductance,
and $\text{Cl}^-/\text{HCO}_3^-$ exchange all represent potential pathways
for basolateral exit (6). Application of serosal DIDS
sharply diminished both unidirectional $\text{Cl}^-$ fluxes (Fig. 3C),
pointing toward $\text{Na}^+-\text{HCO}_3^-$ cotransport and $\text{Cl}^-/\text{HCO}_3^-$
exchange, the prominent stilbene-sensitive elements on the basolateral
membrane, having key roles in cecal $\text{Cl}^-$ transport (Fig. 7).

The absence of DRA impacted net $\text{Cl}^-$ absorption not only
via a reduction in $J_{\text{m}}$, but also $J_{\text{sm}}$ (Table 1), reflecting a
simultaneous involvement of DRA in both absorptive and
secretory $\text{Cl}^-$ pathways. Since $J_{\text{sm}}$ was unaffected by the
absence of $\text{HCO}_3^-/\text{CO}_2$ in both WT and DRA-KO cecum (Fig.
5) this reduced the likelihood that $\text{Cl}^-$ was exiting across the
apical membrane via DRA directly (i.e., in exchange for
$\text{HCO}_3^-$). An alternate apical exit pathway for $\text{Cl}^-$ could be via
a conductive channel such as the cystic fibrosis transmembrane
conductance regulator (CFTR). The apparent involvement of
DRA in $J_{\text{im}}$, revealed here may be representative of its func-

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Fig. 7. A simple model illustrating the proposed
mechanisms of transepithelial sulfate ($\text{SO}_4^{2-}$) and
chloride ($\text{Cl}^-$) secretion (A) and absorption (B) by
the mouse cecum. 1: For secretion, $\text{SO}_4^{2-}$ from
the serosal bath is accumulated in the cell via basolat-
eral DIDS-sensitive anion exchange driven by ei-
er intracellular $\text{Cl}^-$ or $\text{HCO}_3^-$ (indicated by $A^-$). At
the apical membrane, $\text{SO}_4^{2-}$ exits via DRA in
exchange for mucosal $\text{HCO}_3^-$. 2: The baseline
secretory flux of $\text{Cl}^-$ was supported by an anion
exchanger (AE) at the basolateral membrane cou-
ped to a $\text{Na}^+-\text{HCO}_3^-$ cotransporter (NBC), which
provides $\text{HCO}_3^-$ to drive $\text{Cl}^-$ into the cell. A
potential apical pathway for $\text{Cl}^-$ exit was sug-
gested to be via a conductive channel such as the
$\text{CFTR}$. 3: $\text{SO}_4^{2-}$ absorption was mediated by a
DIDS-sensitive apical mechanism independent of
either DRA or PAT1 and may reflect the activity
of DTDST. Intracellular $\text{SO}_4^{2-}$ exits via another
DIDS-sensitive mechanism at the basolateral
membrane tentatively identified as SAT1. 4: DRA
is exclusively responsible for mucosal $\text{Cl}^-$ uptake
driven by the energy in the outward $\text{HCO}_3^-$ gradi-
ent that is largely being supplied from the serosal
bath by basolateral NBC. There are a number of
pathways whereby $\text{Cl}^-$ can then enter the serosal
bath as described in the text, including DIDS-
sensitive AE.
tional association with CFTR. For example, CFTR interacts with DRA to facilitate HCO₃⁻ secretion in the duodenum (52, 61). In the cecum too, CFTR also offers a specific outward Cl⁻ conductance (4). Since Jᵦᵣᵣ was insensitive to serosal bumetanide (Fig. 6C), but strongly inhibited by serosal DIDS (Fig. 3C), this ruled out the involvement of the basolateral NKCC1 and more likely indicated participation of basolateral Cl⁻/HCO₃⁻ exchange, coupled to Na⁺-HCO₃⁻ cotransport in supplying Cl⁻ to the cell (Fig. 7A).

Iₑ in the DRA-KO cecum. The absence of DRA not only resulted in net Cl⁻ secretion but also substantially reduced Iₑ from zero to −4.29 ± 0.26 μeq·cm⁻²·h⁻¹ (Table 1), a characteristic noted previously for the intestine of DRA-KO mice but on a smaller scale of less than −2 μeq·cm⁻²·h⁻¹ (4, 60, 61). Theionic basis of this current was not formally identified by these studies. Alper and coworkers (4) raised the idea that the negative Iₑ may reflect an increased CFTR-dependent Cl⁻ secretion resulting from activation of basolateral K⁺ channels due to the higher resting pHᵣ in DRA-KO enterocytes. In agreement with this possibility, Table 1 shows that the magnitude of this current was closely matched by net Cl⁻ secretion (−5.58 ± 0.68 μmol·cm⁻²·h⁻¹). However, neither net Jᵦᵣᵣ nor Iₑ were sensitive to serosal bumetanide (Fig. 6, D and E) or DIDS (Fig. 3, D and E), and Iₑ exhibited only partial dependence on extracellular Cl⁻, counting against this current being generated exclusively by net Cl⁻ secretion.

There was also a HCO₃⁻ component to this Iₑ following bilateral substitution of HCO₃⁻/CO₂, which increased the Iₑ from −4.29 ± 0.26 to −3.02 ± 0.16 μeq·cm⁻²·h⁻¹. By comparison, reducing luminal pH and HCO₃⁻ concentration eradicated the Iₑ in the DRA-KO cecum (4). Notably, treatment of WT tissues with serosal DIDS produced an immediate reduction in Iₑ from −0.96 ± 0.46, peaking at −3.84 ± 0.70 μeq·cm⁻²·h⁻¹ (Fig. 3E). This did not appear related to Cl⁻ transport since the Iₑ did not match the timing or magnitude of the changes to the accompanying Cl⁻ fluxes (Fig. 3C). Considering that DIDS will most likely block HCO₃⁻ import to the cell (via Cl⁻/HCO₃⁻ exchange and Na⁺-HCO₃⁻ cotransport), this ΔIₑ may represent an abrupt cessation of net HCO₃⁻ secretion. For example, net HCO₃⁻ secretion by the perfused duodenum of DRA-KO mice in vivo was 33% lower than WT counterparts (52), whereas in vitro the basal Jᵦᵣᵣ decreased to 0.3 μeq·cm⁻²·h⁻¹ from 2.2 μeq·cm⁻²·h⁻¹ in WT tissues (61). For rat cecum also, the presence of 0.5 mM serosal DIDS reduced Jᵦᵣᵣ by almost 2 μeq·cm⁻²·h⁻¹ (11). However, HCO₃⁻ secretion by the WT mouse cecum in vitro was surprisingly insensitive to serosal DIDS despite a corresponding ΔIₑ of ~2.5 eq·cm⁻²·h⁻¹ (30), similar to the present study (Fig. 3E).

**Summary and perspectives.** The present study has revealed a number of novel findings regarding epithelial SO₄²⁻ transport and the involvement of the Cl⁻/HCO₃⁻ exchanger, DRA (Slc26a3). Firstly, the mouse cecum undertakes net transcellular SO₄²⁻ secretion in vitro, contrary to suggestions of absorption by the large intestine. Secondly, analysis of tissues from KO mice helped extend the functional characterization of DRA to reveal that it does indeed transport SO₄²⁻, making a substantial contribution to Jᵦᵣᵣ alongside LAT1 (Slc26a6). Thirdly, DRA was proposed to be a DIDS-sensitive SO₄²⁻/HCO₃⁻ exchanger, while operating simultaneously as the primary DIDS-resistant Cl⁻/HCO₃⁻ exchanger that was exclusively responsible for net Cl⁻ absorption.

The overall significance of net SO₄²⁻ secretion by the cecum requires further study. As a site of prominent microbial fermentation in the large intestine, SO₄²⁻ secretion would provide substrate for resident gut bacteria that require SO₄²⁻ for normal growth and metabolism (23). Intestinal secretion may also offer a pathway for excess SO₄²⁻ excretion that would support the regulatory role of the kidney in SO₄²⁻ homeostasis. For example, downregulation of DRA in pregnancy may serve to reduce intestinal SO₄²⁻ secretion and thus help maintain high levels of circulating SO₄²⁻ necessary for fetal development. During mouse gestation levels of DRA mRNA in the ileum were significantly decreased by 30–40% (18). Furthermore, since elevated thyroid hormone levels are directly correlated with serum SO₄²⁻ concentrations during pregnancy (56), this would be compatible with the concomitant decreases in DRA mRNA expression and SO₄²⁻/OH⁻ exchange observed in Caco-2 cells treated with thyroxine (1). Despite these links, mutations of the DRA gene are linked to defective Cl⁻ transport and CCD, with the phenotype exhibited by DRA-KO mice closely resembling the pathophysiology of this disease (46). The present study indicates that normal intestinal SO₄²⁻ secretion will also be affected, but there have been no reported complications arising from disturbances to SO₄²⁻ transport (38, 46).

A complete picture of the precise role for DRA in the pathogenesis of other gastrointestinal diseases has yet to emerge, thus placing emphasis on clarifying its other functions within the epithelium. For example, the diminished expression of DRA has been previously correlated with colon tumor progression (5). Subsequently, DRA was shown to be capable of suppressing tumor growth (12) and to operate as a regulator of cell proliferation in colonic crypts (46). The mechanism(s) behind these functions have not been elucidated, but altered sulfation patterns of key molecules such as glycosaminoglycans and mucins have been implicated in cancer cell biology (29, 48).

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

J.M.W., R.W.F., and M.H. conception and design of research; J.M.W. and R.H.F. performed experiments; J.M.W. analyzed data; J.M.W., R.W.F., and M.H. interpreted results of experiments; J.M.W. prepared figures; J.M.W. drafted manuscript; J.M.W., R.W.F., and M.H. edited and revised manuscript; J.M.W., R.W.F., and M.H. approved final version of manuscript.

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